Appendix I Bylaws
American College of Veterinary Botanical Medicine
Bylaws

ARTICLE I: NAME, NOT-FOR-PROFIT CORPORATION

The name of the corporation shall be the American College of Veterinary Botanical Medicine Inc., hereinafter referred to as ACVBM, or “the College”. The ACVBM has been incorporated under the laws of the State of Delaware as a not-for-profit, tax-exempt organization for the purposes set forth herein, and in the Certificate of Incorporation. The corporation has no members.

ARTICLE II STATEMENT OF OBJECTIVES AND LIMITATIONS

Section A: Mission Statement

The mission of the American College of Veterinary Botanical Medicine is to advance the specialty area of veterinary botanical medicine and to increase the proficiency and competence of veterinarians in the use of medicinal plants. It will do this by establishing requirements for certification, promoting professional education (both undergraduate and postgraduate), promoting research and disseminating new knowledge and providing support to the profession.

Section B: General Purposes

The ACVBM has been founded as a not-for-profit, tax-exempt, voluntary professional certification board and credentialing program dedicated to professional, educational, and scientific purposes, within the meaning of Section 501(c)(6) of the U.S. Internal Revenue Code and regulations, the state of Delaware
Not-For-Profit Corporation Law, and any other applicable successor laws. The purposes and mission of the ACVBM, are subject to the limitations set forth in these Bylaws and in the Certificate of Incorporation, are the establishment, maintenance, evaluation, and administration of professional credentialing programs in the field of veterinary medicine.

Section C: Specific Purposes

Consistent with the ACVBM Certificate of Incorporation and these Bylaws, the ACVBM shall promote the advancement of veterinary practice by identifying to the profession and the public those individuals that have obtained certification in veterinary botanical medicine in one of the following categories: Western botanical medicine or Chinese botanical medicine. To accomplish such purposes the ACVBM shall be operated:

1) To establish and maintain credentialing, certification and ethical standards for veterinary practitioners who excel in botanical medicine and who shall be titled Diplomates.

2) To identify, develop, provide and maintain professional botanical programs, to include but not limited to phytochemistry, phytopharmacology, pharmacognosy, ethnopharmacology, and ethnoveterinary medicine and clinical botanical medicine.

3) To examine and certify veterinarians as specialists in veterinary botanical medicine and facilitate the continued professional development of the Members of the College through development and administration of continuing education programs.

4) To promote the improvement of professional practice standards, scientific inquiry and research into the safe and effective use of botanical medicines for prevention, treatment and control of animal diseases to promote a high quality of life for companion animals and to enhance the wellbeing and productivity of livestock and other production animals.

5) To collaborate with veterinary colleges and other educational institutions that relate to veterinary medicine to encourage and promote the development of graduate veterinary botanical medicine programs, especially in regard to residency training for clinical practice.

6) To identify Diplomates to the public, professionals, other professional organizations and government agencies and other appropriate individuals and bodies.

Section D: Limitations

The purposes and limitations of the ACVBM are restricted as follows:

1. No part of the net earnings of the ACVBM shall inure to the benefit of, or be distributed to, the Board of Directors or Officers, or other private persons, except that the ACVBM shall be authorized to pay reasonable compensation for services rendered and to make payments and distributions in furtherance of, and consistent with, the purposes set forth in these Bylaws and applicable ACVBM policies.
2. The Board of Directors is accountable for and shall have the authority and responsibility to develop, establish, approve, and enforce policies and procedures necessary to implement the goals and requirements of this Article.

ARTICLE III: BOARD OF DIRECTORS

Section A: Power, Duties and Function of the Board of Directors

1. General Authority: The ACVBM shall be governed by the Board of Directors. The Board shall have the duties, functions and power necessary to carry out the objectives and purposes of the corporation. The Board of Directors shall manage, control and supervise the business activities, property and other affairs of the ACVBM. The Board of Directors shall uphold and execute the purposes of the corporation; establish and adopt such policies, rules, and regulations for the conduct of its business, appoint and remunerate agents and employees; disburse funds of the corporation; purchase, lease, sell, transfer, and otherwise convey property; and, or any other lawful activities deemed necessary to further the purposes of the ACVBM, in accordance with the Certificate of Incorporation and these Bylaws, in their present or amended form. The Board of Directors will meet annually and will establish meeting rules, including agendas, frequency, and related procedures.

2. Appointment of Executive Director. An Executive Director may be appointed by the Board and their executive duties and responsibilities will be defined through a contractual agreement between the Board and the Executive Director. A performance review will be conducted annually by the Board.

Section B: Qualifications and Composition of Board of Directors

1. The number of Officers constituting the Board of Directors shall be not less than eight (8) voting members, including at least three (3) Board of Directors members known as Regents, and five (5) Officers. The Directors shall be elected by the Diplomates. Each Officer shall be at least eighteen (18) years of age. The members of the Board of Directors shall be the following: the officers of the College, consisting of the Chair of the Board, President, President-Elect, Vice-President, Secretary/Treasurer; and three (3) Regents, (at-large Directors).

2. All voting Board of Directors members shall maintain Diplomate certification status and shall be in good standing with the ACVBM. The Board of Directors may, at its discretion determine additional qualifications for Board of Directors members, consistent with these Bylaws.

Section C. Ex Officio Members of the Board of Directors

The Board of Directors at its discretion, may appoint an Executive Director of the corporation and an ACVBM representative to the American Board of Veterinary Specialties, who shall be ex-officio, nonvoting members of the Board of Directors. The Board of Directors may appoint other ex-officio, nonvoting members of the Board of Directors, as deemed necessary, on an annual basis.

Section D: Terms of Office
1. The Directors who are Officers of the College shall serve as Directors co-extensively with their service as Officers.

2. All voting Regents, except Officers shall be elected by the Diplomates for a term of three (3) years, and shall serve until his successor has been elected and shall have qualified. No Regent shall be eligible to serve more than two (2) consecutive terms, or six (6) years whichever is greater. At each annual election Directors shall be chosen for a full term, to succeed those whose terms expire, whichever is greater.

ARTICLE IV: MEETINGS OF THE BOARD OF DIRECTORS

Section A. Annual Meeting/Regular Meetings

1. Meetings of the Board may be held at any place within or outside the State of Delaware. Such majority shall be capable of transacting business as may be provided in these Bylaws or under applicable law. Except as otherwise indicated in these Bylaws or by applicable law, the act of a majority of the Board of Directors present at a meeting at which a quorum is present shall be the act of the Board of Directors. The time and place for regular meetings of the Board shall be determined and scheduled by the Board.

2. The Annual Meeting of the Board of Directors shall be at a time and place designated by a majority of the Board of Directors for the transaction of business that comes before the Board of Directors. There shall be at least one (1) other regular meeting of the Board of Directors each year at a place designated by the Board of Directors for the transaction of business. Agendas of all items to be discussed at regular Board of Directors meetings shall be distributed at least two weeks (14) days prior to the meeting.

3. The rules contained in the most recently revised edition of Roberts Rules of Order shall be the parliamentary authority for the conduct of all meetings of the Board of Directors, except as otherwise provided in these Bylaws.

Section B. Special Meetings

Special meetings may be called by the Chair or President, or by a majority of the Board of Directors, or upon the filing of a written special meeting notice stating the object, location, date, and hour of such meeting. Notice of any and all special meetings will be delivered by the Secretary of the College to each Director via email to each Board of Directors member at least ten (10) days prior to the date of the meeting. The Secretary must receive confirmation that the email was received by the recipient in order for the notice to be valid.

Section C. Telephone / Web Conference Meetings

1. The President or Chair may authorize a Board of Directors meeting via telephone/ web conference, or similar form of telecommunications, when deemed necessary, provided that ten (10) days notice of such conference is given to each Board of Directors member by the Secretary. The Secretary must receive confirmation that the email was received by the recipient in order for the notice to be valid.
2. Should an urgent item of business require immediate attention and action by the Board of Directors, a conference may be called without previous notice, so long as all of the Board of Directors members have been contacted and advised of such meeting and the item(s) to be reviewed or acted upon. All Board of Directors members participating in an online/telephone conference meeting must be able to hear, and communicate effectively with, each other. A two-thirds (2/3) roll call vote of the Board of Directors members in attendance will be necessary to carry out a resolution and to authorize Board of Directors action. Participation by such means shall constitute presence in person at the meeting.

Section D: Quorum and Action by the Board

a. A majority is 2/3 of those voting; 20% of the eligible number of members is required to conduct bylaws votes.

b. Any action required or permitted to be taken by the Board or any committee thereof may be taken without a meeting if all the Members of the Board of the committee consent in writing or by electronic transmission to the adoption of a resolution authorizing the action. The resolution and the written consents or electronic transmissions shall be filed with the minutes of the proceedings of the Board of committee.

Section E: Notice and Waiver

The Secretary shall give notice of all regular meetings of the Board of Directors to all Regents and Officers no less than ten days (via email) or more than sixty (60) days (US post) prior to the meeting. Any notice may be waived before or after the date and time stated in the notice. Except as provided herein, the waiver must be in writing, signed by the person entitled to the notice, and delivered to the corporation for inclusion in the minutes, or for filing with the corporate records. A Board of Directors member’s attendance at, or participation in, a meeting shall constitute waiver of any required notice to him or her unless the Board of Directors member shall, at the beginning of the meeting, object to the holding of the meeting or transaction of business at the meeting, and does not thereafter vote for, or assent to, any action taken at the meeting.

Section F. Email Votes

Should a matter arise that requires a vote of the Board of Directors between the Board of Directors meetings, a ballot by email or other appropriate means authorized by the Chair or President, may be taken. A two-thirds (2/3) affirmative vote of the entire voting membership of the Board of Directors shall be necessary to carry any motion, and all members of the Board of Directors must consent, in writing, to the adoption of a resolution authorizing the action. The signed consents, or signed copies, shall be placed in the minutes book of the Board of Directors of Regents. Voting by proxy shall not be permitted.

Section G. Actions of the Board of Directors
Every decision of the Board of Directors shall be by a majority vote, unless otherwise required by law, the policies of the Board of Directors, or these Bylaws. Each Regent and Officer shall be entitled to one (1) vote on any matter coming before the Board of Directors.

Section H. **Nomination of Board of Directors Members**

Recommendations of qualified candidates to be nominated for election to the Board of Directors and Officer positions, consistent with the terms of this Article, shall be submitted to the Chair of the Nominating Committee at least four (4) months prior to the beginning of the fiscal year. The Nominating Committee will select and declare a slate of qualified and appropriate Directors and Officer candidates at least three (3) months prior to the beginning of the fiscal year. The candidate slate will specifically identify the Diplomate(s) nominated for each Director position and the Diplomate(s) nominated for each Officer position. Consistent with rules adopted by the Board of Directors, additional Director candidates for each practice category may be nominated by petition of two (2) Diplomates in good standing and additional candidates for Officer positions may be nominated by petition of one percent (1%) of the all Diplomates in good standing.

Section H. **Election of Board of Directors Members**

All voting Board of Directors members, including Officers, shall be elected by a majority vote of the voting active Diplomates in good standing. Board of Directors members shall be elected by mail ballot, or by any other method designated by the Board of Directors, consistent with rules or procedures established by the Board of Directors. Balloting must be completed before the end of the fiscal year.

Section I. **Director Resignation/Vacancy**

A Director may resign at any time by providing written notice to the President and or the Executive Director. Such resignation shall take effect at the time specified therein, or, if no time is specified, at the time of acceptance as determined by the President or Board of Directors. Vacancies, as they occur on the Board of Directors by resignation, death, incapacity, or the like, shall be filled by appointment by the Board of Directors for the remainder of the term. As otherwise provided by these Bylaws, Officers may resign and Officer positions may be filled.

Section J. **Removal of Directors**

A Director may be removed, for cause, by a two-thirds (2/3) affirmative vote of the Board of Directors at any regular or special meeting of the Board of Directors at which a quorum of the Board of Directors is present, and under rules or procedures approved by the Board of Directors. Officers may be removed by the Board of Directors as otherwise provided by these Bylaws.

Section K. **Limitations and Conduct of the Board of Directors**

The Board of Directors shall be granted the authority to establish policies and procedures specifying Board of Directors conduct and limitations, including but not limited to the following:
1. Compensation for Services. Regents and Officers of the Board of Directors shall not receive any compensation, or other tangible or financial benefit for service on the Board of Directors. However, the Board of Directors may authorize payment by the ACVBM of actual, reasonable expenses incurred by Regents or Officers regarding attendance at Board of Directors meetings and other approved activities.

2. Compensation from ACVBM activities. Regents and Officers of the Board of Directors shall not receive any compensation, or other tangible or financial benefit from any activity of, or related to, the ACVBM, except as reimbursement for actual, reasonable expenses directly associated with such ACVBM element or activity, when authorized by the Board of Directors.

ARTICLE V: OFFICERS

Section A: Officer Titles/Authority

The Officers of the ACVBM shall consist of the Chair of the Board, President, President-Elect, Vice President, and Secretary/Treasurer. The Officers shall be bound by, and be responsible and accountable to, the ACVBM Board of Directors for satisfying resolutions and directives of the Board of Directors, and shall have the authority and accountability conferred and granted by these Bylaws and by the Board of Directors. No individual shall hold more than one elective Officer position at any one time.

Section B: Election and Terms of Officers

The initial Officers shall be elected by the incorporator. Thereafter, the Officers shall be elected by the Diplomates. The Officers shall serve in their elected position for a term of one (1) year. A slate of candidates shall be prepared by the Nominating Committee and submitted to the Diplomates and the Board at least thirty (30) days before the annual meeting. Members may submit recommendations for nominations (with nominee approval) to the Nomination Committee. The candidate receiving a plurality of the votes will be elected to the office. Officers shall take office immediately following the meeting at which they are elected. The Officers shall serve a term of one (1) year. It is expected, but not required, that continuity of administration will be maintained by the President moving up to Chair of the Board, the President-Elect to President, and the Vice-President to President-Elect.

Section C. Duties of the Officers

1. President. The President shall have the authority and responsibilities commonly incident to, and vested in, the corporate offices of Chief Executive Officer consistent with these Bylaw

2. Chair of the Board. The Chair shall preside over all meetings of the Board, call meetings of the Board, act as the spokesman for the Board, perform the usual duties of the Chair

3. President-Elect. The President-Elect shall perform such other duties as the Board of Directors of Regents or the President may designate in the absence or disability of the President, the President-Elect shall serve as acting President, shall have all authority conferred upon the office of President, and shall perform all duties for which the President is responsible for the unexpired portion of the term, or until
the President can resume duties. At the expiration of his or her term, the President-Elect shall succeed to the office of the President.

3. Vice-President. The Vice-President shall perform such other duties as the Board of Directors or the President may, from time to time, designate. In the absence or disability of the President and the President-Elect, the Vice-President shall serve as acting President, shall have all authority conferred upon the office of President, and shall perform all duties for which the President is responsible for the unexpired portion of the term, or until the President or President-Elect can resume duties. The Vice President shall succeed to the office of President-Elect should that office bevacated.

4. Secretary/Treasurer. The Secretary-Treasurer shall be the Chief Financial Officer of the corporation. The Secretary-Treasurer shall have and perform all duties commonly incident to, and vested in, the offices of secretary and treasurer of a corporation, as well as all duties delegated and designated by the Board of Directors or the President including, but not limited to: supervision, safe and secure maintenance of all corporate documents, conduct the correspondence of the College, responsibility for accurate accounting of the minutes of all meetings and the books of the corporation, administration of the fiscal and financial policies of the corporation and ensure the preparation and submission of all other documents required by state or federal authorities, and perform the usual duties of a secretary.

Section D: Officer Resignation/Vacancy

An Officer may resign at any time by providing sixty (60) days written notice to the President, or other authorized representative designated by the Board of Directors, and the Executive Director if retained. Such resignation shall take effect at the time specified therein, or, if no time is specified, at the time of acceptance as determined by the President or Board of Directors. In the event that the office of President becomes vacant, the President-Elect shall assume the office of President for the remainder of the term of office. In the event that any other Officer position becomes vacant, the President shall appoint interim officers to fill such vacant offices until a new Officer is elected by the Board of Directors to serve the unexpired portion of the term at the next scheduled Board of Directors meeting.

Section E. Removal of Officers

The Board of Directors may remove any Officer from office whenever, in its judgment, the best interests of the ACVBM will be served thereby. An Officer of the ACVBM may be removed by a two-thirds (2/3) affirmative vote of the Board of Directors at any regular or special meeting of the Board of Directors at which a quorum is present, and under rules or procedures approved by the Board of Directors.

ARTICLE VI: EXECUTIVE DIRECTOR

Section A. Appointment

The Board of Directors shall have the responsibility and authority to appoint an Executive Director of the ACVBM, who shall act as the Chief Operating Officer and Chief Staff Officer of the ACVBM. The Executive Director shall report to the Board of Directors, and shall be responsible and accountable for the
supervision, control, and management of the ACVBM in its administrative, business, financial, and other operational affairs.

Section B. Authority and Duties

1. At the discretion of the Board, an Executive Director may be retained as a contracted employee of the College. The Executive Director’s authority and responsibilities shall be defined in detail in a contract that is mutually satisfactory and agreed upon between the Executive Director and the Board of Directors.

ARTICLE VII: DIPLOMATES (Members)

Individuals certified by ACVBM shall be known as Diplomates. Individuals seeking certification must apply for membership to the ACVBM. The ACVBM will examine and certify those veterinarians that have demonstrated, by meeting established training and/or experience requirements and by attaining acceptable scores on comprehensive examinations administered by the College, their fitness and ability to practice the specialty. The benefits and procedures for acquiring each classification are governed by the policies and procedures of the Board of Directors. Active Diplomates have fulfilled the requirements set forth for certification by ACVBM and are current on all renewal fees. These members are eligible to vote and hold office.

In all of the following sections of these Bylaws, the term “Diplomate” includes both Charter Diplomate and Diplomate. Before applying for certification by the ACVBM a veterinarian must:

1. Be a graduate of a college of veterinary medicine accredited by the AVMA; or possess a certificate issued by the Commission for Foreign Veterinary Graduates (ECFVG) or are legally qualified to practice veterinary medicine in some state, province, territory, or possession of the United States, Canada, or other country.

2. Be licensed to practice veterinary medicine.

3. Meet the education, training, and experience requirements established by the ACVBM.

4. Demonstrate unquestionable moral character and ethical professional behaviour.

Section A: Classification of Diplomates

Section A: Classification of Diplomates

- There will be three (3) classes of Diplomates and shall consist of the following: Diplomates, Honorary Diplomates, and Emeritus Diplomates. The benefits and procedures for acquiring each classification are governed by the policies and procedures of the Board of Directors.

Section B: Diplomate
Diplomates have fulfilled all the general requirements set forth for certification by ACVBM and are current on all renewal fees. These members are eligible to vote and hold office.

Section C: **Honorary Members**

- Honorary member status may be conferred on a person who has contributed materially to the development of veterinary botanical medicine as to be deserving of special recognition by the College, and must be nominated for Honorary member status by at least two Diplomates.

Section D: **Emeritus Diplomate**

- Emeritus Diplomates have fulfilled the requirements for Diplomate status but are retired from active clinical practice in their specialty category.
  
  1) The Diplomate has been an active member of the ACVBM for at least 25 years or has reached the age of sixty five (65).

  2) The Diplomate has retired from employment in which their ACVBM credentials are required for employment. Income generated from activities associated with their ACVBM credentials through activities such as part-time consulting, teaching, writing, or continuing education is acceptable and will not preclude Emeritus Diplomate status. Emeritus Diplomates are able to vote and hold office, shall be required to pay a renewal fee, but will not be required to recertify.

Section F: **Disability of Diplomate**

1. A Diplomate may request permanent or temporary disabled status by submitting a request therefor to the Board, with a certification that such Diplomate is permanently or temporarily disable. Such request shall be approved if the Diplomate meets the definition of permanent or temporary disability set forth herein. A Diplomate who is approved for temporary disability status must submit an annual request to the Board to maintain such status.

2. For the purposes of these Bylaws “permanent disability” means the permanent inability to engage in veterinary activity as a full-time occupation. For the purposes of these Bylaws, “temporary disability” means the inability, due to a temporary medical disability, to currently engage in veterinary activity as a full-time occupation.

3. A Diplomate who is permanently or temporarily disabled shall have all the rights and all the obligations of Diplomates except they shall be exempt from the payment of annual dues and shall not have the right to vote or be elected as an Officer or Director. A Diplomate who is no longer disabled shall notify the Board and, as of the date of such notification and payment of a portion of the current year’s dues attributable to the remaining portion of the year, shall be restored to Diplomate status.

Section G. **Meetings of Diplomates**
Annual Meeting. The annual meeting of Diplomates for the election of Directors and officers and for such other business of the ACVBM shall meet annually at such a time and place as designated by the Board of Directors.

Section H: Quorum at Meeting of Diplomates

Diplomates entitled to cast one-third of the total number of votes shall constitute a quorum at all meetings of Diplomates for the transaction of any business.

Section I: Termination of Diplomate Status

Before the Board takes action to terminate Diplomate status, the Board shall notify the Diplomate in writing by registered mail of its intended action and the reasons therefor. The Diplomate shall be entitled to present written evidence and to appear before the Board in person, at a date, time and place mutually acceptable to the Board and Diplomate. The Board shall consider all such evidence and shall notify the Diplomate of its decision in writing. One year after termination of Diplomate status under this Section 12, a person shall be entitled to reapply for Diplomate status under Article II Section 3 of these Bylaws. The Board shall terminate a person’s status as a Diplomate in any of the following circumstances:

1. The Diplomate has violated any provision of the Certificate of Incorporation or these Bylaws, as determined by the Board. 2. The Diplomate fails to maintain an acceptable degree of competence in the practice of veterinary botanical medicine, as determined by the Board. 3. The Diplomate has brought discredit upon the ACVBM by unethical conduct, incompetence, fraud or any other reason, as determined by the Board.

Section J. Membership Dues

Annual dues shall be fixed by the Board. Annual dues for each year are due and payable by January 1st of such year. Persons who become Diplomates during a calendar year shall pay a pro rata portion of annual dues for such year, and such pro rata portion is due and payable within thirty (30) days after such person becomes a Diplomate.

ARTICLE VIII: GOVERNANCE

Section A. Autonomy

The Board of Directors shall in all respects be autonomous with respect to: ACVBM credentialing criteria and activities; administration; the conduct of meetings; policies; finances; election and appointment of Committee members and ACVBM representatives; and all other lawful activities.

Section B. Authorization to Act

Except as provided in the Certificate of Incorporation, these Bylaws, or applicable law, no Regent, Officer, employee, agent, or representative of the corporation may act on behalf of the ACVBM, or hold
himself or herself out to the public as authorized to act on behalf of the ACVBM, without the prior, express, written approval of the Board of Directors.

ARTICLE XI: COMMITTEES

Standing Committees Standing committees of the College shall consist of the Executive Committee, Nominating Committee, the Credentials and Residency Committee, the Examination Committee, the Appeals Committee, the Professional Botanical Program Committee and such other committees as may be designated by the Board. Committee members shall each serve a term of three (3) years, unless otherwise provided by the Board or these Bylaws.

Section A. Executive Committee

1. Composition. The Executive Committee shall be composed of the President, President-Elect, Vice President, Secretary-Treasurer, and Executive Director (should one be retained). All Executive Committee members shall be voting members of the Committee, with the exception of the Executive Director.

2. General Authority, Duties and Limitations. The Executive Committee may act for the Board of Directors between meetings of the Board of Directors, or as otherwise authorized by the Board of Directors. The Executive Committee shall not, however, have the power to: remove a Regent or Officer; fill vacancies in the Board of Directors or in any committee; determine and fix compensation for any individuals for serving on the Board of Directors or any committee; sell corporate assets; amend, repeal, or adopt Bylaws; or, amend or repeal any resolution of the Board of Directors. All proceedings and actions of the Executive Committee shall be recorded and reported to the Board of Directors at the next meeting of the Board of Directors.

3. Meetings of the Executive Committee. The Executive Committee shall meet at least once each calendar year, and otherwise at the direction of the President.

4. Actions by the Executive Committee. Unless contrary to applicable law or these Bylaws, the actions of the Executive Committee shall constitute the actions of the Board of Directors between meetings of the Board of Directors, unless subsequently rescinded or withdrawn by the Board of Directors.

Section B. Nominating Committee

1. Composition. The Nominating Committee shall consist of at least two members appointed by the board. All members must be in good standing with the ACVBM.

4. General Authority and Duties. The Nominating Committee shall oversee and supervise the nominating process for members of the Board of Directors, and shall establish appropriate procedures and rules for the selection and presentation of qualified candidates to active ACVBM Diplomates for election.

Section C. Credentials and Residency Committee (CRC)
1. Composition: The Credentials and Residency Committee shall be composed of at least two members. All Committee members must maintain Diplomate certification status and be in good standing with the ACVBM.

2. Appointment. The President shall appoint, with the approval of the Board of Directors, the Chair of the CRC. The Board of Directors shall appoint and replace the members of the CRC, consistent with rules or procedures established by the Board of Directors.

3. Terms of Office. CRC members shall serve for a term of three (3) years.

4. General Authority and Duties. The CRC shall be responsible for: establishing candidate credential eligibility criteria in accordance with these By Laws and overseeing establishment of Residency programs; and other related activities.

Section D. Examination Committee

1. Composition. The Examination Committee shall consist of three (3) members appointed by the Board of Directors. All Examination Committee members must maintain Diplomate certification status and be in good standing with the ACVBM.

2. Appointment. The President shall annually appoint, with the approval of the Board of Directors, the Chair of the Examination Committee.

3. Terms of Office. Examination Committee members shall serve for a term of three (3) years.

4. General Authority and Duties. The Examination Committee shall define the examinations for diplomate status in both subject matter and distribution of questions and type of exam and will write, monitor and grade the Diplomate Certification Examination. It shall make recommendations to the Board regarding competence of those candidates who have completed the Diplomate Certification Examination. Under the direction of the Board of Directors, the Examination Committee shall perform its duties and annually report its findings to the Board of Directors.

Section E. Appeals Committee

1. Composition. The Appeals Committee shall consist of between three (3) members appointed by the Board. An appointed member of the committee will serve as Chair. All Appeals Committee members must maintain Diplomate certification status and must be in good standing with the ACVBM.

2. Appointment. The committee will elect the Chair. An Appeals Committee member may not be a member of the Credentials and Residency Committee or Examination Committee. If a member has a conflict of interest in a specific appeal, the Chair of the Board shall appoint a temporary member to consider that appeal.

3. Terms of Office. Appeals Committee members shall serve for a term of three (3) years.
4. General Authority and Duties. The Appeals Committee shall consider whether correct administrative procedures have been followed in the decisions made by the Credentials Committee and Examination Committee. The Chair of this committee will call a meeting to review an appeal and notify the Chair of the Board and the Executive Director of the results. The decision of the Appeals Committee shall be final and there shall be no appeals there from.

Section F. Professional Program Committee

1. Composition. The Professional Program Committee shall consist of at least two members. All Professional Botanical Program Committee members must maintain Diplomate certification status and must be in good standing with the ACVBM.

2. Appointment. The committee will elect the Chair when there are two (2) or more equally senior members.

3. Terms of Office. Professional Botanical Program Committee members shall serve for a term of three (3) years.

4. General Authority and Duties. The Professional Botanical Program Committee shall determine the content of, and make all necessary plans and arrangements for the RVS Botanical Medicine conference.

Section H. Changes in Standing Committees and Additional Committees

The membership of the standing committees may be increased and other committees may be appointed as the need arises. The Board of Directors may authorize and supervise additional committees; from time to time to perform such functions as may be determined by the Board of Directors. The President shall annually appoint, with the approval of the Board of Directors, the Chair of all standing or special committees, sub-committees or divisions, as may be required by these Bylaws, or as may be deemed necessary.

ARTICLE XII: ADMISSION TO ACVBM SPECIALTY TRAINING PROGRAM

Section A. Eligibility for Admission

To be eligible to enrol in an approved ACVBM specialty training program, all applicants must meet the following criteria:

1. Graduate of a college of veterinary medicine (accredited/or listed by the American Veterinary Medical Association (AVMA)), or possess a certificate issued by the Commission for Foreign Veterinary Graduates (ECFVG) or are legally qualified to practice veterinary medicine in some state, province, territory, or possession of the United States, Canada, or other country.

2. Complete one year of clinical veterinary internship training, a residency in another discipline, or 5 years of active veterinary clinical practice.

3. Meet the education, training and experience requirements established by the ACVBM.
4. Demonstrate unquestionable moral character and ethical professional behavior

5. Legally able to practice at the residency training site (e.g., veterinary medical license and other state or federal requirements

ARTICLE XIII: ACVBM SPECIALTY TRAINING PROGRAMS

Terminology for Training Programs: the word resident is used when referring to veterinarians who are registered in an ACVBM Specialty approved training program. Residents include veterinarians that are registered in full-time and part-time residency training programs. A veterinarian who is applying for registration of a training program with ACVBM is called an applicant. A veterinarian that has completed an ACVBM approved residency training program and whose credentials have been approved is known as a candidate and they are then eligible to take the Diplomate Certification Examination. There will be two residency programs offered by the ACVBM: Standard and Alternate Programs, they must be approved by the Credentials and Residency Committee (CRC) in advance. Residency training programs are the foundation for training of future diplomates of the ACVBM.

Section A. Standard Residency Training Program (SR-TP)

1. The Standard Residency program will be a minimum of 3 years in duration and will be conducted at an AVMA accredited veterinary medical school.

2. Candidates must have completed an approved Residency Program and completed all other requirements including Credentials and publication, before sitting for the certification examination. The CRC and the ACVBM Executive Board approve both Residency Training Programs.

3. An annual training report is to be submitted to the Executive Secretary to monitor the progress of an ACVBM Specialty resident by the supervising Diplomate. The report serves to review the progress and training of all residents to prepare them for the credentials application process.

4. Application for program approval must be submitted no later than ninety (90) days after starting the program. The trainee must register with the Secretary no later than ninety (90) days after beginning the training program using forms available from the Secretary/Treasurer. A response to the application for an SR-TP shall be issued no later than one hundred twenty (120) days after the application is submitted. A response may be approval, disapproval, request for further information or clarification, or indication of program changes required for approval. Upon approval of an SR-TP by the Board, the program shall not be subject to any future additional requirements that may be imposed with respect to such program by the College.

Section B. Denial of Standard Residency Training Program

1. An applicant who’s SR-TP was denied may appeal this decision within thirty (30) calendar days of the postmarked date of the notification. The appeal must be made in writing to the Secretary/Treasurer and shall include a statement of the grounds for reconsideration and appropriate documentation.
2. Upon receipt of an appeal, the Secretary/Treasurer shall notify the Chair of the Board, the Chair of the Training Program Evaluation Committee, and the Chair of the Appeals Committee. The Chair of the Board shall submit to the Appeals Committee documentation indicating the reasons for denial of the SRTP, including, but not limited to, the complete application package of the institution and all available documentation pertaining to the Training Program Evaluation Committee’s review of the application and recommendations to the Board.

3. The Appeals Committee shall review the appeal and provide a recommendation to the Board no later than thirty (30) calendar days after receiving all the documentation from the Chair of the Board.

4. The Board shall render a decision on the appeal after receiving the recommendation of the Appeals Committee and shall notify the appellant of the decision no later than fifteen (15) calendar days after receipt of the recommendation of the Appeals Committee.

Section C. **Alternate Residency Training Program (AR-TP)**

1. The Alternate Residency Program is proposed by the Resident and must meet the same requirements of a Standard Residency but part or the entire program is outside of an AVMA accredited veterinary medical school. The Alternate Program can be up to 6 years in duration.

2. Candidates must have completed an approved Residency Program and completed all other requirements including Credentials and publication, before sitting for the certification examination. The CRC and the ACVBM Executive Board approve both Residency Training Programs.

3. Applicants must complete a minimum of three (3) years of training in an AR-TP, reviewed and recommended by the Training Program Evaluation Committee and Credentials Committee and approved by the Board as equivalent in training and experience to a Standard Residency Training Program. The program will include clinical, teaching and research activities, and at least twelve (12) months of on-clinic time in veterinary botanical medicine under the supervision of a Diplomate of the College, submission of a ACVBM case log, standards to be determined by the Credentials Committee, that was maintained throughout the residency program and one (1) peer reviewed publication.

3. An annual training report is to be submitted to the Executive Secretary by the supervising Diplomate to monitor the progress of an ACVBM Specialty resident. The report serves to review the progress and training of all residents to prepare them for the credentials application process. Maximum time permitted to complete an AR-TP is 6 years.

4. Application for program approval must be submitted no later than ninety (90) days after starting the program using forms available from the Secretary/Treasurer. A response to the application for an AR-TP shall be issued no later than one hundred twenty (120) days after the application is submitted. A response may be approval, disapproval, request for further information or clarification, or indication of program changes required for approval. Upon approval of an AR-TP by the Board, the program shall not be subject to any future additional requirements that may be imposed with respect to such program by the College.
Section D. Denial of Alternate Residency Training Program.

1. An applicant who’s AR-TP was denied, may appeal this decision within thirty (30) calendar days of the postmarked date of the notification. The appeal must be made in writing to the Secretary/Treasurer and shall include a statement of the grounds for reconsideration and appropriate documentation.

2. Upon receipt of an appeal, the Secretary/Treasurer shall notify the Chair of the Board, the Chair of the Training Program Evaluation Committee, and the Appeals Committee. The Chair of the Board shall submit to the Appeals Committee documentation indicating the reasons for denial of the AR-TP, including, but not limited to, the complete application package of the applicant and all available documentation pertaining to the Training Program Evaluation Committee's review of the application and recommendations to the Board, except that such documentation shall be redacted to preserve anonymity of the appellant.

3. The Appeals Committee shall review the appeal and provide a recommendation to the Board no later than thirty (30) calendar days after being appointed and receiving necessary documentation.

4. The Board shall render a decision on the appeal after receiving the recommendation of the Appeals Committee and shall notify the appellant of the decision no later than fifteen (15) calendar days after receipt of the recommendation of the Appeals Committee.

ARTICLE XIV: DIPLOMATE EXAMINATION AND CERTIFICATION

Section A. Diplomate Certification Examination (DCE) –

The DCE is based on a job/task analysis

1. The DCE will be offered only once per year at a time and place designated by the Board and as determined by the Examination Committee and in accordance with these Bylaws. The candidate must pass all sections of the examination no later than six (6) consecutive years after his or her eligibility to sit for the examination was determined.

2. To be eligible to sit for the Diplomate Certification Examination, an applicant must:

   a. Have graduated from a college or school of veterinary medicine approved by the AVMA or possess a certificate issued by the Educational Commission for Foreign Veterinary Graduates (ECFVG)

   b. Be legally qualified to practice veterinary medicine in any state or country

   c. Complete one year of clinical veterinary internship training, a residency in another discipline, or 5 years of active veterinary clinical practice

   d. Have met the education, training, and experience requirements established by the ACVBM

   e. Have demonstrated unquestionable moral character and ethical professional behavior
f. Have completed a recognized certificate program: Standard or Alternate.

g. Submit to the Credentials Committee one (1) publication that has been published in the peer reviewed literature within the past 3 years in which botanical medicine was the primary medical treatment used. Each publication shall pass acceptability criteria as determined by the Credentials Committee.

h. Submit three (3) recommendation letters: one from an active diplomate of ACVBM and two from other ABVS specialists

i. Meet all other residency requirements.

J. Submit required fees and application

3. Applicants deemed eligible by the Board to sit for the DCE shall be notified of the date and format of the examination no later than one hundred twenty (120) calendar days before the date of the examination.

4. The certifying examination for the ACVBM Specialty will test whether the candidate can perform at the level expected of an entry-level specialist in veterinary botanical medicine.

5. The contents of the examination will be determined by the Examination Committee.

6. Examination Dates: Candidates taking the examination for the first time shall take all parts in the same year. Candidates for the examination shall be required to submit questions for future examinations as instructed prior to the examination.

7. Minimum score. The minimum score as determined by the Examination Committee and approved by the Board must be achieved on each section in order to pass the examination. Candidates shall be sent written notification of the results of the examination no later than thirty (30) calendar days after the examination. All candidates shall be given such notification on the same day. Certificates will be issued indicating that the individual is a diplomate of the American College of Veterinary Botanical Medicine. The certificates will not be time limited.

8. Candidate failing one section of the exam. A candidate failing one section of the exam may re-take that section at the next scheduled examination without fulfilling other prerequisites. The candidate must submit a letter of intent to the Secretary/Treasurer and pay the examination fee for only that failed section of the examination. Provided, however, that if it has been more than six (6) years since the candidate was first deemed eligible to take the examination, he or she must submit a new application with all required documentation and fees, including new case reports, to the Secretary/Treasurer for review by the Credentials Committee and approval by the Board, and if deemed eligible, must re-take the entire Diplomate Certification Examination.

9. Candidate failing to pass one or more sections. A candidate that fails any section of the examination a second time must re-take the entire examination. The candidate must submit a letter of intent, updated
curriculum vitae, and examination fee to the Secretary/Treasurer. Provided, however, that if it has been more than six (6) years since the candidate was first deemed eligible to take the examination, he or she must submit a new application with all required documentation and fees, including new case reports, to the Secretary/Treasurer, for review by the Credentials Committee and approval by the Board, and if deemed eligible, must re-take the entire Diplomate Certification Examination.

Section B. **Failure to Pass Diplomate Certification Examination**

1. Candidates failing to pass the Diplomate Certification Examination may appeal this decision within thirty (30) calendar days of the postmarked date of notification. The request for appeal must be made in writing to the Secretary/Treasurer and shall include a statement of the grounds for reconsideration and appropriate documentation. The Secretary/Treasurer shall notify the Chair of the Board, the Chair of the Examination Committee, and the Chair of the Appeals Committee.

2. The Chair of the Board shall submit to the Appeals Committee a written statement of the reasons for the failure of the candidate. The Chair of the Examination Committee shall submit to the Appeals Committee the examination and scores of the candidate, the complete list of scores of all candidates on that examination, and a statement as to the criteria used for the Committee's recommendation for success or failure, except that such documentation shall be redacted to preserve anonymity of both the appellant and the other candidates.

3. The Appeals Committee shall review the appeal and render a recommendation to the Board no later than thirty (30) calendar days after being appointed. The Board shall render a decision on the appeal upon consideration of the recommendation of the Appeals Committee and notify the appellant of the decision no later than fifteen (15) calendar days after receipt of the recommendation of the Appeals Committee.

4. If an appeal is unsuccessful and the appellant wishes to reapply to sit for the Diplomate Certification Examination, the deadline for application shall be extended three months from its designated date.

Section C. **Certification by the ACVBM**

1. Candidates achieving a passing score on all sections of the Diplomate Certification Examination. Candidates which pass all sections of the DCE shall be reviewed by the Examination Committee and a recommendation shall be made by such committee to the Board.

2. After consideration of the recommendation by the Examination Committee, the Board shall determine whether to grant Diplomate status to the candidate. Such determination shall be made by the Board no later than sixty (60) calendar days after a candidate sits for the examination.

3. Diplomate certificates shall be issued to successful candidates by the Board no later than ninety (90) calendar days after Diplomate status is granted.

Section D. **Diplomate Certification Examination Denial of Eligibility**
1. An applicant denied eligibility to sit for the Diplomate Certification Examination may appeal this decision within thirty (30) calendar days of the postmarked date of the notification. The appeal must be made in writing to the Secretary/Treasurer and shall include a statement of the grounds for reconsideration and appropriate documentation.

2. Upon receipt of an appeal, the Secretary/Treasurer shall notify the Chair of the Board, the Chair of the Credentials Committee and the Chair of the Appeals Committee. The Chair of the Board shall submit to the Appeals Committee documentation indicating the reasons for denial of eligibility to sit for the examination, including, but not limited to, the complete application package of the applicant and all available documentation pertaining to the Credential Committee's review of the application and recommendations to the Board, except that such documentation shall be redacted to preserve anonymity of the appellant.

3. The Appeals Committee shall review the appeal and provide a recommendation to the Board no later than thirty (30) calendar days after receiving all necessary documentation.

4. The Board shall render a decision on the appeal upon the recommendation of the Appeals Committee and notify the appellant of the decision no later than fifteen (15) calendar days after receipt of the recommendation of the Appeals Committee.

Section E. Maintenance of Diplomate Status

To maintain active Diplomate status, a Diplomate of the ACVBM is required to obtain 30 hours of botanical medicine continuing education (CE) credits per year.

ARTICLE XV: FISCAL YEAR and FEES

The fiscal year of the corporation shall be determined by the Board of Directors. The Board of Directors is authorized to change and fix the fiscal year as it deems appropriate from time to time. 1. All funds of the College shall be deposited from time to time in such bank or banks as the Board of Directors may select.

2. Annual dues for Diplomats are due and payable January 1 of each year.

3. Applicants shall pay the prescribed fee to the College to sit for the Diplomate Certification Examination. This fee is non-refundable and payable each time the examination is repeated.

4. The annual operating budget for the College will be prepared by the Secretary/Treasurer under the direction of the President and with the assistance of the Executive Director. The budget shall be approved by the Board of Directors.

ARTICLE XVI: INDEMNIFICATION

In accordance with and to the maximum extent permitted under the GCL, in the event any person who is or was a Regent, Officer, employee, trustee, authorized representative, or agent of the ACVBM, acting in good faith and in a manner he reasonably believed to be in the best interests of the ACVBM has been
made party, or is threatened to be made a party, to any threatened, pending or completed action or proceeding by reason of being a representative, whether civil, criminal, administrative, or investigative (other than an action or proceeding by or in the right of the corporation), such representative may be indemnified against reasonable expenses and liabilities, including attorney fees, actually and reasonably incurred, judgments, fines, and amounts paid in settlement in connection with such action or proceeding. Where the representative was successful in defending the action, indemnification is mandatory.

Section A. Determination of Proper Indemnification

Unless ordered by a court, discretionary indemnification of any representative shall be approved and granted only when consistent with the requirements of applicable law, and upon a determination that indemnification of the representative is proper in the circumstances because the representative has met the applicable standard of conduct required by law and in these Bylaws.

ARTICLE XVII: AMENDMENTS

Section A. Amendments to the Certificate of Incorporation

1. The Board of Directors shall adopt a resolution setting forth a proposed amendment to the Certificate of Incorporation and declaring its advisability. These Bylaws may amended by a vote of the Diplomates in good standing, provided that proper written notice of proposed Bylaw change(s) with recommendations by the Board of Directors is given to each eligible Diplomate at least thirty (30) days prior to the counting of the ballots.

2. A two-thirds (2/3) affirmative vote of the Diplomates voting is required in favor of such amendment and if passed a certificate thereof shall be executed, acknowledged and filed and shall become effective in accordance with the GCL.

3. Proper written notice under this Article shall be a copy of the text of the proposed amendment, including any relevant explanatory materials, whether transmitted by mail, facsimile transmission, or other appropriate means. Notice by mail shall be deemed sufficient if sent to the last Post Office address furnished to the Executive Director or Secretary-Treasurer.

ARTICLE XVIII: ADOPTION OF BYLAWS

The American College of Veterinary Botanical Medicine Inc., will organize under the laws of the incorporated State on the gate of incorporation. These Bylaws were adopted by the ACVBM Board of Directors, and became effective in 2015.

ARTICLE XIX: AMERICAN BOARD OF VETERINARY SPECIALITIES (ABVS) REPRESENTATIVE

The duties of the ABVS Representative shall be determined by the Board of Directors and shall include, but are not limited to, the following: attend all regular and special meetings of the ABVS as the official representative of the College; inform the Board and membership of all actions of the ABVS, especially
those having a direct impact on the College; assist as needed in the preparation of annual and other reports of the College to the ABVS; and provide a summary of all ABVS meetings in a timely fashion to the Secretary/Treasurer. If the ABVS Representative is unable to attend an ABVS meeting, a representative designated by the Board shall serve as the Alternate Representative to the ABVS.
Appendix II Policies and Procedures
Policies and Procedures

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1. OVERVIEW

I. Mission Statement
The mission of the American College of Veterinary Botanical Medicine is to advance the specialty area of veterinary botanical medicine and to increase the proficiency and competence of veterinarians in the use of medicinal plants. It will do this by establishing requirements for certification, promoting professional education (both undergraduate and postgraduate), promoting research and disseminating new knowledge and providing service and support to the profession.

II. Historical Perspective
The ACVBM was formally established in 2014 following an inaugural meeting regarding its development in 2004 with a sub-committee of the Veterinary Botanical Medicine Association. The Veterinary Botanical Medicine Association has been providing an annual examination and process for industry recognition of veterinary botanical knowledge and skills since 2003. The exam is independent, as VBMA itself does not offer courses or course content. The ACVBM has been incorporated under the laws of the state of Delaware as a non-profit organization. See appendix 1 (BYLAWS). The College will serve the international veterinary community and veterinarians from all countries will be invited to apply for board certification through the American College of Veterinary Botanical Medicine.

2. ORGANIZATIONAL STRUCTURE

I: General Authority Board of Directors
The Board of Directors shall govern the ACVBM. The Board shall have the duties, functions and power necessary to carry out the objectives and purposes of the corporation. The Board of Directors shall manage, control and supervise the business activities, property and other affairs of the ACVBM. The Board of Directors shall uphold and execute the purposes of the corporation; establish and adopt such policies, rules, and regulations for the conduct of its business, appoint and remunerate agents and employees; disburse funds of the corporation; purchase, lease, sell, transfer, and otherwise convey property; and, or any other lawful activities deemed necessary to further the purposes of the ACVBM, in accordance with the Certificate of Incorporation and these Bylaws, in their present or amended form. The Board of Directors will meet annually and will establish meeting rules, including agendas, frequency, and related procedures.

II. Specific Authority.
   a. The Board of Directors will also oversee publications concerning certification and will be responsible for staffing and management of resources to conduct the programs and activities of ACVBM. The Board of Directors will develop procedures for grievance, appeals, and disciplinary processes. The Board of Directors shall have the authority and control over all matters related to certification and other authorized, lawful activities. Standards for the development and administration of a program of certification of veterinary specialists will be established and overseen by the Board of Directors. The Board’s powers and duties with respect to certification of veterinary botanical specialists shall include, without limitation, the following: 1) Examination content, administration of examinations; and the establishment of cut scores and passing points. 2) Review and approval of Certification Examinations prepared by the Examination Committee, and establishment of minimum passing standards of performance for such Certification Examinations.
b. Determination of eligibility of candidates for Certification Examination, upon consideration of the recommendation of the Credentials Committee.

c. Maintain residency program

d. Review of recommendations of the Examination Committee as to the candidates who passed the Certification Examination and formal acceptance of candidates and issuance of certificates. Pass point will be determined by modified Angoff method.

e. Appointment of Executive Director. The Board may appoint an Executive Director and their executive duties and responsibilities will be defined through a contractual agreement between the Board and the Executive Director. A performance review will be conducted annually by the Board.

III. Qualifications and Composition of Board of Directors

a. The number of Officers constituting the Board of Directors shall be not less than eight (8) voting members, including at least three (3) Board of Directors members known as Regents, and five (5) Officers. Diplomates shall elect the Directors. Each Officer shall be at least eighteen (18) years of age.

b. The members of the Board of Directors shall be the following: the officers of the College, consisting of the Chair of the Board, President, President-Elect, Vice-President, Secretary/Treasurer; and three (3) Regents, (at-large Directors). All voting Board of Directors members shall maintain Diplomate certification status and shall be in good standing with the ACVBM. The Board of Directors may, at its discretion determine additional qualifications for Board of Directors members, consistent with these Bylaws.

c. All voting Board of Directors members shall maintain Diplomate certification status and shall be in good standing with the ACVBM. The Board of Directors may, at its discretion determine additional qualifications for Board of Directors members, consistent with ACVBM Bylaws.

IV. Election and Terms of Officers

The initial Officers were elected by the incorporator. Thereafter, the Officers shall be elected by the Diplomates. The Officers shall serve in their elected position for a term of one (1) year. A slate of candidates shall be prepared by the Nominating Committee and submitted to the Diplomates and the Board at least thirty (30) days before the annual meeting. Members may submit recommendations for nominations (with nominee approval) to the Nomination Committee. The candidate receiving a plurality of the votes will be elected to the office. Officers shall take office immediately following the meeting at which they are elected. The Officers shall serve a term of one (1) year. It is expected, but not required, that continuity of administration will be maintained by the President moving up to Chair of the Board, the President-Elect to President, and the Vice-President to President-Elect.

V. Duties of the Officers

President

The President shall have the authority and responsibilities commonly incident to, and vested in, the corporate offices of Chief Executive Officer consistent with these Bylaws, including, but not
limited to: the authority and responsibility to satisfy the directives of the Board of Directors; designation and appointment of ACVBM representatives (subject to Board of Directors approval), the role of presiding officer at all meetings of the ACVBM and the Board of Directors; the direction of other Officers, and the administration of the affairs of the corporation according to the Certificate of Incorporation, these Bylaws, and the policies adopted by the Board of Directors. The President shall manage the day-to-day operations of the ACVBM and serve as Chair of the Nominating Committee. If an Executive Director has been retained, the President shall be responsible for day-to-day communications with the Executive Director. At the expiration of his or her term, the President shall succeed to the office of the Chair of the Board.

**Chair of the Board**
The Chair shall preside over all meetings of the Board, call meetings of the Board, act as the spokesman for the Board, perform the usual duties of the Chair and serve as the development officer for the College. If the President, President-Elect, and Vice-President are unable to perform the duties of President, the Chair (immediate past president) shall serve as acting President, shall have all authority conferred upon the office of President, and shall perform all duties for which the President is responsible for the unexpired portion of the term, or until another Officer can resume duties.

**President-Elect**
The President-Elect shall perform such other duties as the Board of Directors of Regents or the President may designate and serve as Chair for the Professional Botanical Program Committee. In the absence or disability of the President, the President-Elect shall serve as acting President, shall have all authority conferred upon the office of President, and shall perform all duties for which the President is responsible for the unexpired portion of the term, or until the President can resume duties. At the expiration of his or her term, the President-Elect shall succeed to the office of the President.

**Vice-President**
The Vice-President shall perform such other duties as the Board of Directors or the President may, from time to time, designate. In the absence or disability of the President and the President-Elect, the Vice-President shall serve as acting President, shall have all authority conferred upon the office of President, and shall perform all duties for which the President is responsible for the unexpired portion of the term, or until the President or President-Elect can resume duties. The Vice-President shall succeed to the office of President-Elect should that office be vacated, serve as Chair of the Credentials Committee, and perform such other duties as shall be assigned by the President.

**Secretary/Treasurer.**
The Secretary-Treasurer shall be the Chief Financial Officer of the corporation. The Secretary-Treasurer shall have and perform all duties commonly incident to, and vested in, the offices of secretary and treasurer of a corporation, as well as all duties delegated and designated by the Board of Directors or the President including, but not limited to: supervision, safe and secure maintenance of all corporate documents, conduct the correspondence of the College, responsibility for accurate accounting of the minutes of all meetings and the books of the corporation, administration of the fiscal and financial policies of the corporation and ensure the preparation and submission of all other documents required by state or federal authorities, and perform the usual duties of a secretary.

**Officer Resignation/Vacancy**
An Officer may resign at any time by providing sixty (60) days written notice to the President, or other authorized representative designated by the Board of Directors, and the Executive Director if retained. Such resignation shall take effect at the time specified therein, or, if no time is specified, at the time of acceptance as determined by the President or Board of Directors. In the event that the office of President becomes vacant, the President-Elect shall assume the office of President for the remainder of the term of office. In the event that any other Officer position becomes vacant, the President shall appoint interim officers to fill such vacant offices until a new Officer is elected by the Board of Directors to serve the unexpired portion of the term at the next scheduled Board of Directors meeting.

Removal of Officers
The Board of Directors may remove any Officer from office whenever, in its judgment, the best interests of the ACVSMR will be served thereby. An Officer of the ACVSMR may be removed by a two-thirds (2/3) affirmative vote of the Board of Directors at any regular or special meeting of the Board of Directors at which a quorum is present, and under rules or procedures approved by the Board of Directors.

Executive Director
The Board of Directors shall have the responsibility and authority to appoint an Executive Director of the ACVBM, who shall act as the Chief Operating Officer and Chief Staff Officer of the ACVBM. The Executive Director shall report to the Board of Directors, and shall be responsible and accountable for the supervision, control, and management of the ACVBM in its administrative, business, financial, and other operational affairs.

Authority and Duties
a. At the discretion of the Board, an Executive Director may be retained as a contracted employee of the College. The Executive Director’s authority and responsibilities shall be defined in detail in a contract that is mutually satisfactory and agreed upon between the Executive Director and the Board of Directors. In general, the Executive Director shall be the chief administrative officer of the College with the responsibility to manage the affairs of the College in keeping with the policies, programs and budget as established by the Board. The Executive Director shall recommend and participate in the formation of new policies and shall make decisions within the existing policies approved by the Board. The Executive Director may delegate responsibilities and authority necessary to effectively manage the College but may not relinquish accountability to the Board.
b. If retained, the Executive Director shall have the authority and duty to implement all policies of the corporation, and will report to the Board of Directors. The Executive Director shall have the authority to: hire and dismiss employees and other personnel of the corporation, including consultants, contractors, counsel, and the like; legally bind the corporation and sign on its behalf contracts, checks, drafts, notes, mortgages, leases, and other legal documents, without limitation by reason of specification; receipt of correspondence to the College and distribution to appropriate Diplomates; assist the Secretary and Chair in the performance of their duties. The Executive Director shall perform such other duties as may be designated by the Board of Directors.

3. COMMITTEES OF THE ACVBM

Standing committees of the College shall consist of the Executive Committee, Nominating Committee, the Credentials and Residency Committee, the Examination Committee, the Appeals
Committee, the Professional Botanical Program Committee and the Board may designate such other committees as needed. Committee members shall each serve a term of two (3) years, unless otherwise provided by the Board.

I. Executive Committee

   a. Composition: The Executive Committee shall be composed of the President, President-Elect, Vice-President, Secretary-Treasurer, and Executive Director (should one be retained). All Executive Committee members shall be voting members of the Committee, with the exception of the Executive Director.

   b. General Authority, Duties and Limitations: Obtain funding for the corporation, develop a time line according to ABVS submission guidelines, appoint a secretary/treasurer for the ACVBM and develop a meeting schedule for the Board. The Executive Committee may act for the Board of Directors between meetings of the Board of Directors, or as otherwise authorized by the Board of Directors. The Executive Committee shall not, however, have the power to: remove a Regent or Officer; fill vacancies in the Board of Directors or in any committee; determine and fix compensation for any individuals for serving on the Board of Directors or any committee; sell corporate assets; amend, repeal, or adopt Bylaws; or, amend or repeal any resolution of the Board of Directors. All proceedings and actions of the Executive Committee shall be recorded and reported to the Board of Directors at the next meeting of the Board of Directors.

   c. Meetings of the Executive Committee: The Executive Committee shall meet at least once each calendar year, and otherwise at the direction of the President. Any member of the Executive Committee may request that an Executive Committee meeting be convened to conduct specific business. Such requests shall be communicated to the President, who may call a meeting if appropriate and necessary.

   d. Notice of Executive Committee meetings shall be given to all Committee members at least five (5) days prior to such meeting, unless the President determines that a shorter notice period is appropriate under the circumstances. Executive Committee meetings shall be conducted in person or via telephone conference at a date and time determined by the President, so long as all participants can communicate and effectively participate. Minutes shall be kept of all Executive Committee meetings, and such minutes shall be promptly circulated to the Board of Directors and maintained with the corporate minutes of the Board of Directors.

   e. Actions by the Executive Committee: Unless contrary to applicable law or the ACVBM Bylaws, the actions of the Executive Committee shall constitute the actions of the Board of Directors between meetings of the Board of Directors, unless subsequently rescinded or withdrawn by the Board of Directors.

II. Nominating Committee

   a. Composition: The Nominating Committee shall consist of two (2) members appointed by the Board, none of who shall be current Regents or Officers. All Nominating Committee members must maintain Diplomate certification status and be in good standing with the ACVBM.
b. Appointment: The President shall appoint, with the approval of the Board of Directors, the Chair of the Nominating Committee. The Board of Directors shall appoint and replace the members of the Nominating Committee consistent with rules or procedures established by the Board of Directors.

c. Terms of Office: Nominating Committee members shall serve for a term of three (3) years.

d. General Authority and Duties: The Nominating Committee shall oversee and supervise the nominating process for members of the Board of Directors, and shall establish appropriate procedures and rules for the selection and presentation of qualified candidates to active ACVBM Diplomates for election. Under the direction of the Board of Directors, the Nominating Committee shall perform its duties and annually report its findings to the Board of Directors.

III. Credentials and Residency Committee (CRC).

a. Composition: The Credentials and Residency Committee shall be composed of a Chair and a representative from each botanical medicine practice category (Chinese, Western, Ayurvedic or other). All CRC members must maintain Diplomate certification status and be in good standing with the ACVBM. The CRC member from each professional practice category shall serve as the Vice-Chair of the CRC for that practice category.

b. Appointment. The President shall appoint, with the approval of the Board of Directors, the Chair of the CRC. The Board of Directors shall appoint and replace the members of the CRC, consistent with rules or procedures established by the Board of Directors.

c. Terms of Office. CRC members shall serve for a term of three (3) years.

d. General Authority and Duties. The CRC, shall be responsible for:
   i. establishing candidate credential eligibility criteria in accordance with these By Laws;
   ii. reviewing ACVBM certification applications;
   iii. approving applicants who meet the certification eligibility criteria;
   iv. identifying to the Board of Directors those applicants who are deemed eligible and ineligible for examination;
   v. establish criteria for residencies in veterinary botanical medicine and oversee the establishment and development of new residency programs in veterinary botanical medicine.
   vi. develop and inform diplomats of continuing education opportunities relevant to veterinary botanical medicine;
   vii. develop a list of publications and encourage new initiatives that will provide advanced training in veterinary botanical medicine.

e. The Board of Directors shall make the final decision in each case on eligibility to sit for the Diplomate Certification Examination. Under the direction of the Board of Directors, the CRC shall perform its duties and annually report its findings to the Board of Directors.

IV. Examination Committee
a. Composition. The Examination Committee shall consist of three (3) members appointed by the Board of Directors. All Examination Committee members must maintain Diplomate certification status and be in good standing with the ACVBM. The Examination Committee member from each professional practice category shall serve as the Vice-Chair of the Examination Committee for that practice category.

b. Appointment. The President shall annually appoint, with the approval of the Board of Directors, the Chair of the Examination Committee. The Board of Directors shall appoint and replace the members of the Examination committee consistent with rules or procedures established by the Board of Directors.

c. Terms of Office. Examination Committee members shall serve for a term of three (3) years.

d. General Authority and Duties. The Examination Committee shall:

   i. define the examinations for diplomate status in both subject matter and distribution of questions and type of exam (is multiple choice, short answer, practical etc);
   ii. will write the examination;
   iii. administer, monitor and grade the Diplomate Certification Examination.
   iv. it shall make recommendations to the Board regarding competence of those candidates who have completed the Diplomate Certification Examination.
   v. under the direction of the Board of Directors, the Examination Committee shall perform its duties and annually report its findings to the Board of Directors.

V. Appeals Committee

a. Composition. The Appeals Committee shall consist of between three (3) members appointed by the Board. An appointed member of the committee will serve as Chair. All Appeals Committee members must maintain Diplomate certification status and must be in good standing with the ACVBM.

b. Appointment. The committee will elect the Chair. An Appeals Committee member may not be a member of the Credentials and Residency Committee or Examination Committee. If a member has a conflict of interest in a specific appeal, the Chair of the Board shall appoint a temporary member to consider that appeal.

c. Terms of Office. Appeals Committee members shall serve for a term of three (3) years, which shall be staggered to ensure that two (2) to three (3) members are replaced each year.

d. General Authority and Duties. The Appeals Committee shall consider whether correct administrative procedures have been followed in the decisions made by the Credentials Committee and Examination Committee. The Chair of this committee will call a meeting to review an appeal and notify the Chair of the Board and the Executive Director of the results of that review within thirty (30) days of notification of the appeal. The decision of the Appeals Committee shall be final and there shall be no appeals there from.

VI. Professional Program Committee
a. Composition. The Professional Program Committee shall consist of at least 2 members appointed by the Board. All Professional Botanical Program Committee members must maintain Diplomate certification status and must be in good standing with the ACVBM.

b. Appointment. A board appointed member of the committee will serve as Chair.

c. Terms of Office. Professional Botanical Program Committee members shall serve for a term of three (3) years.

d. General Authority and Duties. The Professional Botanical Program Committee shall determine the content of, and make all necessary plans and arrangements for the RVS Botanical Medicine conference.

e. The Examination Committee has determined that examination questions will be submitted by all members of the organizing committee in multiple-choice format and will cover core knowledge of botanical medicine that is common to all species and common botanical theory including pharmacognosy, principles and pharmacology; then a concentration in the areas of veterinary botanical medicine for equine, production and small animals; and the scientific aspects of either Chinese or western botanical medicine. The examinations will be paper or computer-based and will be administered at a site and on a date determined by the ACVBM Board of Directors. The examinations will be administered and monitored by the Curriculum/Education/Examination Committee. Test questions will be marked and evaluated by the Curriculum/Education/Examination Committee.

f. At the present time funding is based on an initial donation, membership dues and conference dues. Future funding will be based on application fees, examination fees, conference fees and membership dues.

VIII. Changes in Standing Committees and Additional Committees

The membership of the standing committees may be increased and other committees may be appointed as the need arises. The Board of Directors may authorize and supervise additional committees; from time to time to perform such functions as may be determined by the Board of Directors. The President shall annually appoint, with the approval of the Board of Directors, the Chair of all standing or special committees, sub-committees or divisions, as may be required or as may be deemed necessary.

X. Guidelines for Meetings of Diplomates

Annual/Regular Meetings: The annual meeting of Diplomates for the election of Directors and officers and for such other business of the ACVBM shall meet annually at such a time and place as designated by the Board of Directors:

a. The Annual Meeting of the Board of Directors shall be at a time and place designated by a majority of the Board of Directors for the transaction of business that comes before the Board of Directors. There shall be at least one (1) other regular meeting of the Board of Directors each year at a place designated by the Board of Directors for the transaction of business. Agendas of all items to be discussed at regular Board of Directors meetings shall be distributed at least two weeks (14) days prior to the meeting.
b. Meetings of the executive committee: Any member of the Executive Committee may request that an Executive Committee meeting be convened to conduct specific business. Such requests shall be communicated to the President, who may call a meeting if appropriate and necessary. Notice of Executive Committee meetings shall be given to all Committee members at least five (5) days prior to such meeting, unless the President determines that a shorter notice period is appropriate under the circumstances. Executive Committee meetings shall be conducted in person or via telephone conference at a date and time determined by the President, so long as all participants can communicate and effectively participate. Minutes shall be kept of all Executive Committee meetings, and such minutes shall be promptly circulated to the Board of Directors and maintained with the corporate minutes of the Board of Directors.

Special Meetings: Special meetings of Diplomates may be called by the Board in accordance with the procedures set for in the Delaware General Corporation Law (“GCL”) at such a time and place as designated by the Board of Directors. Notice of any and all special meetings will be delivered by the Secretary of the College to each Director via email to each Board of Directors member at least ten (10) days prior to the date of the meeting. The Secretary must receive confirmation that the email was received by the recipient in order for the notice to be valid.

XI. Notice of Meetings

Written notice of the annual and special meetings of Diplomates shall be given by the Board and shall state the place, date and hour of the meeting and, unless it is the annual meeting, shall indicate that it is being issued by or at the direction of the person or persons calling the meeting. Notice of a special meeting shall also state the purpose(s) for which the meeting is called. A copy of the notice shall be given, personally or by mail, to each Diplomate entitled to vote at the meeting. The Secretary shall give notice of all regular meetings of the Board of Directors to all Regents and Officers no less than ten days (via email) or more than sixty (60) days (US post) prior to the meeting. If a meeting is adjourned, notice of such adjourned meeting shall be given, if required.

XII. Protocol for Committee Meetings

Committee meetings will typically be scheduled during the annual board meeting. The Standing committees of the College shall consist of the Executive Committee, Nominating Committee, the Credentials Committee, the Examination Committee, the Appeals Committee, the Professional Botanical Program Committee, the Training Program Evaluation Committee, and such other committees as may be designated by the Board. Committee members shall each serve a term of three (3) years, unless otherwise provided by the Board.

3. ADMINISTRATIVE

Reimbursement Policies and Procedures: The Credentials application fee and the DCE fees are non-refundable and payable each time the credentials application is submitted and the examination is repeated.

Record Retention and Maintenance: It is the duty of the Secretary/Treasurer to retain all records and maintain them in satisfactory fashion.
Membership Renewal Fees: Membership renewal is due at the first of January of each calendar year in the amount of $150.

4. ACVBM RESIDENCY PROGRAM

The residency program of the ACVBM was established to provide a pathway for fulfilling the requirements necessary to submit credentials and sit for the certifying examination. A residency meets the eligibility criteria for ACVBM certification.

I. Objective

The objective of the ACVBM approved residencies is to promote expertise and proficiency in a RVS by providing guidance and instruction through a structured residency program.

II. ACVBM Residency Program Summary

a. There will be two residency programs offered by the ACVBM: Standard and Alternate Programs, they must be approved by the Credentials and Residency Training Committee (CRC) in advance

b. The Standard Residency program will be a minimum of 3 years in duration and will be conducted at an AVMA accredited veterinary medical school.

c. The Alternate Residency Program is proposed by the Resident and must meet the same requirements of a Standard Residency but part or the entire program is outside of an AVMA accredited veterinary medical school. The Alternate Program can be up to 6 years in duration.

d. Candidates must have completed an approved Residency Program and completed all other requirements including Credentials and publication, before sitting for the certification examination. The CRC and the ACVBM Executive Board approve both Residency Training Programs.

III. Goals of the Residency Training Programs

a. Development of a high level of clinical skills and expertise in the field of veterinary botanical medicine

b. Development of a critical understanding and working knowledge of the current veterinary and human literature related to botanical medicine and proficiency in literature review and the synthesis and clinical application of new information

c. Development of critical thought processes and the use of a problem-based approach to patient care

d. Demonstration of an ability to teach, communicate and effectively present information;

e. Demonstration of exceptional ethical standards and the ability to act as a professional role model

f. Demonstration of having made a contribution to the science of veterinary botanical medicine

IV. Residency Application Requirements: The applicants must be veterinarians and meet the following requirements:
a. Graduate of a college of veterinary medicine (accredited/or listed by the American Veterinary Medical Association (AVMA)), or possess a certificate issued by the Commission for Foreign Veterinary Graduates (ECFVG) or are legally qualified to practice veterinary medicine in some state, province, territory, or possession of the United States, Canada, or other country
b. Complete one year of clinical veterinary internship training, a residency in another discipline, or 5 years of active veterinary clinical practice
c. Meet the education, training and experience requirements established by the ACVBM
d. Demonstrate unquestionable moral character and ethical professional behavior
e. Legally able to practice at the residency training site (e.g., veterinary medical license and other state or federal requirements

V. Residency Application Process
a. The Resident applicant and his/her Primary Advisor must submit the ACVBM Residency Application and Registration Form to the Credentials and Residency Committee, which ensures the Resident will meet the minimum requirements for the Residency’s board certification within a six-year period. The application includes the following items: Who is involved in the residency; How the residency is organized; Where the training will take place; How each of the requirements for the Residency will be met and a time frame for completion.

b. Upon receiving approval from the Credentials and Residency Committee, the program may be initiated. Then, the Resident Application and Registration Form (Appendix I) must also be submitted and registered with the ACVBM office within 30 days of program initiation.

VI. Time for Completion

The total minimum time required for completion of a residency will be determined by the Primary Advisor of the residency and the case load available at the Training Site. All residents must complete, the entire residency training program within a maximum of 6 years and no less than 3 years.

VII. Residency Training Programs

Standard Residency Program

The residency program will be conducted at an AVMA accredited veterinary medical school. Residents must be enrolled in a residency program of a minimum of three years in length, approved in advance by the CRC.

Alternate Residency Program

The Resident proposes an Alternate Residency Program that must meet the same requirements of a Standard Residency but part or the entire program is outside of an AVMA accredited veterinary medical school. The Alternate Program can be up to 6 years in duration and is approved in advance by the CRC.

Residency training programs are the foundation for training of future diplomates of the ACVBM. The components of both the Standard and Alternate residency training program include:
a) One (1) Primary Advisor  
b) Two (2) Co-Advisors  
c) Resident  
d) Other requirements of a residency-training program are listed in this document  

Advisory Committee  

The establishment of an Advisory Committee for each Resident is required. Each committee should consist of one Primary Advisor and two co-advisors. The two co-advisors will be selected by the Primary Advisor and may assist in the selection of trainees and supervision of the training program. The co-advisor can be an ACVBM Diplomate, or a specialist from other specialty colleges as well as veterinary clinicians with pertinent PhD credentials, as deemed appropriate by the CRC.  

Primary Advisor  

a. Primary Advisor must be a Diplomate of the ACVBM. The Primary Advisor is directly responsible for the selection of Resident applicants, clinical guidance and mentorship of Residents and collaborates in the assessment and evaluation of Residents with two (2) co-advisors. In the first instance before Diplomates of the ACVBM, Primary Mentors can include Diplomates with training in botanical medicine; DVMs with MS degrees or PhDs in botanical medicine related area or as approved by ACVBM  
b. The Primary Advisor evaluates and approves all relevant forms and documents as well as the training site(s) as suitable for the program  
c. The Primary Advisor also ensures the Resident has completed all requirements of the program of the ACVBM and when the Resident submits the Credentials Application the Primary Advisor is asked to attest in writing that all training requirements have been satisfactorily completed  
d. The Primary Advisor may serve only 3 Residents at one time  

Resident  

a. Will need to commit time, travel and money toward their training  
b. Can choose a Standard Residency program or an Alternate Residency program  
a. Agrees to all program and credential requirements and meets deadlines for submission of fees and relevant documents  
b. Will provide timely progress reports and evaluations throughout the training period for the CRC to review  
c. Must meet the requirements of: a one year of clinical veterinary internship training, a residency in another discipline, or 5 years of full time, veterinary clinical practice  

VIII. Residency Program Description  

An acceptable Botanical Medicine Residency is 100 weeks of training within 3 to 6 years, allows the individual seeking diplomat status time to obtain sufficient knowledge and clinical skills to meet the credentials required to take the ACVBM certifying examination. It includes a minimum of 60 weeks of botanical medicine, 24 weeks of independent study, research, teaching and writing, and 16 weeks of immersion in specialty veterinary practice. If an adequate number of
cases, personnel or facilities are not available at the Program site, then the Primary Advisor is responsible for determining how the requirements should be fulfilled. The CRC must approve such arrangements in advance. Letters of commitment for the provision of off-site training must be submitted to the CRC.

Training Site
The Residency training program can take place at a University, or Practice where the Resident will provide primary patient care appropriate to their level of training. The Resident’s Primary Advisor must provide direct or indirect supervision appropriate to the Resident’s abilities (e.g. direct supervision for new Residents).

Infrastructure
The clinical setting must include advanced diagnostics to enable complete case evaluation and diagnosis as well as follow up of case outcomes. The following is required to be available in the primary training hospital: clinical pathology capabilities including CBC, serum chemistries, urinalysis, cytology and parasitology. The Resident must be able to refer internally or externally for advanced diagnostic procedures or therapeutic interventions (such as CT, MRI, radiation oncology, scintigraphy, specialized surgeries, endoscopy, etc.), if they aren't available in house. The Resident must have access to a botanical medicine pharmacy and botanical products and equipment for dispensing. The Resident must have access to a veterinary and or medical library. The library should have access to conventional as well as veterinary botanical medicine journals. The Primary Advisor should develop a required and recommended reading list.

A Training Week
For the purposes of the residency, a training week is defined as a minimum of 40 hours of logged immersion time. The start day for a Resident’s training week will remain the same day of the week as the first day of their residency.

Direct Supervision
The Resident and Primary Advisor are participating together, concurrently managing cases in clinical practice. The Primary Advisor is physically available for consultation. The minimal direct supervision should be at least 30 training weeks.

Indirect Supervision
The Resident and the Primary Advisor, although participating together, are not concurrently physically involved in clinical practice. To qualify for indirect supervision, the Resident and Primary Advisor must have direct contact (Skype, phone, web, other) for at least 3 hours per week, with consultation available on a daily basis by phone and email (or comparable effective communication platforms). The maximum indirect supervision time should be no longer than 30 training weeks.

Supervision of Remote Consultation
Botanical medicine experience may be gained through extramural opportunities to work in other specialties or institutions directly related to botanical medicine, such as botanic pharmacology and practice. In this case, the Primary Advisor and Resident work closely together yet are not on-site together. In cases where the Primary Advisor and Resident are not physically working in the same location, regular and significant direct communication is required. Depending upon the level of supervision, such experience may be considered as either directly or indirectly supervised.

Training Experiences
Some credentialing requirements must be completed on-site; some may be completed off-site such as the advanced botanical medicine CE courses approved by the RACE and the ACVBM.

Part-Time Experience
Part-time experience is permitted, where cumulative experiences over time may accrue for a block of time. This experience should be outlined on the Application Form, that is pre-approved by the Primary Advisor. It is the Residents’ responsibility to document their experiences with an activity log that is reviewed and signed by the Primary Advisor.

IX. Botanical Medicine Clinical Training

This requirement consists of a minimum of 60 weeks immersion in botanical medicine practice, which is supervised by the Primary Advisor or a co-advisor.

a. A minimum of 30 weeks of clinical experience with direct ACVBM diplomate supervision:
   - Direct supervision is defined as the ACVBM diplomate being at the same location as the Resident during the required time
b. No more than 30 weeks of clinical experience with indirect ACVBM diplomate supervision:
   - The diplomate and Resident must establish a monthly time to interact and discuss the month’s clinical activity related to botanical medicine. This contact may be via phone or web conference (such as Skype or equivalent)

X. Specialty Rotations Training

This requirement consists of 16 weeks of immersion in specialty veterinary practice under the direct supervision of a Diplomate in that specialty area. There must be a minimum of 4 or more specialties (minimum 2 or more weeks per specialty) negotiated with supervisor including but not limited to:

a. Clinical Pharmacology:
b. Nutrition:
c. Internal Medicine in general (equine, canine, or feline):
d. Internal Medicine in Oncology
e. Internal Medicine in Cardiology
f. Internal Medicine in Neurology
g. Veterinary Dermatology
h. Veterinary Behavior
i. Veterinary Exotics
j. Veterinary Rehabilitation
i. Theriogenology
j. Other as approved by Principle advisor and ACVBM

XI. Veterinary Botanical Medicine Study or Practice

This requirement consists of 24 weeks of independent study on topics related to botanical medicine or independent immersion in botanical medicine practice. Independent study or practice is intended to allow development of independent thought, peer mentoring and teaching skills, focused study in specialized facets of botanical medicine, further elective rotations, further
supervised or independent rotations in botanical medicine, cross species training or human botanical medicine training.

The Resident Primary Advisor is responsible for designing this requirement to meet the needs of the individual Resident and is responsible for ensuring that the Resident is receiving adequate training during this time. Continuing education that is intensely focused on a specialized facet of botanical medicine may be logged concurrent with the independent study requirement on an individual basis at the discretion of the Credentials and Residency Committee; however, general continuing education requirements may not be logged concurrently with independent study time.

The Resident, along with the support of their Primary Advisor, must ensure that all independent study requirements are met and logged within the required term of the residency training program. Residents are expected to make timely regular progress in completing these requirements. The biannual report should reflect this progress.

a. A minimum of 10 weeks must be spent conducting Research and manuscript preparation.
b. A minimum of 14 weeks of individualized rotations must be performed, including but not limited to:
   - Rotations at a veterinary medicine practice that concentrates on the Resident’s species of interest. The veterinary medicine rotation must be with a person that has received advanced training in veterinary botanical medicine
   - Rotation with the alternate species
   - Special rotation at a human botanical clinic
   - Vacation: Up to two weeks per year of vacation time may be counted toward this requirement, with a maximum of 6 weeks allowable

XII. Graduate-Level Veterinary Botanical Medicine Training Course and National or International Level Continuing Education Related to Veterinary Botanical Medicine

Education at graduate-level Veterinary Botanical Medicine Training Courses (TC) are required. These TC courses are approved in the first instance by the Primary Advisor. A Resident must submit the TC Application (Appendix II) to be approved by the ACVBM.

XIII. Manuscript Requirement

In addition to the time outlined for research/clinical investigation and manuscript preparation, the Resident must publish at least one (1) manuscript in the field of botanical medicine in order to be accepted to sit for the ACVBM certification examination as below:

a. The research must be the result of the Residency Resident’s work
b. The residency applicant must be the first author on the manuscript
c. The manuscript must follow a scientific approach, including a clearly stated hypothesis or objective, an appropriate description of techniques (including statistical analysis), a report of the results, and a discussion. Communications, case reports, review papers, book chapters, etc. are not acceptable
d. The manuscript must be written in English
e. The date of publication cannot be more than five years old by the deadline for credentials submission. A letter of acceptance can be used to proof publication
f. The journal must be peer reviewed. These criteria are to be fulfilled by the time of manuscript submission and documentation and must be included with the Resident’s
credentials packet, showing that the criteria are met at the time of manuscript submission

XIV. Oral Presentations

Residents are required to present a minimum of 3 multimedia presentations over the course of their program covering topics related to Botanical Medicine. These presentations must be at least 20 minutes in duration and presented to an audience of their peers. At least one diplomate of any specialty college must be present. These are required to be recorded in the Residency Master Log and verified by the ACVBM Diplomate.

XV. Teaching Requirements

Trainees must be involved in teaching students, interns, residents, technicians, veterinarians, producers, and owners. Teaching will include formal and informal clinical tutorials, such as daily rounds, case discussions, as well as appropriate involvement in lecture courses and seminars.

a. A Resident in a university setting is expected to help teach students about botanical medicine. A Resident in a specialty practice with rotating interns and Residents of other disciplines should have some expectation of clinical instruction. A Resident in a non-specialty hospital setting will provide clinical instruction to veterinarians of the practice that hosts that Resident.

b. Trainees will regularly attend and participate in seminars, rounds and case conferences in botanical medicine and other applicable specialty rounds, seminars, etc.

XVI. Other Scholarly Activities

Formal Study: Residents must undertake graduate level training in Botanical Medicine (Curriculum and learning objectives) approved by the ACVBM Credentials and Residency Committee. This may include pharmacognosy, pharmacology and toxicology.

XVII. Journal Study

The Resident is expected to be familiar with journal articles published over the last 5 years pertaining to veterinary medicine as well as reviews and meta-analyses pertaining to Phytomedicine in general.

Other references and study material for the Diplomate Examination can be found at Appendix V.

XVIII. Written Documentation of Progress

Successful completion of a residency in the Specialty of Botanical Medicine may be achieved by successfully completing any residency-training program that has attained approval by the CRC and by meeting all training requirements prior to the start of training. The Primary Advisor will be responsible for documenting that the training has occurred as specified. Completion of all requirements should be documented and/or submitted semi-annually or yearly:

a. Biannually - Biannual Progress Report Form (due February 1) – Appendix III
   • All Residents must submit a Biannual Progress Report. The Credentials and Residency Committee will evaluate the Biannual Progress Report. Recommendations and requirements will be forwarded to the Resident and their Resident Primary Advisor if needed
The Resident Primary Advisor and two co-advisors must sign attesting to satisfactory completion of individual immersion training weeks, experience, and skills requirements in order for credit to be granted.

The Resident and the Primary Advisor are responsible for ensuring that the review form is complete.

b. Yearly - The Resident must provide written documentation including CE Course Form (Appendix II), Biannual Progress Report (Appendix III), Annual Progress Report (Appendix IV) forms and Master Log (Appendix V) to the diplomate’s Primary Advisor that the listed requirements have been completed. The Primary Advisor is responsible for verifying that all of these requirements have been met prior to submission of credentials for board certification. An Annual Progress Report (Appendix IV) and a copy of the Master Log must be signed by the Primary Advisor and submitted to the ACVBM College Secretary by August 1 each year for review by the CRC.

1) CE Course Form
2) Biannual Progress Report
3) Annual Progress Report
4) Master Log is named using the following format: Last Name. MasterLog.xls

XIX. Expected Skills and Knowledge of ACVBM Diplomates

The ACVBM Diplomate is expected to be competent in three (3) areas of botanical medicine: service, teaching and research. To do so, they must have skills to botanical medicine, the ability to incorporate those skills in an integrative medicine setting, and a knowledge base related to botanical medicine. Unique skills include veterinary botanical medicine principles and practices, specialised veterinary botanical medical care, and the preparation and presentation of botanical veterinary case studies and botanical research findings for publication. The latter includes the ability to present the application of the studies in an integrated practice. Skills can be divided into the following primary categories: cognitive, communication and education, clinical, research and regulatory. Subsets of each area are listed in Residency Program.

5. CREDENTIALS EVALUATION

I. Credentials Submission

The credentials submission form must be completed and signed by the Resident. Residents are required to submit all credentialing materials required by the ACVBM and are the same for a formal residency applicant and an alternate path applicant. The credentials submission form along with the Master Log, Annual Progress reports, one peer-reviewed publication, three recommendation letters (one from an active diplomate of ACVBM, and two from other ABVS specialists) must be submitted to the College Secretary at secretary@acvbm.org for review by the CRC to establish eligibility for taking the board certification examination.

II. Credentials Fees and Deadlines

A Resident should submit all the required documents before August 1 each year, with a $350 credential submission fee. All application materials will become the property of the ACVBM.

III. Credentials Evaluation and Notification
A Resident will be notified by the CRC regarding the acceptance of their credential materials for
the College board-certification examination at least 120 days prior to the examination. All
application materials will become the property of the ACVBM

IV. Credentials Confidentiality Policy

All information or material received or generated by the ACVBM in connection with the
certification of a veterinarian will be kept confidential and will not be released unless release is
authorized by the veterinarian or required by law. Applicants are forbidden from contacting any
members of the ACVBM Credentialing or Examination Committee except the Chair of the
Credentials Committee during the certification process.

6. APPLICATION FOR BOARD CERTIFICATION

I. General Eligibility Criteria: To be eligible for ACVBM certification, veterinarians must have:

   a. Graduated from a college or school of veterinary medicine approved by the AVMA or
      possess a certificate issued by the Educational Commission for Foreign Veterinary
      Graduated (ECFVG)

   b. Be legally qualified to practice veterinary medicine in any state or country

   c. Have met the education, training, and experience requirements established by the
      ACVBM

   d. Have demonstrated unquestionable moral character and ethical professional behavior

II. Veterinarians must have completed a recognized certificate program

   a. Standard Residency Program: The residency program will be conducted at an AVMA
      accredited veterinary medical school. Residents must be enrolled in a residency program of a
      minimum of three years in length, approved in advance by the CRC

   b. Alternate Residency Program: An Alternate Residency Program that meets the same
      requirements of a Standard Residency but part or all of the program is outside of an AVMA
      accredited veterinary medical school. The Alternate Program can be up to 6 years in duration and
      is approved by the CRC

III. Application Process

   a. All residency requirements must be met: the Master Log, Annual Progress reports and
      one peer-reviewed publication that has been published in the peer-reviewed literature
      within the past 3 years, in which botanical medicine was the primary medical treatment
      used

   b. A completed Credentials Submission Form and required fees

   c. Three (3) recommendation letters: one from an active diplomate of ACVBM and two
      from other ABVS specialists
d. All of the above must be submitted to the college secretary, at acvbm@listserv.uga.edu by August 1 each year.

e. The College will notify the Resident at least 120 days prior to the examination.

7. DIPLOMATE CERTIFICATION EXAMINATION (DCE)

The DCE is based on a job/task analysis, developed by surveying members of the ACVBM organizing committee as well as other veterinarians that use herbal medicine in their practice. The survey identified skills and knowledge specifically needed for the practice of veterinary botanical medicine, as well as how often each area is used in practice.

The certifying examination for the ACVBM Specialty will test whether the candidate can perform at the level expected of an entry-level specialist in veterinary botanical medicine. The DCE may include but is not limited to: The history of botanical medicine in context of contemporary practice, understanding the language of botanical medicine terminology and concepts, botanical medicine resources and research evidence based approaches, philosophy and principles of botanical medicines and Materia Medica. General botanical medical principles common to all species: herbal therapeutics in practice (of the gastrointestinal system, cardiovascular system, integumentary system, respiratory, hematologic system, musculoskeletal system, nervous system, endocrine system, etc), clinical strategies, botanical medicine case analysis and diagnosis, development of therapeutic treatment plans and prognosis, integration with conventional medicine, pharmacology, drug herb interactions and adverse effects, pharmacognosy, ethnoveterinary, ethnobotanical medicine, zoopharmacognosy, manufacturing, processing and dispensing of botanical medicines, veterinary herbal pharmacy management.

The examination will cover the following areas:

a. General botanical medicine (all candidates are required to take this section)

b. Principles and Practices

c. Clinical botanical medicine

d. Botanical identification of medicinal plants.

I. Examination Structure

The main body of the exam will consist of multiple choice questions. The examinations will be paper or computer based and Test questions will be marked and evaluated by the Examination Committee.

IV. Examination Schedule

The candidate must pass all sections of the examination no later than six (6) consecutive years after his or her eligibility to sit for the examination was determined. The exam will be administered at a site and on a date determined by the ACVBM Board of Directors, but no more often than once a year. Candidates must apply to sit for the exam no less than 3 months before the scheduled time of the examination. If no applications are submitted by that time, the examination will not be offered that year.

V. Examination procedures
a. Written examinations will reflect the professional competence and knowledge base expected of a diplomate of the ACVBM.
b. After approval of credentials, candidates will have a period of 120 days available for examination preparation prior to taking the certification test.
c. If a candidate’s credentials are denied and an appeal is filed, the ACVBM will review this appeal and inform the candidate of their decision no later than 90 days prior to the examination date.
d. Candidates will receive an outline of the exam content and exam format prior to the exam.
e. Candidates will be informed prior to the examination of the passing score, or, if this is not determined in advance, the method of setting the passing score. The passing score may be adjusted lower but not higher after administering the exam. The minimum passing score will be determined by the Examination Committee and approved by the Board.
f. Candidates shall be sent written notification of the results of the examination no later than thirty (30) calendar days after the examination. All candidates shall be given such notification on the same day.
g. Candidates who do not successfully pass the examination, will, upon request, be provided with an explanation of the deficiencies that prevented their passing the examination. This procedure will be published by the ACVBM organization prior to the examination.
h. All candidates will be informed of their remaining eligibility and reapplication procedures.
i. ACVBM will avoid personal conflict, or the appearance of conflict, that could affect results of examinations.
j. The ACVBM will accommodate reasonable requests from applicants with documented disabilities for special test considerations in accordance with the Americans with Disabilities Act (ADA).

VI. Grading

The examination is a multiple choice exam and the final grade will be the percent correct out of the total number of questions. The passing score will be 70%, or less based on the modified Angoff method. If the score determined by the Angoff method is less than 70%, that score shall be the passing score for that year. Paper tests will be graded by the examination committee, according to a master answer sheet. Tests given by computer will be computer-scored.

VII. Notification of Results

Candidates will be notified of the results no more than one month after they take the exam.

8. DIPLOMATE three (3) classes of Diplomates and shall consist of the following: Diplomates, Honorary Members, and Emeritus Diplomates. The benefits and procedures for acquiring each classification are governed by the policies and procedures of the Board of Directors.

a. Diplomates: Have fulfilled all the general requirements set forth for certification by ACVBM and are current on all renewal fees. These members are eligible to vote and hold office
b. **Honorary Members**: Honorary member status may be conferred on a person who has contributed materially to the development of veterinary botanical medicine as to be deserving of special recognition by the College, under the following provisions: A person must be nominated for Honorary Member status by at least two Diplomates.

c. **Emeritus Diplomates**: Have fulfilled the requirements for Diplomate status but are retired from active clinical practice in their specialty category. A Diplomate may request Emeritus Diplomate status by submitting a written application to the Board. Such application shall be granted provided the Diplomate meets the following requirements:

   i. The Emeritus Diplomate has been an active member of the ACVBM and has reached the age of sixty five (65)

   ii. The Diplomate has retired from employment in which their ACVBM credentials are required for employment. Income generated from activities associated with their ACVBM credentials through activities such as part-time consulting, teaching, writing, or continuing education is acceptable and will not preclude Emeritus Diplomate status.

   iii. Emeritus Diplomates are able to vote and hold office, shall be required to pay a renewal fee, but will not be required to recertify.

d. **Initial Period of ACVBM Organization**

In order to fulfill the organizational requirements of the ACVBM, ACVBM organizational members who are recognized veterinary herbalists and who have materially contributed to the committee are permitted to undertake the Diplomate Certification Examination. They must meet the following prerequisites and provide evidence to be assessed by the Credential and Residency Committee. This procedure must be undertaken within 5 years of ACVBM RVS status.

Candidates must provide evidence to the Credentialing committee of:

- Ten (10) or more years of experience in veterinary botanical medicine, with not less than 75% of professional time devoted to the practice
- Experience of teaching/lecturing/research in the specialty for ten (10) years and have contributed substantially to the development of the specialty
- Authorship of significant publications resulting from research or practice in botanical medicine as determined by the Board or a professorship in the specialty of botanical medicine at a college or school of veterinary medicine
- Ability and willingness to mentor potential new ACVBM Diplomate candidates
- Evidence of advanced training in botanical medicine and have demonstrated competency through teaching, research, or practice the specialty to which most of the individuals professional time is devoted.

**Maintenance of Diplomate Status**

To maintain active Diplomate status, a Diplomate of the ACVBM is required to obtain 30 hours of botanical medicine continuing education (CE) credits per year. These credits may be obtained from both veterinary and botanical medicine meetings, at least fifty (50)
percent of the CE units must be obtained from veterinary botanical medicine CE. A point system will be used as outlined by AVMA where points may be accrued in a variety of ways including continuing education attendance or presentations, publications, serving on committees. An honor system of compliance requires Diplomates to self-declare completion of requirements each year with membership renewal. A log book will be submitted. Each year ACVBMA will audit 5% of members, requiring specific evidence supporting requirements. Diplomates will be provided with standard CPE Log Books and Guidelines for completion.

9. APPEALS PROCESS

a. Candidates failing to pass the Diplomate Certification Examination may appeal this decision within thirty (30) calendar days of the postmarked date of notification. The request for appeal must be made in writing to the Secretary/Treasurer and shall include a statement of the grounds for reconsideration and appropriate documentation. The Secretary/Treasurer shall notify the Chair of the Board, the Chair of the Examination Committee, and the Chair of the Appeals Committee.

b. The Chair of the Board shall submit to the Appeals Committee a written statement of the reasons for the failure of the candidate. The Chair of the Examination Committee shall submit to the Appeals Committee the examination and scores of the candidate, the complete list of scores of all candidates on that examination, and a statement as to the criteria used for the Committee's recommendation for success or failure, except that such documentation shall be redacted to preserve anonymity of both the appellant and the other candidates.

c. The Appeals Committee shall review the appeal and render a recommendation to the Board no later than thirty (30) calendar days after being appointed. The Board shall render a decision on the appeal upon consideration of the recommendation of the Appeals Committee and notify the appellant of the decision no later than fifteen (15) calendar days after receipt of the recommendation of the Appeals Committee.

d. If an appeal is unsuccessful and the appellant wishes to reapply to sit for the Diplomate Certification Examination, the deadline for application shall be extended three months from its designated date.

e. An applicant denied eligibility to sit for the Diplomate Certification Examination may appeal this decision within thirty (30) calendar days of the postmarked date of the notification. The appeal must be made in writing to the Secretary/Treasurer and shall include a statement of the grounds for reconsideration and appropriate documentation.

f. Upon receipt of an appeal, the Secretary/Treasurer shall notify the Chair of the Board, the Chair of the Credentials Committee and the Chair of the Appeals Committee. The Chair of the Board shall submit to the Appeals Committee documentation indicating the reasons for denial of eligibility to sit for the examination, including, but not limited to, the complete application package of the applicant and all available documentation pertaining to the Credential Committee's review of the application and recommendations to the Board, except that such documentation shall be redacted to preserve anonymity of the appellant.
g. The Appeals Committee shall review the appeal and provide a recommendation to the Board no later than thirty (30) calendar days after receiving all necessary documentation.
Appendix III Support for Residency
October 17, 2107

To Whom This May Concern:

I am happy to express my support for the establishment of a residency program at Cornell University to train applicants to enable them to qualify for membership in there proposed American College of Veterinary Botanical Medicine. There is an increasing interest in complimentary medicine by veterinary student as well as clients. Use of herbs is an important part of complimentary treatments and advanced training in their use should be available.

Sincerely,

[Signature]

Robert B. Hillman, DVM, DACT
Senior Clinician Emeritus
October 25, 2017

American Board of Veterinary Specialists
American Veterinary Medical Association
ATTN: ABVS
1931 N. Meacham Road, Suite 100
Schaumburg, IL 60173-4360

Dear Committee Members:

I am writing this letter in support of the development of the American College of Veterinary Botanical Medicine (ACVBM). Here at Oklahoma State University’s Boren Veterinary Medicine Teaching Hospital there are several ACVIM, ACVS, ACVSMR, and ACT diplomates who utilize botanicals in our practice of clinical veterinary medicine. We are in the support of the establishment of the ACVBM and support their goals of the development of greater and specialized clinical skills and expertise in the field of veterinary botanical medicine and greater contributions to the science of veterinary botanical medicine.

Please know that should the AVBS approve the establishment of this ACVBM speciality, and OSU administration approve, which we expect, that we would support the exploration and development of a residency program here.

Please do not hesitate to call me for more information should you have any questions or concerns.

Sincerely,

G. Reed Holyoak, DVM, PhD, DACT
Bullock Professor of Theriogenology
reed.holyoak@okstate.edu
405-744-8475
Dear Dr Fougere and committee members:

I am writing this letter in strong support of a specialty in Botanical Medicine. I am a strong proponent of this venture and I wholeheartedly support a residency in this discipline. In addition, I would be happy to serve as a mentor in any way I can, and residents would be welcome in my practice for rotations.

If you have any questions or concerns, please contact me: ccolitzacvo@gmail.com

Warmly,

Carmen MH Colitz, DVM, PhD
Diplomate, American College of Veterinary Ophthalmologists
November 2, 2017

American Board of Veterinary Specialists
American Veterinary Medical Association
ATTN: ABVS
1931 N. Meacham Road, Suite 100
Schaumburg, IL 60173-4360

Dear Committee Members:

This letter is to confirm my interest in developing an ACVBM residency program at the University of Tennessee College of Veterinary Medicine in the future, pending the approval of the UTCVM administration. I have read the residency training standards and understand the resources and support required for such a residency program. I believe that we have the required resources and already have several successful residency programs at our institution. I am residency coordinator for our anesthesia residency program so understand what is required for a successful program. In addition to myself, several clinicians in other specialties (ACVIM, ACVS, ACVSMR) utilize botanicals in their clinical practice at the University of Tennessee and are also pursuing valuable research into the use of botanicals in veterinary medicine. If I can provide any further information, please do not hesitate to contact me at cegger@utk.edu or 865-755-8186.

Sincerely,

Christine M Egger, DVM, MVSc,
CVA, CVH, Diplomate, ACVAA
Head, Section of Anesthesiology,
Critical Care and Emergency Medicine,
Professor, Anesthesia, Analgesia, and Integrative Medicine.
Dear Committee Members:

I am writing this letter in support of the development of the American College of Veterinary Botanical Medicine (ACVBM). There are several ACVIM, ACVS, and ACT diplomates who utilize botanicals in the small and large animal hospitals of the College of Veterinary Medicine at the University of Florida. We support the establishment of the ACVBM and specifically their goal to develop improved and specialized clinical skills and expertise in the field of veterinary botanical medicine.

Please know that should the AVBS approve the establishment of this ACVBM specialty, and the University of Florida administration approves, that we would support the exploration and development of a residency program here.

Please do not hesitate to call me for more information should you have any questions or concerns.

Sincerely,

Margaret M. Sleeper VMD, DACVIM (cardio)  
margaretmssleeper@ufl.edu
October 31, 2017

To whom it may concern:

My name is Carolina Medina and I am a rehabilitation specialist at Coral Springs Animal Hospital. I have also been certified in acupuncture and herbs for the past 12 years. I fully support the American College of Veterinary Botanical Medicine to become recognized as a specialty in our profession. Coral Springs Animal Hospital is a high-end referral center where we train interns, specialty interns, and residents. We offer the following specialty services: internal medicine, oncology, cardiology, dermatology, ophthalmology, neurology, critical care, radiology, surgery, and rehabilitation. We are fully equipped with digital radiology, ultrasonography, CT, MRI, fluoroscopy, endoscopy, and other advanced diagnostic and therapeutic services. I would be willing to take on a resident should the American College of Veterinary Botanical Medicine receive approval by the ABVS as a recognized veterinary specialty.

Sincerely,

Carolina Medina DVM, CVA, CVCH, Diplomate American College of Veterinary Sports Medicine & Rehabilitation
Coral Springs Animal Hospital
2160 N. University Drive
Coral Springs, FL 33071
Tel: 954-753-1800
Fax: 954-753-5120
www.coralspringsanimalhosp.com
November 8, 2017

American Board of Veterinary Specialties
American Veterinary Medical Association
Attn: ABVS
1931 Meacham Rd, Suite 100
Schaumburg, IL 60173-4360

Dear Committee Members,

I am writing a letter of support of the development of the American College of Veterinary Botanical Medicine (ACVBM). I work at NCSU and have been using botanicals in practice in our clinical veterinary medicine. We additionally have a pharmacist at our college who is deeply interested in and helps with the prescription of botanicals for veterinary care. We are in support of developing greater specialized clinical skills and expertise as well as enhancing the contributions as well as scientific studies in veterinary botanical medicine. I am also working on becoming certified in veterinary botanical medicine.

If AVBS approves the establishment of this ACVBM specialty, and NCSU administration approve, we hope that we could develop a residency program, or at minimum support other residency programs through rotations through our service in zoo and exotic animals and our pharmacy on the treatment of a variety of animals through botanicals.

Please do not hesitate to contact me if you have any additional questions. Thank you for your consideration of this specialty.

Sincerely,

Tara M. Harrison, DVM, MPVM, DACZM, DACVPM, DECZM (ZHM), CVA
APPENDIX IV Residency Guidelines
American College of Veterinary Botanical Medicine

ACVBM Residency Guidelines
Candidates for the Diplomate Certification in Veterinary Botanical Medicine Examination must have completed an approved Residency Program and met all other requirements including Credentials and publication. The Residency Training Programs including both Standard and Alternate are approved by the American College of Veterinary Botanical Medicine (ACVBM) Credentials and Residency Committee (CRC) and Executive Board.

1. **THE GOALS OF THE RESIDENCY TRAINING PROGRAMS**
   a. Development of a high level of clinical skills and expertise in the field of veterinary botanical medicine
   b. Development of a critical understanding and working knowledge of the current veterinary and human literature related to botanical medicine and proficiency in literature review and the synthesis and clinical application of new information
   c. Development of critical thought processes and the use of a problem-based approach to patient care
   d. Demonstration of an ability to teach, communicate and effectively present information;
   e. Demonstration of exceptional ethical standards and the ability to act as a professional role model
   f. Demonstration of having made a contribution to the science of veterinary botanical medicine

2. **TIME FOR COMPLETION**

   The total minimum time required for completion of a residency will be determined by the Primary Advisor of the residency and the case load available at the Training Site. All residents must complete, the entire residency training program within a maximum of 6 years and no less than 3 years.

3. **PRIMARY ADVISOR**
   a. Primary Advisor must be a Diplomate of the ACVBM. The Primary Advisor is directly responsible for the selection of Resident applicants, clinical guidance and mentorship of Residents and collaborate in the assessment and evaluation of Residents with 2 co-advisor
   b. The Primary Advisor evaluates and approves all relevant forms and documents as well as the training site(s) as suitable for the program
   c. The Primary Advisor also ensures the Resident has completed all requirements of the program of the ACVBM and when the Resident submits the Credentials Application the Primary Advisor is asked to attest in writing that all training requirements have been satisfactorily completed
   d. The Primary Advisor may serve only 3 Residents at one time
   e. The Primary Advisor must be familiar with, and understand the training program guidelines and credentialing requirements, and must be willing and able to guide and evaluate a Resident’s progress in the areas of clinical training, teaching and research
   f. In the first instance before Diplomates of the ACVBM, Primary Mentors can include Diplomates with training in botanical medicine; DVMs with MS degrees or PhDs in botanical medicine related area or as approved by ACVBM

4. **REQUIREMENTS FOR THE RESIDENCY APPLICANTS**

   This is the totality of the program requirements to be completed before taking the Certification Exam. The applicants must be veterinarians and meet the following requirements:
a. Graduate of a college of veterinary medicine (accredited/or listed by the American Veterinary Medical Association (AVMA)), or possess a certificate issued by the Commission for Foreign Veterinary Graduates (ECFVG) or are legally qualified to practice veterinary medicine in some state, province, territory, or possession of the United States, Canada, or other country
b. Complete one year of clinical veterinary internship training, a residency in another discipline, or 5 years of active veterinary clinical practice
c. Meet the education, training and experience requirements established by the ACVBM
d. Demonstrate unquestionable moral character and ethical professional behavior
e. Legally able to practice at the residency training site (e.g., veterinary medical license and other state or federal requirements)

5. APPLICATION PROCESS
a. The Resident applicant and his/her Primary Advisor must submit the ACVBM Residency Application and Registration Form (Appendix I) to the Credentials and Residency Committee, which ensures the Resident will meet the minimum requirements for the Residency’s board certification within a six-year period. The application includes the following items: Who is involved in the residency; How the residency is organized; Where the training will take place; How each of the requirements for the Residency will be met; A time frame for completion.
b. Upon receiving approval from the Credentials and Residency Committee, the program may be initiated. Then, the Resident Application and Registration Form (Appendix I) must also be submitted and registered with the ACVBM office within 30 days of program initiation.

6. RESIDENCY TRAINING PROGRAMS

Residency training programs are the foundation for training of future diplomates in the ACVBM. The components of both the Standard and Alternate residency training program include: 1) one Primary Advisor; 2) two Co-Advisors; 3) Resident; 4) Other requirements of a residency training program are listed in this document.

Advisory Committee:
The establishment of an Advisory Committee for each Resident is required. Each committee should consist of one Primary Advisor and two co-advisors. The two co-advisors will be selected by the Primary Advisor and may assist in the selection of trainees and supervision of the training program. The co-advisor can be an ACVBM Diplomate, or a specialist from other specialty colleges as well as veterinary clinicians with pertinent PhD credentials, as deemed appropriate by the CRC.

Resident:
The Resident undertaking a residency program in Veterinary Botanical Medicine:
  a. Will need to commit time, travel and money toward their training
  b. Can choose a Standard Residency program or an Alternate Residency program
  c. Will agree to all program and credential requirements and meet deadlines for submission of fees and relevant documents
  d. Will provide timely progress reports and evaluations throughout the training period for the CRC to review
  e. Must meet the requirements of: a one year of clinical veterinary internship training, a residency in another discipline, or 5 years of full time, veterinary clinical practice
Training Site:
The Residency training program can take place at a University, or Practice where the Resident will provide primary patient care appropriate to their level of training. The Resident’s Primary Advisor must provide direct or indirect supervision appropriate to the Resident’s abilities (e.g. direct supervision for new Residents).

Infrastructure:
The clinical setting must include advanced diagnostics to enable complete case evaluation and diagnosis as well as follow up of case outcomes. The following is required to be available in the primary training hospital: clinical pathology capabilities including CBC, serum chemistries, urinalysis, cytology and parasitology. The Resident must be able to refer internally or externally for advanced diagnostic procedures or therapeutic interventions (such as CT, MRI, radiation oncology, scintigraphy, specialized surgeries, endoscopy, etc.), if they aren't available in house.

The Resident must have access to a botanical medicine pharmacy and botanical products and equipment for dispensing.

The Resident must have access to a veterinary and or medical library. The library should have access to conventional as well as veterinary botanical medicine journals. The Primary Advisor should develop a required and recommended reading list.

A Training Week:
For the purposes of the residency, a training week is defined as a minimum of 40 hours of logged immersion time. The start day for a Resident’s training week will remain the same day of the week as the first day of their residency.

Direct Supervision:
The Resident and Primary Advisor are participating together, concurrently managing cases in clinical practice. The Primary Advisor is physically available for consultation. The minimal direct supervision should be at least 30 training weeks.

Indirect Supervision:
The Resident and the Primary Advisor, although participating together, are not concurrently physically involved in clinical practice. To qualify for indirect supervision, the Resident and Primary Advisor must have direct contact (Skype, phone, web, other) for at least 3 hours per week, with consultation available on a daily basis by phone and email (or comparable effective communication platforms). The maximum indirect supervision time should be no longer than 30 training weeks.

Supervision of Remote Consultation:
Botanical medicine experience may be gained through extramural opportunities to work in other specialties or institutions directly related to botanical medicine, such as botanic pharmacology and practice. In this case, the Primary Advisor and Resident work closely together yet are not on-site together. In cases where the Primary Advisor and Resident are not physically working in the same location, regular and significant direct communication is required. Depending upon the level of supervision, such experience may be considered as either directly or indirectly supervised.

Training Experiences:
Some credentialing requirements must be completed on-site; some may be completed off-site such as the advanced online botanical medicine CE courses approved by the RACE and the ACVBM.

Part-Time Experience:
Part-time experience is permitted, where cumulative experiences over time may accrue for a block of time. This experience should be outlined on the Application Form, that is pre-approved by the Primary Advisor. It is the Residents’ responsibility to document their experiences with an activity log that is reviewed and signed by the Primary Advisor.

**Standard Residency:**

a. The residency program will be conducted at an AVMA accredited veterinary medical school
b. Residents must be enrolled in a residency program of three years in length, approved in advance by the CRC

**Alternate Residency Program:**

a. The Resident proposes an Alternate Residency Program that meets the same requirements of a Standard Residency but part or all of the program is outside of an AVMA accredited veterinary medical school
b. The Alternate Program can be up to 6 years in duration and is approved by the CRC

7. **PROGRAM REQUIREMENTS**

An acceptable Botanical Medicine Residency is 100 weeks of training within 3 to 6 years, allows the individual seeking diplomate status time to obtain sufficient knowledge and clinical skills to meet the credentials required to take the ACVBM certifying examination. It includes a minimum of 60 weeks of botanical medicine, 24 weeks of independent study, research, teaching and writing, and 16 weeks of immersion in specialty veterinary practice.

If an adequate number of cases, personnel or facilities are not available at the Program site, then the Primary Advisor is responsible for determining how the requirements should be fulfilled. The CRC must approve such arrangements in advance. Letters of commitment for the provision of off-site training must be submitted to the CRC.

**A. Botanical Medicine Clinical Training**

This requirement consists of a minimum of **60 weeks** immersion in botanical medicine practice, which is supervised by the Primary Advisor or a co-advisor.

a. A minimum of 30 weeks of clinical experience with direct ACVBM diplomate supervision:
   - Direct supervision is defined as the ACVBM diplomate being at the same location as the Resident during the required time
b. No more than 30 weeks of clinical experience with indirect ACVBM diplomate supervision:
   - The diplomate and Resident must establish a monthly time to interact and discuss the month’s clinical activity related to botanical medicine. This contact may be via phone or web conference (such as Skype or equivalent)

**B. Specialty Rotations Training**

This requirement consists of **16 weeks** of immersion in specialty veterinary practice, as negotiated with Primary Advisor in 4 or more specialties (2 or more weeks per specialty) including but not limited to the following areas:

- Clinical Pharmacology
- Nutrition
- Internal Medicine in general (equine, canine, or feline)
Internal Medicine in oncology
Internal Medicine in cardiology
Internal Medicine in neurology
Veterinary Exotics
Veterinary rehabilitation
Veterinary Behavior
Theriogenology
Veterinary Dermatology

C. Veterinary Botanical Medicine Study or Practice
This requirement consists of 24 weeks of independent study on topics related to botanical medicine or independent immersion in botanical medicine practice. Independent study or practice is intended to allow development of independent thought, peer mentoring and teaching skills, focused study in specialized facets of botanical medicine, further elective rotations, further supervised or independent rotations in botanical medicine, cross species training or human botanical medicine training.

The Resident Primary Advisor is responsible for designing this requirement to meet the needs of the individual Resident and is responsible for ensuring that the Resident is receiving adequate training during this time. Continuing education that is intensely focused on a specialized facet of botanical medicine may be logged concurrent with the independent study requirement on an individual basis at the discretion of the Credentials and Residency Committee; however, general continuing education requirements may not be logged concurrently with independent study time.

The Resident, along with the support of their Primary Advisor, must ensure that all independent study requirements are met and logged within the required term of the residency training program. Residents are expected to make timely regular progress in completing these requirements. The biannual report should reflect this progress.

a. A minimum of 10 weeks must be spent conducting Research and manuscript preparation
b. A minimum of 14 weeks of individualized rotations must be performed, including but not limited to:
   - Rotations at a veterinary medicine practice that concentrates on the Resident’s species of interest. The veterinary medicine rotation must be with a person that has received advanced training in veterinary botanical medicine
   - Rotation with the alternate species
   - Special rotation at a human botanical clinic
   - Vacation: Up to two weeks per year of vacation time may be counted toward this requirement, with a maximum of 6 weeks allowable

D. Graduate-Level Veterinary Botanical Medicine Training Course and National or International Level Continuing Education Related to Veterinary Botanical Medicine
Education at graduate-level Veterinary Botanical Medicine Training Courses (TC) are required. These TC courses are approved by the Primary Advisor. A Resident must submit the TC Application (Appendix II) to be approved by the ACVBM.
E. Manuscript Requirement
In addition to the time outlined for research/clinical investigation and manuscript preparation, the Resident must publish at least one manuscript in the field of botanical medicine in order to be accepted to sit for the ACVBM certification examination as below:

a. The research must be the result of the Residency Resident’s work
b. The residency applicant must be the first author on the manuscript
c. The manuscript must follow a scientific approach, including a clearly stated hypothesis or objective, an appropriate description of techniques (including statistical analysis), a report of the results, and a discussion. Communications, case reports, review papers, book chapters, etc. are not acceptable
d. The manuscript must be written in English
e. The date of publication cannot be more than five years old by the deadline for credentials submission. A letter of acceptance can be used to proof publication
f. The journal must be peer reviewed. These criteria are to be fulfilled by the time of manuscript submission and documentation and must be included with the Resident’s credentials packet, showing that the criteria are met at the time of manuscript submission

F. Oral Presentations
Residents are required to present a minimum of 3 multimedia presentations over the course of their program covering topics related to Botanical Medicine. These presentations must be at least 20 minutes in duration and presented to an audience of their peers. At least one diplomate of any specialty college must be present. These are required to be recorded in the Residency Master Log and verified by the ACVBM Diplomate.

G. Teaching Requirements
Trainees must be involved in teaching students, interns, residents, technicians, veterinarians, producers, and owners. Teaching will include formal and informal clinical tutorials, such as daily rounds, case discussions, as well as appropriate involvement in lecture courses and seminars.

a. A Resident in a university setting is expected to help teach students about botanical medicine. A Resident in a specialty practice with rotating interns and Residents of other disciplines should have some expectation of clinical instruction. A Resident in a non-specialty hospital setting will provide clinical instruction to veterinarians of the practice that hosts that Resident
b. Trainees will regularly attend and participate in seminars, rounds and case conferences in botanical medicine and other applicable specialty rounds, seminars, etc.

H. Other Scholarly Activities:
Formal Study: Residents must undertake graduate level training in Botanical Medicine (Curriculum and learning objectives) approved by the ACVBM Credentials and Residency Committee. This may include pharmacognosy, pharmacology and toxicology. Statistics?

Journal Study: The Resident is expected to be familiar with journal articles published over the last 5 years pertaining to veterinary medicine as well as reviews and meta-analyses pertaining to Phytomedicine in general.

Other references and study material for the Diplomate Examination can be found at Appendix V.
8. WRITTEN DOCUMENTATION

Successful completion of a residency in the Specialty of Botanical Medicine may be achieved by successfully completing any residency-training program that has attained approval by the CRC and by meeting all training requirements prior to the start of training. The Primary Advisor will be responsible for documenting that the training has occurred as specified. Completion of all requirements should be documented and/or submitted semi-annually or yearly:

a. Biannually - Biannual Progress Report Form (due February 1) – Appendix III
   - All Residents must submit a Biannual Progress Report. The Credentials and Residency Committee will evaluate the Biannual Progress Report. Recommendations and requirements will be forwarded to the Resident and their Resident Primary Advisor if needed
   - The Resident Primary Advisor and two co-advisors must sign attesting to satisfactory completion of individual immersion training weeks, experience, and skills requirements in order for credit to be granted
   - The Resident and the Primary Advisor are responsible for ensuring that the review form is complete

b. Yearly - The Resident must provide written documentation including CE Course Form (Appendix II), Biannual Progress Report (Appendix III), Annual Progress Report (Appendix IV) forms and Master Log (Appendix V) to the diplomate’s Primary Advisor that the listed requirements have been completed. The Primary Advisor is responsible for verifying that all of these requirements have been met prior to submission of credentials for board certification. An Annual Progress Report (Appendix IV) and a copy of the Master Log must be signed by the Primary Advisor and submitted to the ACVBM College Secretary by August 1 each year for review by the CRC.
   1) CE Course Form
   2) Biannual Progress Report
   3) Annual Progress Report
   4) Master Log should be named using the following format – LastName.MasterLog.xls

9. CREDENTIALS SUBMISSION

This form must be completed and signed by the Resident. This form along with the Master Log, Annual Progress reports, one peer-reviewed publication, three recommendation letters (one from an active diplomate of ACVBM, and two from other ABVS specialists), and a $350 credential submission fee must be submitted to the College Secretary at secretary@acvbm.org for review by the CRC to establish eligibility for taking the board certification examination. The deadline for this submission is set for August 1 each year. All application materials will become the property of the ACVBM.

10. EXPECTED SKILLS AND KNOWLEDGE OF ACVBM DIPLOMATES

The ACVBM Diplomate is expected to be competent in 3 areas of botanical medicine: service, teaching and research. To do so, they must have skills to botanical medicine, the ability to incorporate those skills in an integrative medicine setting, and a knowledge base related to botanical medicine. Unique skills include veterinary botanical medicine principles and practices, specialised veterinary botanical medical care, and the preparation and presentation of botanical veterinary case studies and botanical research findings for publication. The latter includes the ability to present the application of the studies in an integrated practice. Skills can be divided into the following primary categories: cognitive, communication and education, clinical, research and regulatory. Subsets of each area are listed in the tables below.
A. Skills

Cognitive skills
- Ability to apply knowledge and skills of botanical medicine in service, research, teaching
- Use critical thinking to review, analyze, consolidate and synthesize clinical problems and identify and provide integrated solutions to complex problems
- Integrate contemporary veterinary medicine and botanical medicine principles

Communication and education skills
- Ability to communicate knowledge, research, application to other Diplomates, veterinarians, and the general public
- Communication of basic concepts and treatment plans to veterinary clients
- Ability to demonstrate application of botanical medical knowledge/skills in veterinary practice
- Ability to demonstrate the integration of veterinary botanical medicine and current practice
- Presentation and research studies to veterinarians and Diplomates

Clinical skills
- Integrate veterinary botanical medicine safely and effectively within the context of contemporary animal health care
- Apply in-depth knowledge of botanical medicine *materia medica* and the principles of veterinary botanical medicine within clinical practice
- Use a comprehensive and in-depth understanding of veterinary botanical medicine knowledge in a professional practice for the following:
  - Obtain animal patient health history, including a botanical medicine perspective
  - Conduct a clinical exam on a patient including botanical medical principles of assessment and diagnosis
  - Initiate, plan, implement and evaluate the botanical medicine treatment strategy for a range of common veterinary conditions

Research skills
- Search and apply traditional, and scientific information sources to research subject
- Critically evaluate and integrate knowledge from a range of sources to prepare case
- Prepare veterinary case studies for publication
- Prepare and present in-depth veterinary botanical medicine research findings

Regulatory
When prescribing veterinary botanical medicines demonstrate knowledge of, regulatory framework, health and safety, labelling requirements, scheduled herbs and principles of manufacturing

B. Knowledge Base

Concepts and history
- The principles and practices of veterinary botanical medicine practice
- The history and development of botanical medicine in a veterinary context
- The cultural and traditional lines of evidence used in botanical medicine
- The integration of these approaches with current practices in veterinary medicine
Materia medica

- Plant and botanical medicine identification
- Application of botanical medicine across species
- The actions, indications, and active principles of the main botanical medicines used for each system/condition
- Constituent phytochemicals and their actions
- Indications and contraindications for botanical medicine
- The principles of formulation (including dosage and duration of treatment)
- Herb-drug interactions and side effects
- Plant toxicology, intoxications and adverse events
- Dosage principles
- Chemical and physical incompatibilities of botanical medicines
- Botanical medicine practice
- Sources of botanical medicines for use in veterinary practice
- Preparation of botanical medicines for topical, rectal and oral administration
- Manufacturing processes for basic botanical medicine preparations
- Knowledge of major botanical medicine categories (single herbs and formulas) with respect to their medicine indications
- Basic requirements for veterinary botanical medicine pharmacy
- Factors that affect botanical medicines in preparation and storage
- Recording requirements and procedures for botanical medicines
- Items required for a basic veterinary botanical pharmacy
- Preparation of veterinary botanical medicine for dispensing in practice
- Legal requirements relating to the prescription of medicine
- OHS hazards and controls
- Good manufacturing practice and quality control in botanical medicine
- Recent issues and events affecting the industry

Treatment strategies

Veterinary Botanical Medicine treatment strategies applied to veterinary practice for a range of conditions in each of the following categories:
- immune conditions including infectious disease and autoimmune diseases
- cancer
- cardiopulmonary disorders
- endocrine and reproductive disorders
- ear, nose and eye disorders
- gastrointestinal disorders
- hepatobiliary disorders
- musculoskeletal disorders
- skin diseases
- renal and urinary disorders
- behavior conditions
- neurological conditions

Research

- Professional development activities, opportunities and options available
- Relevant legislation and codes of ethics or practice standards
- Knowledge of research methodologies commonly used in veterinary medicine research
• Knowledge of recent research affecting the integrative veterinary medicine industry
• Knowledge of relevant reference works and information sources

Ethics
• Conservation of botanical medical plant species
• Biodiversity and sustainability of endangered plant species and plant medicine substitutes

11. ACVBM DIPLOMATE CERTIFICATION EXAMINATION (DCE)

Once all requirements have been met, the Master Log, Annual Progress reports, one peer-reviewed publication that has been published in the peer-reviewed literature within the past 3 years, in which botanical medicine was the primary medical treatment used, a completed Credentials Submission Form (Appendix VI), three recommendation letters (one from an active diplomate of ACVBM and two from other ABVS specialists), and a $350 credential submission fee must be submitted to the college secretary, at acvbm@listserv.uga.edu, for review by the CRC to establish eligibility for taking the board certification examination.

A Resident should submit all the above required documents before August 1 each year. A Resident will then be notified by the CRC regarding the acceptance of their credential materials for the College board-certification examination at least 120 days prior to the examination.

The DCE is based on a job/task analysis, developed by surveying members of the ACVBM organizing committee as well as other veterinarians that use herbal medicine in their practice. The survey identified skills and knowledge specifically needed for the practice of veterinary botanical medicine, as well as how often each area is used in practice.

The examination will consist of four (4) parts:

a. General botanical medicine (all candidates are required to take this section)
   b. Principles and Practices
   c. Clinical botanical medicine
   d. Botanical identification of medicinal plants.

The Modified Angoff Method is used to determine the passing score. More information is available from the Examination Committee for those who wish to take the exam.

12. APPENDICES

Appendix I. Residency Application and Registration Form
Appendix II. Training Course and CE Application Form
Appendix III. Residency Biannual Progress Report Form
Appendix IV. Residency Annual Progress Report
Appendix V. Master Log (Excel Worksheet)
Appendix VI. Residency Credentials Submission Form
The ACVBM Residency Committee provides this form to assist the Residency Training Program Advisors to determine if their proposed residency program will meet the ACVBM requirements.

This form is to be completed and returned to the Secretary of the ACVBM College.

The Resident must sign the signature page and also have this form signed by her/his Primary Advisor and Co-advisors.

**Part One: Information on Residency, Primary Advisor and Co-advisors**

<table>
<thead>
<tr>
<th>First Name</th>
<th>Your Recent Photo (2 inch X 2 inch)</th>
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<tr>
<td>Last Name</td>
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<td>Email</td>
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<td>Mailing Address</td>
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<td>City</td>
<td>State</td>
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<td>Phone</td>
<td>Fax</td>
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<tr>
<td>DVM Degree or Equivalent Degree</td>
<td>Institution and Location</td>
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<tr>
<td>Other Advanced Degree if Applicable</td>
<td>Title of Degree</td>
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<tr>
<td>Current Position</td>
<td>Title of Position</td>
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<tr>
<td>Your Primary Advisor</td>
<td>Name</td>
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<td></td>
<td>Title</td>
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<td></td>
<td>Department:</td>
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<tr>
<td>Hospital or University:</td>
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<td>Street Address:</td>
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<td>City, State, Zip, Country:</td>
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<td>Phone:</td>
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<td>Fax:</td>
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<tr>
<td>Signature by your Primary Advisor</td>
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</tbody>
</table>

**Your Co-Advisor I**

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<th>Name:</th>
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<td>Title:</td>
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<td>Department:</td>
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<td>Hospital or University:</td>
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<td>Phone:</td>
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<td>Fax:</td>
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<tr>
<td>Signature by your Co-Advisor</td>
<td></td>
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</tbody>
</table>

**Your Co-Advisor II**

| Name:                   |                      |
| **Title:** |  |
| **Department:** |  |
| **Hospital or University:** |  |
| **Street Address:** |  |
| **City, State, Zip, Country:** |  |
| **Email:** |  |
| **Phone:** |  |
| **Fax:** |  |
| **Signature by your Co-Advisor** |  |
Part Two: Signature Page by Resident

I hereby register my residency with the American College of Veterinary Botanical Medicine in accordance with its rules and guidelines, as published in the college’s Constitution and Bylaws, and Residency Guidelines. I have read the current Residency Program Guidelines as adopted by the American College of Veterinary Botanical Medicine. I understand that any false information that I provide or other evidence of fraud on my part will adversely affect my residency training and/or acceptance of my Credentials Application and may be reason for termination of my residency program and/or permanent disqualification of my application.

I further covenant and agree:
(i) to indemnify and hold harmless the American College of Veterinary Botanical Medicine and each and all of its members, regents, officers, examiners and agents from and against any liability whatsoever in respect of any act or omission in connection with this registration, applications, credentials, examinations, the grades on such examinations and/or the granting or issuance of or failure to grant or issue a certificate to me, and
(ii) that any certificate, which may be granted and issued to me shall be and remain the property of the American College of Veterinary Botanical Medicine.

Resident Signature ______________________________

Date ______________________________
Part Three: Residency Training Program Plan

The plan includes: Who is involved in the residency, how the residency is organized, where the training will take place and how each of the requirements for the Residency will be met.

A. Physical location of the residency training program

<table>
<thead>
<tr>
<th>Site</th>
<th>Name of Institution</th>
<th>Address</th>
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<tbody>
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<td>Primary site:</td>
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<tr>
<td>Secondary site:</td>
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<td>Other sites:</td>
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</tbody>
</table>

B. 60-week Botanical Medicine Clinical Training Plan

This requirement consists of a minimum of 60 weeks immersion in a botanical medicine practice, that is supervised by the Primary Advisor or a co-advisor

<table>
<thead>
<tr>
<th>Clinical Experience</th>
<th>Number of training weeks</th>
<th>Supervisor and location</th>
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</thead>
<tbody>
<tr>
<td>Direct supervision by ACVBM diplomate</td>
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<tr>
<td>Indirect supervision by ACVBM diplomate</td>
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</table>

C. Specialty Rotations Training Plan

This requirement consists of 16 weeks of immersion in a specialty veterinary practice within 4 or more specialties (2 or more weeks per specialty) that must be directly supervised by a diplomate. The Specialty Rotations Program must be approved by the Primary Advisor and relevant to the candidate.

<table>
<thead>
<tr>
<th>Specialty Rotations</th>
<th>Supervisor</th>
<th>Time Frame for completion</th>
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<tbody>
<tr>
<td>Clinical Pharmacology</td>
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<tr>
<td>Nutrition</td>
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<td>Internal Medicine</td>
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<td>Oncology</td>
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<td>Cardiology</td>
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<td>Neurology</td>
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<td>Theriogenology</td>
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<td>Dermatology</td>
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<td>Behavior</td>
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<td>Rehabilitation</td>
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<td>Exotics</td>
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<td>Other:</td>
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</tbody>
</table>
## D. Time frame for completion

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Time Frame for completion</th>
<th>Location</th>
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</thead>
<tbody>
<tr>
<td>60-week Botanical Medicine Clinical Training</td>
<td></td>
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<tr>
<td>16-week Specialty Rotations Training</td>
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<tr>
<td>24 weeks of Independent Study</td>
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<tr>
<td>120 CE hours in Courses Related to Botanical Medicine</td>
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<tr>
<td>Oral Presentations</td>
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<tr>
<td>Research published in a peer reviewed journal</td>
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<tr>
<td>Teaching</td>
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</table>
APPENDIX II AMERICAN COLLEGE OF VETERINARY BOTANICAL MEDICINE
TRAINING COURSE (TC) AND CONTINUE EDUCATION (CE) APPLICATION FORM

- Education at a graduate-level Veterinary Botanical Medicine Course (TC) are required and the TC must be approved by the ACVBM. This form should be completed and returned to the ACVBM College.
- The TC courses and CE program/seminar(s)

<table>
<thead>
<tr>
<th>Title of the training course and CE program/seminar</th>
<th>Name of the institution that offers this course</th>
<th>Dates and locations of the course that is offered</th>
<th>Class hours or CE hours</th>
<th>Signed by the institution that offers the course</th>
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Signature by the ACVBM Credential and Residency Committee

Sign:_______________________________________ Date:_________________
APPENDIX III AMERICAN COLLEGE OF VETERINARY BOTANICAL MEDICINE
RESIDENT BIANNUAL PROGRESS REPORT FORM

- All Residents must submit a Biannual Progress Report. The Credentials and Residency Committee will evaluate the Biannual Progress Report. Recommendations and requirements will be forwarded to the Resident and their Resident supervisor as needed
- The Resident Primary Advisor and two co-advisors must sign, attesting to satisfactory completion of individual immersion training weeks, experience, and skills requirements, in order for credit to be granted
- The Resident and the Resident Primary Advisor are responsible for ensuring that the review form is complete

Part One Personnel Information and Signature

Date to Submit this Biannual Progress Report Form:____________________

Date the residency started:__________________

Signed by the Resident:_____________________

Resident’s Contact Information

<table>
<thead>
<tr>
<th>Name:</th>
<th>Practice or University:</th>
<th>Address:</th>
<th>City, State, Zip:</th>
<th>Country:</th>
<th>Phone:</th>
<th>E-mail:</th>
</tr>
</thead>
</table>

Current year of residency training (please circle): 1st year 2nd year 3rd year Other:

Projected Date of Training Program Completion:

Resident Primary Advisor’s (Diplomate; Oversees Resident’s daily activities) Contact Information

<table>
<thead>
<tr>
<th>Name:</th>
<th>Practice or University:</th>
<th>Address:</th>
<th>City, State, Zip:</th>
<th>Country:</th>
<th>Phone:</th>
<th>E-mail:</th>
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</table>

Overall Impression (Approval or Disapproval) on the 6-month training of this Resident:
### The First Resident Co-Advisor's Contact Information

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<th>Name:</th>
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<tbody>
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<td>Practice or University:</td>
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<td>E-mail:</td>
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Overall Impression (Approval or Disapproval) on the 6-month training of this Resident:  

Signed by the 1st co-advisor

### The Second Resident Co-Advisor's Contact Information

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<th>Name:</th>
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<td>E-mail:</td>
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</table>

Overall Impression (Approval or Disapproval) on the 6-month training of this Resident:  

Signed by the 1st co-advisor
Part Two Instructions

- This part must be completed by the Primary Resident Advisor
- Mark the box at the appropriate level of Resident progression or accomplishment in their training program for each of the listed items below.

### 1. Program Requirements

<table>
<thead>
<tr>
<th></th>
<th>Unacceptable</th>
<th>Needs Improvement</th>
<th>Average</th>
<th>Above Average</th>
<th>Excellent</th>
<th>N/A</th>
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<tbody>
<tr>
<td>Clinic schedule</td>
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<tr>
<td>Participation in rounds or journal club</td>
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<tr>
<td>Progress in Teaching</td>
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<td>Progress in presentation</td>
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<tr>
<td>Progress in research project</td>
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<td>Progress toward publication</td>
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<td>Other comments</td>
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### 2. Knowledge Base

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<th>Unacceptable</th>
<th>Needs Improvement</th>
<th>Average</th>
<th>Above Average</th>
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<tr>
<td>Basic scientific knowledge</td>
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<tr>
<td>General knowledge of Botanical Medicine</td>
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<tr>
<td>Clinical knowledge of Botanical Medicine</td>
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<tr>
<td>Awareness of current literature</td>
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<td>Feedback from other departments</td>
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<td>Feedback from</td>
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### 3. Clinical Abilities

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<th>Unacceptable</th>
<th>Needs Improvement</th>
<th>Average</th>
<th>Above Average</th>
<th>Excellent</th>
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<tbody>
<tr>
<td>History taking</td>
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<td>Physical examination skills and assessment</td>
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<td>Formulating Differential Diagnoses</td>
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<td>Identifying relevant Botanical Medicine diagnosis</td>
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<td>Development of Botanical Medical treatment plans</td>
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<td>Patient care and compassion</td>
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<td>Patient follow up</td>
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<td>Other comments</td>
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### 4. Clerical and Managerial Skills

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<th>Unacceptable</th>
<th>Needs Improvement</th>
<th>Average</th>
<th>Above Average</th>
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<tbody>
<tr>
<td>Support of hospital procedures and policies</td>
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<tr>
<td>Completeness of medical records</td>
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<tr>
<td>Responding to correspondence or contacts</td>
<td></td>
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</table>
### 5. Interpersonal skills

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<th></th>
<th>Unacceptable</th>
<th>Needs Improvement</th>
<th>Average</th>
<th>Above Average</th>
<th>Excellent</th>
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<tbody>
<tr>
<td>Attitude and communication with in-house veterinarians</td>
<td></td>
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<tr>
<td>Attitude and communication with RDVMs</td>
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<tr>
<td>Attitude, communication and ability to relate to clients</td>
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<tr>
<td>Attitude and communication with staff</td>
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<tr>
<td>Attitude, communication and interaction with other departments</td>
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<tr>
<td>Ability to handle emergencies or stressful situations</td>
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<td>Professional behavior and appearance</td>
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<td>Leadership qualities</td>
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<td>Teamwork and receptive to feedback</td>
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<td>Recognizes limitations</td>
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<td>Ability to multitask</td>
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<td>Willingness to ask for help</td>
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<tr>
<td>Positive aspects of Resident’s performance, including improvements since last evaluation (if applicable)</td>
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<tr>
<td>Comments or suggestions for improvement in the Resident’s performance or progress toward completion of their training program</td>
<td></td>
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<tr>
<td>Short-term goals (please include timeframe for completion)</td>
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<tr>
<td>Long-term goals (please include timeframe for completion)</td>
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</tbody>
</table>
APPENDIX IV  AMERICAN COLLEGE OF VETERINARY BOTANICAL MEDICINE  

RESIDENT ANNUAL PROGRESS REPORT FORM

- The Resident and the Primary Advisor must complete and sign this form.
- The completed form must be submitted to the ACVBM College by August 1 each year for review by the Credentialing and Residency Committee along with Training Course and CE Application Form (Appendix II), Biannual (Appendix III) and Master Log (Appendix V)

Date to Submit this Annual Progress Report Form:____________________

Date the residency started:______________________

Projected Date of completion for the Resident:______________

<table>
<thead>
<tr>
<th>Annual Assessment by a Resident</th>
<th>Comments on Primary Advisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE Course</td>
<td></td>
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<tr>
<td>Teaching</td>
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<tr>
<td>Oral Presentation</td>
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<tr>
<td>Research Project</td>
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<tr>
<td>Publications</td>
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</tr>
<tr>
<td>Signed by Resident</td>
<td></td>
</tr>
<tr>
<td>Signed by Primary Advisor</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX V AMERICAN COLLEGE OF VETERINARY BOTANICAL MEDICINE MASTER LOG
This is an electronic excel file to record activities throughout training period. This is available by contacting ACVBM.
APPENDIX VI AMERICAN COLLEGE OF VETERINARY BOTANICAL MEDICINE RESIDENCY CREDENTIALS SUBMISSION FORM

- This form must be completed and signed by a Resident.
- This form along with the Master Log, Annual Progress reports, one peer-reviewed publication, three recommendation letters (one from an active diplomate of ACVBM, and two from other ABVS specialists), and $350 Credential Submission Fee must be submitted to the College Secretary at secretary@acvbm.org for review by the Credentials and Residency Committee to establish eligibility for taking the board certification.
- The deadline for this submission is August 1 each year.
- All application materials will become the property of the American College of Veterinary Botanical Medicine.

<table>
<thead>
<tr>
<th>First Name:</th>
<th>Last Name:</th>
<th>Your Recent Photo (2 inch X 2 inch)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Email:</td>
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<tr>
<td>Mailing Address:</td>
<td></td>
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<td>City, State, Zip:</td>
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<td>Country:</td>
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<td>Cellular Phone:</td>
<td>Fax:</td>
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<tr>
<td>Graduate of Veterinary Medicine Degree</td>
<td>Degree (such as DVM):</td>
<td>Institution and Location:</td>
</tr>
<tr>
<td>Graduation Year:</td>
<td>Veterinary medical license (State or Province, Country):</td>
<td>License number:</td>
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<tr>
<td>Credential Submission Fee ($350)</td>
<td>Credit Card</td>
<td>Type: Number: Expiration date:</td>
</tr>
<tr>
<td>Check</td>
<td>Mailing the check to the ACVBM College 9002 Sunset Drive; Colden, NY 14033</td>
<td></td>
</tr>
<tr>
<td>Your 1&lt;sup&gt;st&lt;/sup&gt; reference (must be an active ACVBM diplomate)</td>
<td>Name:</td>
<td></td>
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<td>Credential:</td>
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<td>Contact Information:</td>
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<tr>
<td>Your 2&lt;sup&gt;nd&lt;/sup&gt; reference (must be an active diplomate of other ABVS specialty)</td>
<td>Name:</td>
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<td>Credential:</td>
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<td>Contact Information:</td>
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<tr>
<td>Your 2&lt;sup&gt;nd&lt;/sup&gt; reference (must be an active diplomate of other ABVS specialty)</td>
<td>Name:</td>
<td></td>
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<tr>
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<td>Credential:</td>
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<tr>
<td></td>
<td>Contact Information:</td>
<td></td>
</tr>
</tbody>
</table>

You will receive acknowledgement of receipt of your submitted credential materials from the ACVBM College Secretary within 14 days of submission. You will then be notified by the Credentials and Residency Committee regarding the acceptance of your credential materials for the College board-certification examination at least 120 days prior to the examination.

**All candidates are required to sign the following agreement at the time of credentials submission:**

I hereby apply to the American College of Veterinary Botanical Medicine for examination in accordance with its rules and herewith enclose the application fee. I also hereby agree that, prior to or subsequent to my sitting the board-certification examination, the Board of Directors may investigate my standing as a veterinarian, including my reputation for complying with the standards and ethics of the profession.

Signature: ____________________________

Date: ____________________________
APPENDIX V Curriculum Vitae of the ACVBM’s Organizing Committee Members

1. Signe Beebe Current President Elect ACVBM
2. Erin Bannink
3. Ihor Basko Current Chairperson of the ACVBM
4. Joe Castro
5. Carmen Colitz
6. Curtis Wells Dewey
7. Connie DiNatale
8. Barbara Fougere Current President of the ACVBM
9. Joyce Harman Vice President the ACVBM
10. Reed Holyoak
11. Hubert Karreman
12. Cynthia Lankenau Secretary/Treasurer of the ACVBM
13. Steve Marsden Board member of the ACVBM
14. Richard Palmquist Board member
15. Donna Raditic
16. Nancy Scanlan
17. Justin Shmalberg
18. Rob Silver
19. Susan Wynn advisor to the ACVBM Board
20. Huisheng Xie Board member of the ACVBM

(Note, These CVs have been abridged to fit two pages each, full CVS are available on request)
Signe E Beebe DVM
Integrative Veterinary Center
5524 Elvas Avenue
Sacramento, CA 95819
DOB 4/25/57

Education
- 2007 Chi Institute of Chinese Veterinary Medicine, Reddick, FL, Chinese Veterinary Herbology
- 2006 University of Pretoria South Africa, Centre for Wildlife Management, South Africa, Ecosystems and Wildlife Management Course
- 2006 Chi Institute of Chinese Veterinary Medicine, Reddick, FL, Veterinary Tuina
- 2005 Chi Institute of Chinese Veterinary Medicine, Reddick, FL, 7th Annual TCVM conference, Chinese Veterinary Food Therapy
- 2004 University Of Tennessee School of Veterinary medicine, Canine Rehabilitation and Physical Therapy course, Knoxville, TN
- 2002-2003 Chinese Veterinary Herbal Medicine Course (basic and advanced), Albuquerque NM
- 2000-2001 Chi Institute of Chinese Veterinary Medicine, Reddick, FL, Veterinary Acupuncture
- 1988 Purdue University School of Veterinary Medicine, DVM. W. Lafayette, IN
- 1981-1982 Kansas State University, Major Animal Science &amp; Industry, Manhattan, KS
- 1981 University of Hawaii AA, Oahu, Hawaii

Professional Activities
Lecturer/Instructor since 2000 for numerous organizations and institutions including most recently:
- 2016 10th Annual China Medical Chinese Veterinary Medicine Seminars, Basel, Switzerland
- 2016 Qi Academy, Gangelt, Germany
- 2015 ACVBM and the AHVMA Augusta, Georgia
- 2015 Annual IVAS Congress on Herbal medicine Nova Scotia, Canada
- 2015 Lotus Institute of Integrative Medicine, City of Industry California
- 2015 Annual China Medical Chinese Veterinary Medicine Seminars, Basel, Switzerland
- 2014 Annual China Medical Chinese Veterinary Medicine Seminars, Basel, Switzerland
- 2014 Western Veterinary Conference, Las Vegas, Nevada
- 2013 AHVMA Kansas City, Kansas
- 2013 International Symposium on Veterinary Chinese Herbology, Bioethicus Institute and University Sao Paulo State, Botucatu, Brazil
- 2013 Annual China Medical Chinese Veterinary Medicine Seminar: China Medical, Basel, Switzerland

Scientific Organizations
American Veterinary Medical Association

Professional Organizations
- World Association of Traditional Chinese Medicine Board Member
- American Journal of Traditional Chinese Veterinary Medicine Associate editor
- LOTUS Institute of Integrative Medicine, faculty
- Veterinary Botanical Medical Association
- American Holistic Veterinary Medical Association
- Institute of Traditional Chinese Medicine
Offices held
- Current Chair, American College of Veterinary Botanical Medicine
- 2015 President, American College of Veterinary Botanical Medicine
- 2014-2015, President, American College of Veterinary Botanical Medicine

Bibliography (selected)
- 2011 Beebe S, Salewski M, Chen J, Chen T, Chinese Herbal Formulas for Veterinarians, City of Industry, California: Art of Medicine Press
- 2010 Beebe, S, Traditional Chinese Medicine Treatment of Anemia, American Journal of Traditional Chinese Medicine, Vol.5 No.1, February 2010
- 2009 Beebe, S, Treatment of Congestive Heart Failure with Conventional Pharmaceuticals plus Acupuncture, Chinese Herbal Medicine and Food Therapy in a Toy Poodle Dog, American Journal of Traditional Chinese Medicine, Vol.4 No.2, August
Erin O’Neil Bannink DVM, DACVIM (Oncology)
Integrative Veterinary Center
5524 Elvas Avenue
Sacramento, CA 95819

Education
- Animal Cancer and Imaging Center, May 2004-October 2006 Resident, Medical Oncology Rochester Hills, MI
- Animal Cancer and Imaging Center, 2003-2004 Intern, Medical Oncology Rochester Hills, MI
- Michigan State University, 1998-2002 Doctor of Veterinary Medicine College of Veterinary Medicine East Lansing, MI
- Graduate Diploma in Veterinary Chinese Herbal Medicine College of Integrative Veterinary Medicine, 2015

Professional Activities
- Specialist, Oncology and Complementary and Alternative Medicine, January 2007-present Oakland Veterinary Referral Services Bloomfield Hills, MI
- Resident, Medical Oncology, 2004-2006 Animal Cancer and Imaging Center Rochester Hills, MI
- Intern, Medical Oncology, 2003-2004 Animal Cancer and Imaging Center Rochester Hills, MI
- Education of Oncology and Alternative Medicine Clerkship externs from the Michigan State University College of Veterinary Medicine junior and senior class Oakland Veterinary Referral Services, 2007-present
- Education of Oncology Clerkship externs from the Michigan State University College of Veterinary Medicine junior and senior class Animal Cancer and Imaging Center, 2003-2006

Scientific Organizations
- American College of Veterinary Internal Medicine Diplomat (Oncology), 2008
- Veterinary Cancer Society, 2003-present
- American Veterinary Medical Association, 2003-present
- Michigan Veterinary Medical Association, 2002-present
- Southeastern Veterinary Medical Association, 2007-present

Professional Organizations
- International Veterinary Acupuncture Society, 2004-present
- American Association of Veterinary Acupuncturists, 2007-present
- American Holistic Veterinary Medical Association, 2007-present

Awards
- Arthur D. Marosi Award, excellence in surgery, Michigan State University, 2002
Bibliography (selected)

- Sauerbrey ML, Obradovich JE, BanninkEO. Single agent chemotherapy with CCNU (lomustine) with or without prednisone for dogs with lymphoma: 15 cases. Proceedings of the Veterinary Cancer Society Annual Conference; October 2006.
Ihor John Basko DVM
6240 Helena Lane
Kapaa, HI 96746
DOB 2/13/1947

Education
- Wayne State University (Biology Major) 1965-1967
- Michigan State University Veterinary School 1968-1971 (DVM)
- University of California Los Angeles (Comparative Eastern Philosophy 1972-1973)
- Institute of Traditional Medicine Santa Cruz, CA (1984-1985) Completed course in Chinese Herbolgy

Professional Activities
- Small Animal and Equine Clinical Practices (1971-current)
  - All Creatures Great & Small Veterinary Services Kapaa, HI
  - Surf Paws Animal Hospital Honolulu, HI
- Herbal and Nutritional Consultant (Industry and Private Sector)
- Lecturer and Educator (Veterinarians, Pet Industry & Pet Parents)
- Research Consultant: Herbs and Supplements
- Heart Research Team / Bypass surgeries & Mitral Valve replacement
- Thoracic surgery training program/ MSU / Lansing General Hospital Lansing, MI (1970 – 1971)
- Author

Scientific Organizations
- American Veterinary Medical Association
- Hawaii State Veterinary Medical Association

Professional Organizations
- American Holistic Veterinary Medical Association
- Veterinary Botanical Medical Association

Honors
- Herbal Educator of the Year Award 2014 VBMA
- The Dr. Carvel Tiekert Life Achievement Award 2014 AHVMA
- Holistic Veterinarian of the Year 1998  AHVMA

Offices held
- Current President-Elect of the ACVBM
- Board of Directors: Veterinary Botanical Medical Association (2010- current)
- Council of Elders / American Holistic Veterinary Medical Association (1998- 2013)
- Research Advisory Board / National Animal Supplements Council (2003-2006)
- President of the Veterinary Botanical Medical Association (2001-2003)
- Board of Directors: American Holistic Veterinary Association (1999-2001)
- President / Hawaii State Veterinary Medical Association (1998-1999)
- Board of Directors / Hawaii State Veterinary Medical Association (1993 –1998)

Bibliography (Selected)
• Healing Your Horse: Alternative Therapies (co-author) 1993
• Cooking For Da Hawaiian Kine Dog 1999
• Fresh Food & Ancient Wisdom: Preparing Healthy Meals For Your Dogs 2010
Jose R Castro, DVM, ECFVG, PhD, Diplomate American Board Veterinary practitioners; Diplomate American Board Veterinary Surgery

University Tennessee

Education

- DACVS Diplomate American College of Veterinary Surgery – Equine Emphasis. February 1, 2012
- DABVP Diplomate American Board of Veterinary Practitioners – Equine Practice. November 16, 2009
- ECFVG Certification, American Veterinary Medical Association. March 19, 2004
- Doctor of Veterinary Medicine and Zootechnology, Central University of Ecuador. Faculty of Veterinary Medicine and Zootechnology (CUE-FVMZ) December 27, 2000

Professional Activities

- Clinical Assistant Professor University Tennessee Equine Field Service
- Veterinary Medical Center-Large Animal Clinical Sciences

Scientific Organizations

- American College of Veterinary Surgeons
- American Board of Veterinary Practitioners
- Veterinary Medical Association of Ecuador
- Ecuadorian Association of Equine Practitioners
- USDA-APHIS-Veterinary Accreditation
- Latin-American Association of Veterinary Emergencies and Critical Care

Professional Organizations

- American Association of Traditional Chinese Veterinary Medicine
- Phi-Zeta-The Honor Society of Veterinary Medicine
- World Council of Traditional Chinese Veterinary Medicine

Honors

- Nominated for the Chancellor’s Excellence in Graduate Mentoring and Advising Award. University of Tennessee. Institute of Agriculture. College of Veterinary Medicine. 2017
- Chi Institute of Chinese Medicine Faculty Scholarship Award. Equine Acupuncture. 2013
- Resident of the Year. University of Tennessee College of Veterinary Medicine. 2003
- Intern of the Year. University of Tennessee College of Veterinary Medicine.

Bibliography (selected)

Carmen Maria Helena Colitz, DVM, PhD, Diplomate ACVO

Education
- The University of Paris, Paris, France, Summer 1985 Cours de Civilisation Française de la Sorbonne
- The University of Tennessee, Knoxville, TN, Spring 1989. Undergraduate
- The University of Tennessee, Knoxville, TN, 1989 to 1993. Doctor of Veterinary Medicine, May 1993
- The University of Tennessee, Knoxville, TN, 1993 to 1996. Doctor of Philosophy in Comparative and Experimental Medicine, 1996
- North Carolina State University, Raleigh, NC, 1996 to 1999. Residency, Comparative Ophthalmology and Post-Doctoral Research Associate

Professional Activities
- Co-owner: Jupiter Pet Emergency and Specialty Center November 2012 to present
- Owner: All Animal Eye Care, Inc January 2013 to present
- Principle, Animal Health Quest Solutions, LLC 2007 to present
- Sole Proprietor, Aquatic Animal Eye Care, LLC Consulting Veterinary Ophthalmologist 2009 to December 2012
- Consulting or Surgical Veterinary Ophthalmologist for zoos and aquariums
- Courtesy Faculty Appointment, Small Animal Clinical Sciences, Aquatic Animal Health
- Adjunct Associate Professor Raleigh, North Carolina 2007 to present
- Veterinary Ophthalmologist West Palm Beach, Florida, January 2007 to December 2009
- Assistant Professor, Comparative Ophthalmology Columbus, Ohio, October 2001 to November 2006
- Assistant Professor, Comparative Ophthalmology September 1999 to September 2001 Louisiana State University School of Veterinary Medicine
- Resident, Comparative Ophthalmology/ Post-Doctoral Research Associate July 1996-99 North Carolina State University College of Veterinary Medicine
- Post Doctoral Research Trainee Fellowship July 1993 to June 1996
- Small Animal Veterinarian August 1993 to June 1996

Scientific Organizations
- American Board of Veterinary Ophthalmologists, Member of the Board, 2012 to 2014.
- The American Veterinary Medical Association, 1993- present.
- American College of Veterinary Ophthalmology, Diplomate, 1999-present.
- American College of Veterinary Ophthalmology, Member of the Board of Regents, 2006 to 2012, President (2010-2011).
- International Association for Aquatic Animal Medicine, 2006 - present.

Professional Organizations
- American Holistic Veterinary Medical Association

Honors
- Herbal Educator of the Year Award 2014 VBMA
- The Dr. Carvel Tieckert Life Achievement Award 2014 AHVMA
Holistic Veterinarian of the Year 1998  AHVMA

Offices held
- OSU Department of Veterinary Clinical Sciences Department Chair, 2006 to present, filled, Dr. Rustin Moore, start date November 2006.
- OSU Graduate Studies Committee, 2004-present.
- OSU Search Committee for Food Animal Faculty Position Summer 2003.
- Editorial Board Member or Ad Hoc Journal Review for:
  - Veterinary Ophthalmology, 1999 - present.
  - Comparative and Veterinary Oncology, 2006.
  - Experimental Eye Research, 2000 - present
  - Veterinary Pathology, 2002 - present.
  - Veterinary Medicine, 2005 to present.
  - Journal of the American Veterinary Medical Association, 2005 to present.
  - Graefe’s Archives of Clinical and Experimental Ophthalmology, 2005 to present.
  - Clinician’s Briefs, 2007 to present.
  - The Veterinary Journal, 2007 to present.
  - Current Eye Research, 2009 to present.
  - Molecular Vision, 2009 to present.

Bibliography (selected)
- 78 Publications or Dissertations including:
- 20 Book Chapters
- 134 Proceedings and Abstracts
Curtis W Dewey, DVM Diplomate - ACVIM (Neurology) Diplomate - ACVS
Cornell University College of Veterinary Medicine

Education

- 1993-1995 University of California, Davis, Residency, Neurology/Neurosurgery
- 1993 University of Georgia, MS, Anatomy
- 1990-1993 University of Georgia, Residency, Small Animal Surgery
- 1989-1990 University of Georgia, Internship, Small Animal Medicine
- 1989 Cornell University College of Veterinary Medicine, DVM
- 1985 Cornell University, BS, Animal Science

Professional Activities

- Associate Professor, Section of Neurology/Neurosurgery Department of Clinical Sciences
  Cornell University College of Veterinary Medicine

Scientific Organizations

- 2004-Present Veterinary Information Network (VIN)
- 1996-Present American College of Veterinary Surgeons
- 1996-Present American College of Veterinary Internal Medicine
- 1993-Present Veterinary Emergency and Critical Care Society
- 1989-Present American Veterinary Medical Association

Professional Organizations

- IVAS
- AATCVM

Honors

- Meritorious Service Award (Long Island Veterinary Specialists)
- 1996 Diplomate, American College of Veterinary Surgery
- 1996 Diplomate, American College of Veterinary Internal Medicine (Neurology)
- 1990 Intern Recognition Award (University of Georgia)
- 1989 Phi Zeta (Cornell University)
- 1989 Pharmacology Faculty Award (Cornell University)

Offices held

- ACVIM (Neurology) Residency Training Committee, 2005-2008 (committee chair 2007-2008);
- ACVIM Taskforce on Neurosurgical Training of Neurology Residents-Committee Member, 2004-present (Chair, 2007-2010);

Bibliography (selected)


Connie Dinatale DVM

742 Clay Street
Winter Park, FL 32789

Education

- Doctor of Veterinary Medicine 1992, University of Florida, Gainesville, FL
- Bachelor of Science, Microbiology 1987, University of Florida, Gainesville, FL
- Herbal Training Course 1999, Chi Institute

Professional Activities

- Practitioner, founder, owner Winter Park 1999- Present
- Associate Allen Shoen and Associates; Osceola Animal Clinic, Tony Weiratha 1993-1999
- Research
  - 1985 - 1987, Small Colon Resection and Anastomosis Surgical Comparisons Under Alan Nixon, D.V.M. and Reid Hanson, D.V.M. University of Florida
  - 1985 - 1988, Research Assistant for Large Animal Surgical Sciences
- Lecturer/ Instructor
  - Chi Institute Clinical Applications of Herbal Medicine 1999 – Present
  - Qi Academy (Germany) 2016
  - American Holistic Veterinary Medical Association 2013, 2012
  - AVMA Conference 2006

Scientific Organizations

- Central Florida Veterinary Medical Association
- American Veterinary Medicine Association

Professional Organizations

- American Holistic Veterinary Medical Association
- Veterinary Botanical Medical Association Central
- American College Veterinary Botanical Medicine

Offices held

- Current President of the ACVBM

Bibliography (Selected)

Barbara Fougere BSc BVMS (Hons)
College Integrative Veterinary Therapies Pty Lt trading as All Natural Vet Care
PO Box 474 Rozelle 2039 NSW 292 Lyons Rd Russell Lea 2046 NSW
DOB 10 September 1964

Education
- Masters Health Science (Herbal Medicine) (University New England 2005)
- Masters Organisational Development and Training (UNE/ Charles Sturt University 1995)
- Graduate Diploma Social Science (University New England 1994)
- Graduate Diploma of Business Management (University New England 1992)
- Graduate Diploma Phytotherapy (Australian College Phytotherapy/University New England 2004)
- Bachelor of Health Science Complementary Medicine (Charles Stuart University 2004)
- Bachelor of Veterinary Medicine and Surgery (Hons) (Murdoch University Veterinary School 1986)
- Bachelor of Science (Murdoch University Veterinary School 1984)
- Graduate Diploma Veterinary Western Herbal Medicine (CIVT 2012)
- Graduate Diploma Veterinary Chinese Herbal Medicine (CIVT 2012)

Professional Activities
- Current President American College of Veterinary Botanical Medicine
- Current Board member Veterinary Botanical Medicine Association
- Past President International Veterinary Acupuncture Association
- Practitioner and Director Integrative Veterinary Services trading as All Natural Vet Care
  Veterinary Hospital which is an offsite training center for Sydney University Veterinary School for student rotations
- Principal and Director of the College of Integrative Veterinary Therapies – registered training organization
  o Teaching 2 year post graduate accredited courses in Veterinary Chinese Herbal Medicine and Veterinary Western Herbal Medicine
- Current external reviewer safety efficacy of complementary medicines Australian Pesticides and Veterinary Medicines Authority (Canberra)
- Lecturer WSAVA, New York Veterinary Conference, NAVC, AHVMA, VIN, AVA, IHS
- Adjunct Alabama Veterinary School Elective in Herbal Medicine

Scientific Organizations
- Chartered Member of the Australian Veterinary Association

Professional Organizations
- National Herbalists Association Australia (Registered Human Herbalist)
- American Holistic Veterinary Medicine Association
- Australian Integrative Veterinary Group
- Australian Holistic Veterinary Group
- Australian College of Nutritional and Environmental Medicine
- American Botanical Council
- College Integrative Veterinary Therapies
- Veterinary Botanical Medicine Association

Honors
- 2010 Practitioner of the Year Award 2010 (AHVMA) for services to Integrative Veterinary Medicine.
- 2011 Educator of the Year Award 2011 (AHVMA) for education in Integrative Medicine.
- Outstanding Leadership Award by the World Association of Traditional Chinese Veterinary Medicine, (2015)
- Petplan Finalist Vet of The Year 2017

Offices held
- Current Vice-President of the American College of Veterinary Botanical Medicine; Chair 2014-2015
- Board Member Australian Holistic Veterinarians 2000-2009.
- Board member Feline Health Research Council Australia 2003-2012
- Policy Council Member for the Australian Veterinary Association 1995-2003
- Therapeutic Advisory Committee veterinary medicines 2000-2004
- Reviewer for the Australian Veterinary Journal (current)

Bibliography (Selected)

- Fougere B, Wynn S Ed Text Book of Veterinary Herbal Medicine 2006
- Fougere B, in Goldstein’s Integrating Alternative Medicine into Veterinary Practice 2008
- Fougere B, in Encyclopedia of Animal Behaviour 2010
Joyce Harmen DVM MRCVS

1214 North Poes Rd.
Flint Hill, VA 22627
Date of Birth: June 22, 1955

Education
- BS 1980 University of Georgia, Animal and Dairy Science
- DVM 1984 Virginia Maryland Regional College of Veterinary Medicine
- MRCVS 1985 Member, Royal College of Veterinary Surgeons University of Bristol College of Veterinary Medicine, England

Professional Activities
- Instructor, lecturer Equine Herbal Medicine College of Integrative Veterinary Therapies 2013-Present
- Instructor, lecture Chi Institute 2002-2014 Reddick, FL
- Teaching/ Nutrition/Herb Company Veterinary Institute for Integrative Medicine
- 1995-present Boulder, Colorado
- CAVM Equine Practice Harmany Equine Clinic, Ltd. 1990 – Present Flint Hill, Virginia
- Equine Practice Dr. B. Furlong & Associates 1987 – 1989 Oldwick, New Jersey
- Research Veterinarian Bioengineering Isler 1986 – 1987 Zurich, Switzerland
- Equine Practice 1986 - 1986 Somerton Veterinary Hospital Curragh, Co. Kildare, Ireland
- Veterinary Research Assistant 1984 – 1986 Animal Health Trust, Suffolk, England

Scientific Organizations
- American Veterinary Medical Association
- American Association of Equine Practitioners

Professional Organizations
- American Holistic Veterinary Medical Association
- College Integrative Veterinary Therapies
- Veterinary Botanical Medicine Association

Offices held
- Current Board Member ACVBM
- 1992 - 1994: Chairman, American Association of Equine Practitioners, Subcommittee on Therapy Options
- 1996 - 1999 Board of Directors, Association of Equine Sports Medicine
- 1996 - 2000 Board of Directors, American Holistic Veterinary Medical Association
- 1996 - 2009 Veterinary Advisory Board, Animal Ambassadors and TTEAM
- 1998 - 1999 President, American Holistic Veterinary Medical Association
- 1999-2000 Member, American Veterinary Medical Association Task Force on CAVM
- 2006-present American Journal of Traditional Chinese Veterinary Medicine advisory board
- 1999-2010 Editorial Board, The Horse (magazine)
- 2004-present Veterinary Advisor to Board, The Holistic Horse (magazine)
- 2005-2007: Board of Directors, Veterinary Botanical Medical Association
- 2006-2007: President, Veterinary Botanical Medical Association
Bibliography (Selected)

Gilbert Reed Holyoak DVM MS PhD Diplomate ACT

Oklahoma State University

Education

- Diplomate American College Theriogenologists 2000
- PhD Infectious Diseases University Kentucky 1992
- DVM Washington State University 1988
- MS Brigham Young University 1984
- BS Brigham Young University 1983

Professional Activities

- Professor, 2016 - present, Veterinary Clinical Sciences Department, Oklahoma State University
- Adjunct Professor, 2014 - present, Faculty, School of International Studies
- Professor & Head, 2012 – 2016 Veterinary Clinical Sciences Department, Oklahoma State University
- Professor, 2009 - 2012, Oklahoma State University; Veterinary Clinical Sciences Department
- Bullock Endowed Professorship, 9/2001 - present, Oklahoma State University; Center for Veterinary Health Sciences
- Associate Professor, 2001 - 2009, Oklahoma State University; Veterinary Clinical Sciences Department
- Veterinary Medical Officer, 6/19 - 7/19, 2003, USDA Exotic Newcastle Disease Task Force, Garden Grove, CA. Strike Team Leader
- Assistant Professor, 1999 - 2001, Oklahoma State University; Veterinary Clinical Sciences Department
- Assistant Professor, 1993 - 1999, Utah State University; Animal, Dairy and Veterinary Sciences Department

Scientific Organizations

- American College of Theriogenologists, Diplomate – 2000
- Society of Theriogenology - member 1993 – present
- Ad Hoc reviewer – Journal of the American Veterinary Medical Association, 1999 - present
- Ad Hoc reviewer – Journal of Equine Veterinary Science, 2002 - present
- Ad Hoc reviewer – OIE Scientific and Technical Review, 2010 - present
- American Association of Equine Practitioners, member 1988 - present
- American Veterinary Medical Association - member 1988 – present
- Oklahoma Veterinary Medical Association – member - 2006 - present

Professional Organizations

- Associate Editor, Basic Science & Research, American Journal of Traditional Chinese Veterinary Medicine, 2013-present; Ad hoc reviewer – AJTCVM, 20010 - present
- American Association of Traditional Chinese Veterinary Medicine, member 2012 - present

Honors
• Visiting Professorship, 5/2015 – 7/2018 College of Life Sciences, Foshan University, Foshan, Guangzhou, PR China
• U.S.U. College of Agriculture, Teacher of the Quarter, Fall 1996.
• U.S.U. College of Agriculture, Teacher of the Quarter, Fall 1993.
• Senior Veterinary Student Scholarship, Washington State University, 1988.

Offices held
• American College of Theriogenologists
  Board of Directors, Vice President 2016-7
  Board of Directors, Secretary 2013-2016
  Examination Committee 2005 – 2011; Chair – 2010
  Trichomoniasis ad hoc committee 2010 - 2013
• American Association of Equine Practitioners
  Table Topics Chair 2008,9,10 (Equine Viral Arteritis)

Research Activities
Oklahoma State University, current research projects include: microbiomics and infectious diseases affecting reproduction; mustang contraceptives, embryo-pathogen/toxin interactions with equine arteritis virus and others; embryo transfer in large animal species including food animals and equine.

Bibliography (Selected)
Hubert Karreman VMD

555 Red Hill Road
Narvon, PA 17555
DOB 9/25/62

Education
- 2008-2009 University of Pennsylvania School of Medicine, Certified Clinical Research
- 1991-1995 University of Pennsylvania School of Veterinary Medicine V.M.D
- 1980-1984 University of New Hampshire College of Life Sciences B.S.

Professional Activities
- 9/13-present The Rodale Institute
  - Conduct research studies with organic dairy farms in region
  - Conduct organic veterinary animal health classes
  - Partner with collaborators on pasture projects
  - Make presentations domestically and internationally
- 2010-present Bovinity Health, Lancaster PA & beyond
  - Natural treatment & products for non-antibiotic treatment of infectious disease
  - Part-time practice in medical treatment of certified organic cattle using non-antibiotic treatments against infectious disease and non-hormonal treatments against infertility via botanical derivatives, biological immuno-modulation, acupuncture, low dose therapeutics and hands-on physical therapy as needed.
  - Educational seminars for veterinary schools & organizations, farmer organizations, with optional in-barn instruction
- 6/95 – 12/09 Veterinary practitioner, full-time: Lancaster County, PA
  - Developed an effective non-antibiotic treatment of infectious disease in dairy cattle by utilizing botanicals and biologics
  - Integrative medicine techniques applied daily: botanical derivatives, biological immuno-modulation, acupuncture, low dose therapeutics and conventional approaches as each case requires.
  - Successful completion of 2005 SARE grant project ONE05-042 to analyze trends and associated management on organic farms using DHIA records in PA.

Scientific Organizations
- American Veterinary Medical Association
- American Association of Bovine Practitioners

Professional Organizations
- Veterinary Botanical Medicine Association

Honors
- 9/2007- 5/2009 Affiliate Assistant Professor, Dept of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH

Offices held
  Write recommendations for regulatory implementation by USDA.
- 5/99-3/01 AVMA Task Force on Alternative and Complementary Medicine, representing Food Animal sector to develop standards for CAVM within AVMA
Bibliography (Selected)

- The Non-Antibiotic Treatment of Infectious Disease. Proceedings 1st Organic Congress of the East Black Sea (Kelkit, Turkey) (June 2013)
- Hubert J. Karreman. Treating Dairy Cows Naturally: Thoughts and Strategies 2nd ed. Acres USA, Austin, TX, 2007 (Hardcover, 412 pgs).
Cynthia Jean Lankenau DVM Grad Dip VCHM Grad Dip VWHM

9002 Sunset Drive,  
Colden, NY 14033  
DOB 11/30/1956

Education

- Cornell University; Agriculture and Life Sciences College; 1974-1977
- Cornell University, College of Veterinary Medicine: 1977-1981; Doctorate of Veterinary Medicine; (DVM, graduated with distinction)
- Herbal training in Chinese Medicine: 1995-1997 through the International Veterinary Acupuncture Society
- Chi Institute: Chinese Herbal Training Class: 2002-2003
- Sage Mountain Herbal Studies: 1994-2004; Herbal Practitioner
- College of Integrative Veterinary Therapies: Graduate Diploma Veterinary Chinese Herbal Medicine: 2009-2011
- College of Integrative Veterinary Therapies: 2012-2015; Grad Dip Veterinary Western Herbal Medicine

Professional Activities

- 1984-1985: Government District Officer: Peace Corps, Malawi, Africa; District Veterinary Officer
- 1993-now: Private Practitioner: 100% alternative modality Practice: Holistic Center for Veterinary Care, Colden, NY
- 2015-now: lecturer for the College of Veterinary Integrative Therapies
- Registered Professional Herbalist by the American Herbal Guild RH(AHG)
- Lecturer:
  - Student Holistic Group at Cornell University College of Veterinary Medicine: 2013 and 2010.
  - World Association of Traditional Chinese Veterinary Medicine, 2016

Scientific Organizations

- American Veterinary Medical Association
- New York State Veterinary Medical Society

Professional Organizations

- Veterinary Botanical Medicine Association
- College Integrative Veterinary Therapies
- American Holistic Veterinary Medical Association

Honors

- Outstanding Leadership Award by the World Association of Traditional Chinese Veterinary Medicine, (2015)
- Excellent Speaker Award, by the World Association of Traditional Chinese Veterinary Medicine, Beijing, China, 2016
Offices held

- American College of Veterinary Botanical Medicine: secretary-treasurer (2014-now)
- Veterinary Botanical Medicine Association: board member (2008-2015) current past president
- American Holistic Veterinary Medical Association: on the Council of Elders (2007-now)
- New York State Veterinary Medical Society: committee head of Complementary and Alternative Medicine: (2012-now)
- New York Complementary and Alternative Medicine Association: founding Board Member: (2012-now)
- World Association of Traditional Chinese Veterinary Medicine (WATCVM): board member (2013-now)
- Peer reviewer of Herbal articles for RAIVE:2014

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- Lankenau, C.; Acute Tendinitis Treated with Chinese Medicine, Journal of the Veterinary Botanical Medical Association, Summer, 2014, PP13-17
- Lankenau, C; YooHoo: A Case of a Retained Pathogen: JVBMA, February, 2014; p.6-13
- Lankenau, C; Treatment of Mastitis in a Jersey Cow with Chinese Herbal Medicine, JVBMA; March, 2011 pp.57-62
- Lankenau, C; Idiopathic Hepatobiliary Disease in an Equine treated with Chinese Herbal Medicine, JVBMA; March 2011; pp. 39-48.
- Lankenau, C; Congenital Cardiac Defect in an Equine Supported with Chinese Herbal Medicine, JVBMA; March, 2011; pp. 11-21.
- Lankenau, C; Practice Pearls; from Clinical Cases; JVBMA; March 2011; pp. 11-14.
- Lankenau, C; Zoopharmacognosy at Work; Journal of Integrative Therapies, (4)2014; p.43.
- Lankenau, Cynthia: Herbal Wiki; Veterinary Botanical Medicine Association; Members Only web-site.
Steve Marsden DVM MSc

8215 102 Street
Edmonton Canada
DOB 9/20/1963

Education
- Doctor of Veterinary Medicine, University of Saskatchewan, 1988
- Naturopathic Physician, National College of Natural Medicine, 1999
- Master of Science in Oriental Medicine, National College of Natural Medicine, 1999
- Licensed Acupuncturist, National Certification Commission for Acupuncture and Oriental Medicine (NCCAOM), USA, 1999
- Diplomate of Chinese Herbology, NCCAOM, 1999
- Graduate Diploma Veterinary Chinese Herbal Medicine 2012

Professional Activities
- Practitioner
- Teacher, lecturer internationally numerous conferences
- Faculty Position
  - College of Integrative Veterinary Therapies, Sydney, Australia
    - Director, Instructor
  - National College of Natural Medicine, Portland, Oregon
    - Director Emeritus
    - Adjunct Faculty Member, Department of Classical Chinese Medicine
- Board Member, American College of Veterinary Herbal Medicine

Scientific Organizations
- Canadian Veterinary Medical Association
- Alberta Veterinary Medical Association
- Canadian Naturopathic Doctors Association
- Alberta Association of Naturopathic Practitioners

Professional Organizations
- Veterinary Botanical Medicine Association
- College Integrative Veterinary Therapies
- American Holistic Veterinary Medical Association
- American Herbalist’s Guild

Honors
- Small Animal Clinician of the Year, Canadian Veterinary Medical Association, 2009
- Teacher of the Year, American Holistic Veterinary Medical Association, 2010

Offices held
- Board Member ACVBM Current
- Board of Directors, National College of Natural Medicine, Portand OR, 2005-14
  - Treasurer, 2012-14
- Director of Continuing Education
  - Edmonton Association of Small Animal Veterinarians, 1989-1994
  - Alberta Veterinary Medical Association, 1989-1992
- Vice-President, Edmonton Humane Society
  - 1989-1994

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- Foundations of Formula Design, AHVMA Press, Maryland, 1999
- New Understandings of the Pathophysiology of Pemphigus Foliaceous, JAHVMA, Jan. 2000
- Textbook of Veterinary Herbal Medicine, Elsevier, New York, 2006 (contributing author)
- Pain Management in Veterinary Practice, Wiley-Blackwell, in press (contributing author)
- Veterinary Clinics of North America: Cancer, in press (contributing author)
Richard Palmquist, DVM

721 Centinela Ave.
Inglewood, CA 90302

Education
- CSU 1983 DVM
- Grad Dip Veterinary Chinese Herbal Medicine (CIVT 2015)

Professional Activities
- Chief of Integrative Medicine, Centinela Animal Hospital, Inglewood, CA
- Teacher, lecturer internationally
- VIN, Alt Med Folder consultant www.vin.org
- CIVT, Faculty
- Nuova Biologics Research Associate: PVX drug development (canine distemper)
- AHVMF president, Past President (first)
- Western University School of Veterinary Medicine: Faculty as Clinical Preceptor rotation site in small animal integrative medicine as part of the 4th year professional degree program.
- Registry of Approved Integrative Veterinary Education (RAIVE) committee member

Scientific Organizations
- AVMA
- CVMA
- SCVMA member and past program chairman for Northbay-Westside chapter

Professional Organizations
- Veterinary Botanical Medicine Association
- College Integrative Veterinary Therapies
- American Holistic Veterinary Medical Association
- AHVMA, Board of Directors, Research committee chairperson, Ethics committee member, member, and speaker
- ACVBM
- WATCVM

Honors
- Excellent speaker trophy 18th Annual TCVM Conference in Beijing, China
- COE Peacemaker Award 2012
- UpJohn Award for Proficiency in Small Animal Medicine

Offices held
- 2016 AHVMF, president and research chair
- Council of Elders and Editorial Committee, AHVMA
- AHVMF, Past President and research chair
- AHVMF president, Past President (first)
- World Association of TCVM (WATCVM) Board of Directors
Bibliography (selected)

- Palmquist RE. Chronic cervical pain and an active scar relieved with essential oils and neuro auricular therapy: a canine case report. JAHVMA. 29(1):16-25.
- NAVC 2013 - Approaching Integrative Veterinary Medicine for your Practice; Canine Cutaneous Hemangiosarcoma: A potentially new approach; and Translational medicine: A model for advancing medical care. Orlando, Florida.
- Perspective: Evidence Based Veterinary Medicine, Evidence Based Practice and Translational Medicine In Pioneering Areas. JAHVMA submitted Feb 2014.
Donna Raditic, DVM DACVN

331 Confederate Drive
Knoxville, TN 37922

Education
- Cornell University Ithaca, New York Bachelor of Science with Honors and Distinction 1982
- Cornell University College of Veterinary Medicine, 1986
- American College of Veterinary Nutrition Alternative Residency Program, 2005-2010
- University of Tennessee College of Veterinary Medicine Residency in Nutrition, 2005-2010
- Bachelor of Science with Honors and Distinctions, 1982

Professional Activities
- Diplomat of America College of Veterinary Nutrition
- Adjunct Assistant Professor, Department of Small Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, 2008-Present
- Assistant Professor, Department of Small Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, November 2012-Present
- Medical Director, 2006-2008 VCA All Caring Animal Hospital Great Barrington, MA
- Veterinarian/Owner/Developer, 1997-2006 All Caring Animal Center, Inc. Great Barrington, MA
- Veterinarian, 1986-1997 Berkshire Veterinary Hospital Pittsfield, MA
  Research 2010 Evaluation of three herbal compounds used for management of lower urinary tract disease in cats. University of Tennessee COE grant
- Research The use of botanical therapy. NHV Inc. In progress

Scientific Organizations
- American Veterinary Medical Association
- Massachusetts Veterinary Medical Association
- New York State Veterinary Association
- American Academy of Veterinary Nutrition
- American Society of Parental and Enteral Nutrition
- Metabolomics Society

Professional Organizations
- Veterinary Botanical Medical Association
- CIVT (College of Integrative Veterinary Therapy)
- American Holistic Veterinary Medicine Association

Offices held
- AHVMA Foundation Professional Advisory Board – 2 years

Bibliography (Selected)
- Raditic DM, ELISA testing for common food antigens in four dry dog foods used in dietary elimination trials.” J Anim Phys Anim Nutr; February 2010
- Raditic DM, Combined parenteral and enteral feeding in a cat. In: Small Animal Clinical

- Raditic D, Remillard RL, Tater KC ELISA testing for Common Food Antigens in Dry Dog Foods Used in Dietary Elimination Trials, Oral abstract for AAVN at ACVIM Forum 2010
- Willis-Mahn C, Raditic D, Tater K, Remillard R, ELISA testing for soy antigens in dry dog foods used in dietary elimination trials. Abstract ACVD and AAVD Research Award, Derm Digest, 2011
Nancy Scanlan DVM

404 N Mt Shasta Blvd, Rm B,
Mount Shasta CA 96067
DOB 10/21/1946

Education
- BS, University of California, Davis (on a National Scholarship Foundation 4-year scholarship) 1968
- Doctor of Veterinary Medicine, University of California, Davis, 1970
- Higher Education Administration Course, Carnegie Mellon (on a Bush Leadership Fellowship) 1982
- MS in Financial Planning (MSFP), Golden Gate University, 2004
- Currently enrolled in MS in Integrative Cancer Therapy program at University of South Florida medical school

Professional Activities
- Lecturer in CAVM
- RAIVE Committee member
- Practitioner
- Writer for numerous journals and magazines

Scientific Organizations
- California Veterinary Medical Association (delegate to their House of Delegates)
- American Animal Hospital Association
- Southern California Veterinary Medical Association

Professional Organizations
- Veterinary Botanical Medicine Association
- College Integrative Veterinary Therapies
- California Holistic Veterinary Medical Association (founder)
- American Holistic Veterinary Medical Association
- Veterinary Botanical Medical Association

Offices held
- American Holistic Veterinary Medical Association (past board member, past president, past executive director, current delegate to AVMA House of Delegates)
- Veterinary Botanical Medical Association (past board member, past president)
- California Veterinary Medical Association (delegate to their House of Delegates)
- California Holistic Veterinary Medical Association (founder)

Bibliography (Selected)
- Complementary Medicine for Veterinary Technicians and Nurses, Wiley, 2011
- Medical Terminology: Building a Vocabulary 1988
- Editor for: Chinese Herbal Formula for Veterinarians, Art of Medicine Press, 2012
Robert Silver DVM MS

7345 N 63rd St;
Longmont, CO 80503
DOB 03-22-1949

Education

- Colorado State University 1974 BS
- Colorado State University 1976 MS
- Colorado State University 1982 DVM

Professional Activities

- Small animal practice
- Instructor: Animal Health Technology program
- Consultant: Technical Writer (Pet food industry; Animal nutraceutical industry; Veterinary Holistic industry)
- Chief Medical Officer (Animal nutraceutical company)
- Post-graduate education presenter (RACE approved speaker)

Professional Organizations

- Veterinary Botanical Medicine Association
- American Holistic Veterinary Medical Association

Offices held

- President, Rocky Mountain Holistic Veterinary Medical Association
- Board of Directors, American Holistic Veterinary Medical Association
- Council of Elders, American Holistic Veterinary Medical Association
- President-elect, Veterinary Botanical Medical Association

Bibliography (Selected)

- Introduction to Veterinary Nutraceuticals (Atlantic Coast Veterinary Conference 2014)
- The Hyperpermeable Bowel and Its Relationship to IBD (The North American Veterinary Conference 2014)
- Quality of Life Instruments in the Evaluation of Multifactorial Integrative Veterinary Medical Protocols (IAHPC 2013)
- Integrative Oncology (Pennsylvania Veterinary Medical Association: Hershey Conference 2013) Using Nutraceuticals to Maintain QoL in Pet Hospice (Atlantic Coast Veterinary Medical Conference 2014)
- Environmental Toxins and Animal Health (Atlantic Coast Veterinary Conference 2014)
- Genetically Modified Foods and Animal Health (Atlantic Coast Veterinary Conference 2014)
- Medicinal Cannabinoids and the Veterinary Patient (American Holistic Veterinary Medical Association 2014)
Justin Shmalberg DVM Diplomate ACVN Diplomate ACVSMR

Small Animal Hospital College of Veterinary Medicine University of Florida

Education

- Diplomate, American College of Veterinary Nutrition and American College of Veterinary Sports Medicine and Rehabilitation
- DVM, University of Wisconsin-Madison, Madison, WI
- BA, University of Kansas, Lawrence, KS

Professional Activities

- Clinical Assistant Professor of Integrative Medicine Medical Director, Small Animal Hospital College of Veterinary Medicine University of Florida current
- Resident, Nutrition, University of Florida College of Veterinary Medicine, Gainesville, FL 2009-2011
- Intern, Acupuncture, University of Florida College of Veterinary Medicine, Gainesville, FL 2008-2009
- Associate, Small Animal General Practice, Gainesville, FL 2009-2010

Scientific Organizations

- American College of Veterinary Nutrition
- American College of Veterinary Sports Medicine and Rehabilitation

Bibliography (Selected)

Susan Wynn DVM, DACVN

BluePearl Veterinary Specialists
455 Abernathy Rd
Sandy Springs, GA 30328
DOB 12/12/1960

Education
- Emory University, Atlanta GA 1978-1982  B.S., biology
- University of Georgia, College of Veterinary Medicine 1983-1987  DVM
- Emory University, Atlanta GA 1993-1996 (post-doctoral fellowship, viral immunology)
- University of Tennessee, College of Veterinary Medicine 2007-2010 Clinical Residency, Small Animal Nutrition
- Chinese Veterinary Herbal Medicine course, Chi Institute, Gainesville, FL 2000-2001
- Basic Medical Herbalism, Living with Herbs Institute, Atlanta, GA 2000-2001
- Professional herbalist (via peer review) by American Herbalist Guild 2002

Professional Activities
- Practitioner, Diplomat Clinical Nutrition  Blue Pearl Veterinary Specialists
- 2005 to present, Adjunct Faculty, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia
- Discussion Board Consultant (Alternative Medicine) Veterinary Information Network 1999 to present
- Faculty and teacher College Integrative Veterinary Therapies post graduate herbal medicine
- Lecturer and teacher numerous conferences

Scientific Organizations
- American College of Veterinary Nutrition
- American Veterinary Medical Association
- Georgia Veterinary Medical Association
- American Academy of Veterinary Nutrition

Professional Organizations
- Veterinary Botanical Medicine Association
- College Integrative Veterinary Therapies
- Veterinary Botanical Medicine Association (founder and lifetime member)
- American Herbalist Guild

Honors
- 2008, National Academy of Sciences, Ad hoc reviewer for Safety of Dietary Supplements for Horses, Dogs and Cats
- 1998, Office of Alternative Medicine, National Institutes of Health, Ad Hoc Review Group
- 1997, Behavioral and Neurosciences Special Emphasis Panel, National Eye Institute, Ad Hoc Review Group
- Expert Panel Membership
- 2004, A Model for Regulating Animal Medical Treatments by Non-veterinarians, Coalition on the Scope of Veterinary Practice, American Society of Veterinary Medical Association Executives, Lakewood, CO.
- 2000, Closed meeting for Advanced Research in Complementary and Alternative Medicine, Wellcome Trust, London
Offices held

- Current Advisor to the ACVBM Board
- 2012-2014: President, VetHeart of Georgia
- 2010 to 2016, District Director, Georgia Veterinary Medical Association
- 2006-2007, President, American Holistic Veterinary Medical Association
- 2000-2004 Secretary/Treasurer, American Academy of Veterinary Nutrition
- 2001 to 2006, founder and executive director, Veterinary Botanical Medicine Association
- 1995 to 2002, Founder, President, Executive Director, Georgia Holistic Veterinary Medical Association

Bibliography (Selected)

Huisheng Xie DVM, MS PhD

Department of Small Animal Clinical Sciences
PO Box 100126 HSC
College of Veterinary Medicine
University of Florida
Gainesville, FL 32610
DOB 10/7/1963

Education
- 1983 Bachelor of Science Degree in Veterinary Medicine (equivalent to DVM), Sichuan College of Animal Sciences and Veterinary Medicine, Rongchang, Sichuan Province, PR China
- 1988 Masters Degree, Veterinary Acupuncture, at the College of Veterinary Medicine, Beijing Agricultural University, PR China
- PhD, University of Florida, Gainesville, FL “Acupuncture for pain control in horses and its mechanism”

Professional Activities
- 1983-1988 Lecturer and Staff Veterinarian: the Beijing Agricultural University, College of Veterinary Medicine. PR China
- 1988-1991 Assistant Professor and Staff Veterinarian: the Beijing Agricultural University, College of Veterinary Medicine. PR China
- 1992-1994 Associate Professor and Staff Veterinarian: the Beijing Agricultural University, College of Veterinary Medicine. PR China
- 1994-1998 Research Fellow Department of Animal Science, University of Florida
- 1999-2004 Lecturer, College of Veterinary Medicine, University of Florida
- 2005-2008 Clinical Assistant Professor, College of Veterinary Medicine, University of Florida
- 2009-now Clinical Associate Professor, College of Veterinary Medicine, University of Florida

Scientific Organizations
- American Association of Equine Practitioner (AAEP)
- American Veterinary Medical Association (AVMA)

Professional Organizations
- American Association of Traditional Chinese Veterinary Medicine (AATCVM)
- China Society of Traditional Chinese Veterinary Medicine, CHINA

Honors (A selection)
- 2014: Fu-xi Award (the highest honor) from the Chinese Association of Traditional Veterinary Science (CATVS), Taiwan
- 2014: Excellent Leadership Award for the World Association of Traditional Chinese Veterinary Medicine (WATCVM), Hebei, China
- 2013: Excellent Speaker Award for the 15th Annual International TCVM conference, World Association of Traditional Chinese Veterinary Medicine (WATCVM), September 11-15, 2013, El Escorial, Madrid, Spain
- 2013: Honorary professorship from South China Agricultural University, Guang-zhou, China
- 2012: Year of the Holistic Teacher from AHVMA, Birmingham, USA
- 2006 Honorary Professorship from Southwest University, Chong-qing, China
Offices held

- Advisor to the ACVBM Board
- American Association of Traditional Chinese Veterinary Medicine (AATCVM) Co-founder and Executive Director from 2006-now
- China Society of Traditional Chinese Veterinary Medicine, CHINAA Advisory Board Director from 2000-now
- Reviewer Journal of the American Veterinary Medical Association (JAVMA) from 2003-now
- Reviewer American Journal of Traditional Chinese Veterinary Medicine (also Executive Director) from 2006-2008

Bibliography (selected)

- Xie H, Pasteur C, Smith L. TCVM for Equine Lameness in Horses-Diagnosis and Treatment. Integrative Veterinary Care. Summer issue: 54-58, 2013
Appendix VI  Outline Of Proposed Examination

The certifying examination for the ACVBM Specialty will test whether the candidate can perform at the level expected of an entry-level specialist in veterinary botanical medicine. The Diplomate certifying Examination (DCE) may include but is not limited to: The history of botanical medicine in context of contemporary practice, understanding the language of botanical medicine terminology and concepts, botanical medicine resources and research evidence based approaches, philosophy and principles of botanical medicines and Materia Medica. General botanical medical principles common to all species: herbal therapeutics in practice (of the gastrointestinal system, cardiovascular system, integumentary system, respiratory, hematologic system, musculoskeletal system, nervous system, endocrine system, etc), clinical strategies, botanical medicine case analysis and diagnosis, development of therapeutic treatment plans and prognosis, integration with conventional medicine, pharmacology, drug herb interactions and adverse effects, pharmacognosy, ethnovenery, ethnobotanical medicine, zoopharmacognosy, manufacturing, processing and dispensing of botanical medicines, veterinary herbal pharmacy management.

The initial contents of the examination have been determined by the Credentials and Education Committee and based upon the Job Task Analysis performed August- October 2017. This Job Task Analysis is expected to be reviewed periodically (every five years).

Each job task was evaluated by a panel of practitioners who were identified as veterinarians with expert level knowledge of botanical medicine. They were asked to evaluate each job task in two ways: how important the task was for the practice of botanical medicine, and how often they used the task in their practice. Evaluations were graded on a sliding scale, from 0 (not important, not used) to 5 (very important, used daily). A numerical score was obtained for each item by adding the two grades, and job tasks were then sorted by their total rank.

The rankings were used to determined the percentage of questions from each general area, as well as specific job tasks, to be included on the exam.

**Job Task Analysis**

Veterinary botanical medicine is oriented to clinical practice. Although practitioners are interested in research, and some participate and publish, the majority of practitioners are invested in incorporating botanical medicine into their practice rather than being in a university setting.

Seven areas of practice have been identified for practitioners of veterinary botanical medicine. While the description of most of these areas will sound familiar to any veterinary practitioner, all of them incorporate botanical medicine for this specific discipline.

The seven areas identified for the practice of veterinary botanical medicine include:

- Patient assessment
- Botanical diagnostic framework
- Providing botanical treatment
- Botany, wildcrafting, manufacture and plant/herb identification
- Facilitation of veterinary botanical medicine practice methods (in the practitioner’s practice and also for others looking to incorporate botanical medicine in their practice)
- Serve as a resource on veterinary botanical medicine for veterinarians and for public policy
- Advance veterinary botanical medicine

Six to twelve specific job tasks were identified for each area, for a total of 62 job tasks. They include:
Patient assessment
- Obtain animal patient health history including history from a botanical medicine perspective
- Elicit additional observations from owner from a botanical medicine perspective
- Conduct a clinical exam on the patient including botanical assessment methods
- Analyse patient health status
- Determine animal disease status and differential diagnosis
- Implement diagnostic testing as appropriate
- Evaluate patient appropriateness for botanical therapy

Botanical Diagnostic Framework
- Evaluate relative strength and progression of disease
- Recognize patterns of disharmony/ differentiation of syndromes according to framework of botanical medicine
- Identify situations/conditions where botanical medicine would produce undesired effects
- State pathophysiology in Western medical terms and botanical medicine terms
- Know clinical indications for using botanical medicine
- Modify botanical formulas and doses according to patient's condition
- Re-evaluate and analyze patients current health status and progress
- Adjust treatment plan based on assessment
- Supply owner education and referral where indicated

Providing Botanical Treatment
- Know herbal terminology
- Recognize commonly used herbs/formulas
- Know species specific pharmacology
- Know phytopharmacological actions of herbs
- Evaluate potential herb drug interactions and modify treatment accordingly
- Know herb properties and qualities
- Know botanical indications based on research
- Know how to integrate botanical medicine with conventional medicine
- Evaluate botanical medicine and drugs for possible toxicological activity
- Know the principles of botanical prescriptions and formulations (including dosage and duration of treatment)
- Plan and provide the botanical medicine treatment strategy
- Support the management of life threatening states with botanical medicine

Botany, Wildcrafting, Manufacture and Plant Identification
- Know endangered plant species and plant medicine substitutes.
- Practitioner awareness of botanical medicine sources including biodiversity and sustainability
- Know appropriate part of herb to use and preparation
- Know the effects of growing conditions on variability and manage this
- Know types of botanical medicine preparations
- Be able to identify common botanical medicines

Facilitate veterinary botanical medicine practice methods
- Use critical thinking and analysis effectively in integrated practice
- Work within veterinary botanical medicine regulatory requirements
- Source botanical medicines for use in veterinary practice
- Prepare botanical medicines for topical, rectal and oral administration
- Facilitate development of professional botanical practice
- Prepare veterinary botanical medicine for dispensing in practice
- Monitor veterinary botanical medicine stock in practice
- Implement reflective learning practices in integrated practice
- Be aware of manufacturing processing of commercial herbs, including GMP
- Be aware of contemporary issues concerning safe botanical products
• Communicate veterinary botanical medicine principles and practices to staff

Serve as resource on veterinary botanical medicine and public policy
• Provide expertise for legislative items and policies
• Be available to consult on regulations
• Know information about botanical medicine history, trends
• Know ethical issues, health and welfare implications
• Identify contemporary botanical medicine issues
• Know local practice acts
• Know federal and state requirements

Advance Veterinary botanical medicine
• Search and critically apply traditional, alternative and scientific information
• Critically evaluate specific research and information
• Apply information to integrated veterinary treatment strategy
• Communicate and collaborate effectively with other Diplomates and veterinarians
• Promote development of education curricula
• Contribute to botanical medicine research
• Educate veterinary and herbal community about herbal medicine
• Communicate with public as expert in botanical medicine issues
• Pursue professional development in botanical medicine
• Communicate botanical medicine case outcomes effectively

The Diplomate Certifying Examination
The Examination Committee has determined that examination questions will be in multiple-choice format and written under the guidelines of the National Board of Medical Examiners (NBME). The initial exam will be written by the examination committee, with the help of members of the organizing committee. Later on, candidates who pass the test will be required to submit three questions, including published references for each question, to add to the databank of questions.

The written examination will consist of four (4) parts.
1. A general botanical medicine section (all candidates are required to take this section)
2. A specific botanical medicine category
3. Veterinary botanical medicine for equine, production and small animals

The examination shall consist of 100 or more questions for sections I-III in a multiple choice format with one correct answer and four distractors. Three (3) minutes will be allotted for each question. Depending on the number of questions, the total examination time may vary. The contents of the examination will be determined by the Credentials and Education Committee.

A proposed outline to be further developed by the Exam Committee is as follows:

Section I
• Pharmacognosy
• Principles
• Phytochemistry
• Pharmacology
• Manufacture
• Dispensing
• Quality
• Safety
• Pharmacy issues with herbs

Section II
The pass point for the exam will be determined using the modified Angoff technique. Questions for the initial examination will be solicited from the organizing committee, as well as from any well-respected veterinary herbalists who are not on the committee. Additional questions will be written by the exam committee, in order to have sufficient questions in each area of the examination, according to the ratio of each area indicated by the job task survey.

Evaluation of exam questions for the initial exam will be done by as many organization committee members as possible who can meet together in person, and no less than five will participate. For later questions, members of the examination committee will be the evaluators. All questions in the exam question data bank will be evaluated to derive a predictive pass/fail value.

The goal of the evaluation is to have a pass point as close to 70% as possible. If the predictive Angoff score for the whole exam is much higher, questions will be substituted to bring the score closer to 70%. If the predictive Angoff score for any specific year is below 70%, the pass point will change to that lower score, for that specific exam only.

Exam examples:
Sample question from each general area of job tasks

One job task was chosen from each area in the job task analysis, and a question was supplied for it. In the following examples, Areas are in italics and job tasks are identified by “Task”

**Patient assessment**
Task: Evaluate patient appropriateness for botanical therapy

Medicinal herbs that may present problems in post-performance drug tests in equines include all of the following EXCEPT 1:

a) *Capsicum frutescens* topically
b) *Valeriana officinalis* root orally
c) *Withania somnifera* root orally
d) *Chamomilla recutita* flower orally

**Botanical Diagnostic framework**
Task: Identify situations/conditions where herbs/formulas would produce undesired effects

Caution should be exercised when prescribing *Hypericum performatum* with cyclosporine in dogs 2

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1 USEF’s Drugs and Medications Guidelines handbook 2010
2 Fukunaga K, Orito K. Time-course effects of St John’s wort on the pharmacokinetics of cyclosporine in dogs: interactions between herbal
because:

a) It will reduce the efficacy of cyclosporine through pharmacokinetic interaction
b) It will enhance the blood plasma levels of cyclosporine through pharmacokinetic interaction
c) It will reduce the efficacy of cyclosporine through pharmacodynamic interaction
d) It will potentially mask depression in canine patients

**Providing treatment**
Task: Know phytopharmacological actions of herbs

A class of constituents common3 to *Withania somnifera, Glycyrrhiza glabra, Tussilago farfara, Althea officinalis* and *Symphytum officinale* that have made them useful in suppression of cough reflex are:

a). terpinols
b). phenols
c). polysaccharides
d). glycosides

Anthraquinone glycosides are found in all except:

a). *Aloe vera*
b). *Aconitum carmichaelii*
c). *Cascara sagrada*
d). *Rheum palmatum*

**Botany, plant/herb ID**
Task: Know endangered plant species and plant medicine substitutes.

You are considering using the following 4 herbs for use in a case:
1. *Berberis vulgaris*
2. *Plantago major*
3. *Panax quinquefolius*
4. *Hydrastis canadensis*

Which two of these herbs are endangered in the wild?

a) 1 and 3
b) 1 and 4
c) 2 and 3
d) 3 and 4

**Facilitate veterinary botanical medicine practice methods**
Task: Be aware of manufacturing processing of commercial herbs, including GMP

In evaluating processing methods for a herbal product which process is the most useful to ensure consistency between batches and product quality?
a. Whether the botanical medicine was extracted and produced under GMP conditions
b. Where the botanical medicine was harvested and in what season and identifying the species
c. Whether organoleptic means were used to identify the species
d. Whether the botanical medicine is sourced from an organic farm and the species identified

**Serve as resource on veterinary botanical medicine and public policy**
Task: Provide expertise for legislative items and policies

*Symphytum officinalis* has a long history of use for wound healing. A veterinarian involved in product...
development has contacted you to ask your advice on label instructions based on the FDA position on the use of *Symphytum officinale* (common comfrey), *S. asperum* (prickly comfrey), and *S. x uplandicum* (Russian comfrey). What is then appropriate policy for use in veterinary medicine?

a. Use orally as directed under the supervision of a veterinarian  
b. Use topically for wound healing only  
c. Use orally as directed and topically on wounds  
d. Use only topically and on unbroken skin

**Advance Veterinary botanical medicine**  
Task: Apply information to integrated veterinary treatment strategy

Which of the following botanical medicines can offset the benefits of spironolactone in the treatment of congestive heart failure or kidney disease or hypokalemia?4

a) *Glycyrrhiza glabra*  
b) *Crataegus oxyacantha*  
c) *Crataegus monogyna*  
d) *Crataegus leavigata*

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Appendix VII Training Programs currently Available

Current Training Programs available in Veterinary Chinese and Western Herbal Medicine range from short courses through to Post Graduate Degrees.

**VBMA**
- 16 basic to advanced webinar Topics in veterinary herbal medicine

**VIN**
- 16 hours Intermediate Herbal Medicine
- 12 hours Introduction to Herbal Medicine
- 10 hours Introduction to Veterinary Chinese Herbal Medicine
- 6 hours Using herbs for Liver and Kidney Disease

**Chi**
- 155 hour Chinese Veterinary Herbal Medicine Program approved by a majority of state boards, provides training Veterinary Chinese Herbalist and this contributes towards a Masters degree in Traditional Chinese Veterinary Medicine.

**IVAS**
- 160 hours Veterinary Chinese Herbal Medicine training
- 500 hours Advanced Veterinary Chinese Herbal Medicine training

**CIVT**
- 1305 hours Graduate Diploma Veterinary Chinese Herbal Medicine (Grad Dip VCHM) accredited post graduate degree competency based training
- 945 hours Graduate Diploma Veterinary Western Herbal Medicine (Grad Dip VWHM) accredited post graduate degree competency based training
- 500 hours Foundation Course in Western Veterinary Herbal Medicine
- 445 hours Advanced Western Veterinary Herbal Medicine
- 120 hours Foundation Course Veterinary Chinese Herbal Medicine
- 40 hours Getting Started Veterinary Chinese Herbal Medicine Introductory course
- 24 hours Essentials of Western Veterinary Herbal Medicine

Samples of Programs:
Chi Institute Veterinary Herbal Medicine Program

The Chinese Herbal Medicine Program is a 155 hour CE Program (Including the Intro to Herbal Medicine course) (approved by a majority of state boards) that trains students in Veterinary Chinese Herbal Medicine. It is presented in five modules, each covering different sets of organ systems and their affiliated health disorders. Each module offers five hours of wet lab, approved by many state boards. All five modules are offered each year, and can be taken either online or on-site. The modules are:

- Respiratory/Cardiovascular
- Gastrointestinal/Spleen
- Liver/Endocrinology
- Kidney/Urinary/Reproductive/Geriatric
- Dermatology/Oncology/Immune-mediated Diseases

The course is intended for small, mixed, and equine vets, and both small and large animals (mostly dogs and horses) are used during case studies and wet labs. General topics covered in each module include herbal medicine, a TCVM approach to Western diseases, and advanced TCVM theories and principles.

For further information:

http://www.tcvm.com/Programs/AdvancedPrograms/HerbalMedicine/Syllabus.aspx

IVAS Herbal Training Programs

IVAS Chinese Herbal Medicine Training

The IVAS Course in VCHM curriculum enables students to gain skills and knowledge in the principles of Veterinary Chinese herbal medicine, including materia medica, classic formula study, dispensary, herb drug interactions and herbal therapeutics with an emphasis on integrative approaches, biomedical understandings of formula and case based learning. Assessment includes case analysis assessment, submission of three case reports and multiple choice assessments. Support is provided by experienced veterinary Chinese herbal practitioners.

The IVAS Course curriculum covers a minimum of 100 herbs and a focused group of about 30 key classical formulas that can be modified, enabling a small pharmacy that meets the main needs of veterinary practice. Attention is paid to avoiding herbs and formulas composed of animal parts or endangered species and knowing relevant substitutions that are acceptable.

The 160 hour IVAS Course is RACE approved for 100 hours and trains graduates to be able to:

1. Explain the basis of how Chinese herbs work from a traditional medicine basis and from a biomedical and pharmacological perspective.
2. Explain how Chinese herbal products are made and therefore be able to ascertain quality issues
3. Take a history, conduct and interpret the necessary diagnostic procedures in order to make a TCM diagnosis by integrating patterns of disharmony with etiological factors and pathological processes and how these aspects interconnect - along with the Western diagnosis
4. Elucidate the treatment principles needed for patients and therefore be able to select an appropriate strategy based upon the actions of Chinese herbal medicine
5. Have the practical skills to dispense Chinese herbal medicine and integrate them with conventional medications
6. Monitor the patient’s condition as a result of treatment, re-evaluate diagnostic information and differential diagnosis and modify treatment strategy as the patient’s condition changes over time.
7. Be aware of safety issues including the potential for side effects (know how to interpret side effects)
8. Be able to integrate Chinese medicine principles with conventional veterinary medicine with emphasis on potential herb drug interactions and manage the risk.
9. Be aware of professional codes of ethics and the practice of veterinary Chinese herbal medicine
10. Be aware of significant research issues

For Further Information:

www.ivas.org

**IVAS Advanced Chinese Herbal Medicine Training**

The IVAS Advanced Course in VCHM curriculum enables students to gain advanced skills and knowledge in the principles of Veterinary Chinese herbal medicine, including materia medica, classic formula study, dispensary, herb drug interactions and herbal therapeutics with an emphasis on integrative approaches, biomedical understandings of formula and case based learning. Assessment includes case analysis assessment, submission of fifteen case reports and multiple choice assessments. Support is provided by experienced veterinary Chinese herbal practitioners.

The IVAS Course curriculum covers therapeutic strategies and some 80 key classical formulas. Attention is paid to avoiding herbs and formulas composed of animal parts or endangered species and knowing relevant substitutions that are acceptable.

The 500 hour IVAS Course trains graduates to be able to:

1. Explain the basis of how Chinese herbs work from a traditional medicine basis and from a biomedical and pharmacological perspective.
2. Explain how Chinese herbal products are made and therefore be able to ascertain quality issues
3. Take a history, conduct and interpret the necessary diagnostic procedures in order to make a TCM diagnosis by integrating patterns of disharmony with etiological factors and pathological processes and how these aspects interconnect - along with the Western diagnosis to an advanced level
4. Elucidate the treatment principles needed for your patients and therefore be able to select an appropriate strategy based upon the actions of Chinese herbal medicine to an advanced level
5. Have the practical skills to dispense Chinese herbal medicine and integrate them with conventional medications to an advanced level
6. Monitor the patient’s condition as a result of treatment, re-evaluate diagnostic information and differential diagnosis and modify treatment strategy as the patient’s condition changes over time.
7. Be aware of safety issues including the potential for side effects (know how to interpret side effects)
8. Deal with complex and critical case cases by integrating conventional and Chinese medicine
9. Be aware of professional codes of ethics and the practice of veterinary Chinese herbal medicine
10. Be current with recent relevant research that supports the evidence base

For Further Information:

www.ivas.org

CIVT Herbal Training Programs

Both of these courses are government accredited as post graduate degrees under the Australian Quality Skills Authority.

10014NAT Graduate Diploma of Veterinary Chinese Herbal Medicine

consists of 8 Modules which are the teaching components. The rest of the Graduate Diploma consists of keeping a reflective case log book of personal experience of the skills practiced; demonstrated professional ability and competence and preparing material and evidence for assessment of competencies.

There are 8 Units of Competency that are core units and are required before full qualification is granted. Students are assessed continuously throughout the training and in the final module submit their portfolio of evidence to CIVT for assessment. Qualified assessors of CIVT assess each final submission.

Units of Competency

<table>
<thead>
<tr>
<th>Unit Code</th>
<th>Unit Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>VETCHM801A</td>
<td>Apply the veterinary Chinese herbal medicine diagnostic framework</td>
</tr>
<tr>
<td>VETCHM802A</td>
<td>Work within veterinary Chinese herbal medicine principles and practices</td>
</tr>
<tr>
<td>VETCHM803A</td>
<td>Plan and provide the veterinary Chinese herbal medicine treatment strategy</td>
</tr>
<tr>
<td>VETCHM804A</td>
<td>Perform the veterinary Chinese herbal medicine assessment</td>
</tr>
<tr>
<td>VETCHM805A</td>
<td>Provide specialised veterinary Chinese herbal medicine care</td>
</tr>
<tr>
<td>VETTCM802A</td>
<td>Operate a veterinary Chinese medicine practice</td>
</tr>
<tr>
<td>VETINT801A</td>
<td>Prepare veterinary case studies for publication</td>
</tr>
<tr>
<td>VETINT802A</td>
<td>Reflect upon integrated veterinary medicine practice</td>
</tr>
</tbody>
</table>

Part-time. 24 months. Expected volume of learning and study including clinical case time over the 24 months- 1310 hours. Participants are required to demonstrate evidence of competencies through assessment processes which comprise activities and assessments to demonstrate knowledge and skills largely based on actual and simulated cases in practice, over the two year period.

For further information:

http://www.civtedu.org/graduate-diploma-veterinary-chinese-herbal-medicin/

10049NAT Graduate Diploma of Veterinary Western Herbal Medicine

consists of 2 Parts, of which the programs - Year 1 Foundations Veterinary Western Herbal Medicine (Modules 1-4) and Year 2 Advanced Veterinary Herbal Therapeutics (Modules 5-8) are the teaching components (non-accredited when taken alone). The additional component of 10049NAT Graduate Diploma of Veterinary Western Herbal Medicine consists of keeping a reflective case log book of
personal experience of the skills practiced, demonstrated professional ability and competence and preparing material and evidence for assessment of competencies.

All 6 Units of Competency are core units and are required before full qualification is granted. Students are assessed continuously throughout the training and in the final module submit their portfolio of evidence to CIVT for assessment. Qualified assessors of CIVT assess each final submission.

**UNITS OF COMPETENCE**

<table>
<thead>
<tr>
<th>UNIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>VETWHM801A - Work within a veterinary Western Herbal Medicine framework</td>
</tr>
<tr>
<td>VETWHM802A - Work within veterinary Western Herbal Medicine principles and practices</td>
</tr>
<tr>
<td>VETWHM803A - Operate a veterinary Western Herbal Medicine pharmacy</td>
</tr>
<tr>
<td>VETWHM804A - Perform the veterinary Western Herbal Medicine assessment</td>
</tr>
<tr>
<td>VETWHM805A - Plan and provide the veterinary Western Herbal Medicine treatment strategy</td>
</tr>
<tr>
<td>VETWHM806A - Prepare and present veterinary Western Herbal Medicine research findings</td>
</tr>
</tbody>
</table>

Part-time. 24 months. Expected volume of learning and study including clinical case time over the 24 months- 945 hours. Participants are required to demonstrate evidence of competencies through assessment processes which comprise activities and exercises based on actual and simulated cases in practice over the two year period.

For further information:

Appendix VIII Examples of recent journal articles
Effects of cranberry extract on prevention of urinary tract infection in dogs and on adhesion of *Escherichia coli* to Madin-Darby canine kidney cells

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**OBJECTIVE**  
To determine effects of cranberry extract on development of urinary tract infection (UTI) in dogs and on adherence of *Escherichia coli* to Madin-Darby canine kidney (MDCK) cells.

**ANIMALS**  
12 client-owned dogs (in vivo experiment) and 6 client-owned dogs (in vitro experiment).

**PROCEDURES**  
12 dogs with a history of recurrent UTI received an antimicrobial (n = 6) or cranberry extract (6) orally for 6 months. Dogs were monitored for a UTI. For the in vitro experiment, cranberry extract was orally administered to 6 dogs for 60 days. Voided urine samples were collected from each dog before and 30 and 60 days after onset of extract administration. Urine was evaluated by use of a bacteriostasis assay. An antiadhesion assay and microscopic examination were used to determine inhibition of bacterial adherence to MDCK cells.

**RESULTS**  
None of the 12 dogs developed a UTI. The bacteriostasis assay revealed no zone of inhibition for any urine samples. Bacterial adhesion was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results for urine samples obtained before extract administration. Microscopic examination revealed that bacterial adherence to MDCK cells was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results after culture with urine samples obtained before extract administration.

**CONCLUSIONS AND CLINICAL RELEVANCE**  
Oral administration of cranberry extract prevented development of a UTI and prevented *E. coli* adherence to MDCK cells, which may indicate it has benefit for preventing UTIs in dogs. (*Am J Vet Res* 2016;77:421–427)

Urinary tract infections are associated with a temporary or permanent breach in host defense mechanisms that allows virulent microbes to adhere, multiply, and persist within the urinary tract. Infections can be confined to a single site within the urogenital tract, such as the renal pelvis (pyelonephritis), ureter (ureteritis), bladder (cystitis), urethra (urethritis), prostate gland (prostatitis), or vagina (vaginitis), or can be found at multiple sites.\(^1\) Although fungi and viruses also infect the urinary tract, UTIs are most commonly caused by bacteria such as *Escherichia coli*, which is the most common uropathogen.\(^2\)\(^-\)\(^4\)

Cranberries consist primarily of water (88%), but they also contain organic acids (including salicylate), fructose, high amounts of vitamin C (200 mg/kg of fresh berries), flavonoids, proanthocyanidins, catechins, and triterpinoids.\(^4\)\(^-\)\(^5\) The *E. coli* strains that cause UTIs have proteinaceous macromolecules (fimbriae) that facilitate adhesion of bacteria to uroepithelial cells in the urinary tract. In vitro and in vivo studies\(^5\)\(^-\)\(^6\) indicate that cranberry products prevent bacterial adhesion to cells in the wall of the urinary tract.

Studies\(^5\)\(^-\)\(^7\) indicate that the consumption of cranberry extract can prevent UTIs in women. However, there is a paucity of studies on the benefits of cranberries for prevention of UTIs in dogs. Therefore, the objectives of the study reported here were to investigate the effect of cranberry extract on the development of UTIs in dogs with a history of recurrent UTIs and to evaluate effects of urine obtained from dogs provided cranberry extract on adhesion of *E. coli* to MDCK cells.

**Materials and Methods**

**Animals**  
The in vivo experiment involved 12 client-owned female dogs, each of which had at least 3 UTIs during...
the preceding year. All dogs were confirmed to have recovered from the most recent UTI, as determined on the basis of results of urinalysis and bacterial culture of a urine sample.

The in vitro experiment involved 6 client-owned dogs (4 mixed-breed dogs, 1 Pug, and 1 Shih Tzu). There were 3 neutered males and 3 spayed females. Age of the dogs ranged from 7 to 11 years (mean, 8.9 years), and body weight ranged from 6.5 to 20.5 kg (mean, 13.6 kg). All 6 dogs were considered healthy at the time of enrollment, as determined on the basis of the medical history and results of a complete physical examination (no signs of urinary tract disease).

Owners provided consent for inclusion of the dogs in the study. All dogs received care in accordance with institutional animal care and use committee guidelines.

**Experimental design**

**In vivo experiment**—Dogs were allocated into 2 groups (6 dogs/group). One group comprised 4 Schnauzers and 2 Toy Poodles (3 spayed and 3 sexually intact; age of the dogs ranged from 7 to 14 years [mean, 9.8 years], and body weight ranged from 2.8 to 7.4 kg [mean, 5.6 kg]). These dogs received cephalixin (20 mg/kg, PO, q 12 h for 14 days). The second group comprised 4 Schnauzers and 2 Chihuahuas (4 spayed and 2 sexually intact; age of the dogs ranged from 5 to 12 years [mean, 8.0 years], and body weight ranged from 2.5 to 7.3 kg [mean, 5.3 kg]). Dogs in the second group received powdered cranberry extract daily for 6 months. The powder was mixed with food and administered to each dog at the morning meal. The amount of cranberry extract provided to each dog was the dose specified on the product (1 g for dogs < 25 kg and 2 g for dogs ≥ 25 kg). The first day of administration of cephalixin or cranberry extract was designated as day 1.

Dogs were monitored throughout the experiment. Blood samples and voided urine samples were collected from each dog immediately before onset of cephalixin or cranberry extract administration and then once per month for 6 months. Once each month, a complete physical examination, hematologic examination, biochemical analysis, urinalysis, and bacterial culture of a urine sample were performed.

**In vitro experiment**—Dogs received powdered cranberry extract (1 g for dogs < 25 kg and 2 g for dogs ≥ 25 kg) daily for 60 days (1 day before administration of cranberry extract was designated as day 0). The powder was mixed with food and administered to each dog at the morning meal. Voided urine samples were collected from each dog immediately before onset of cranberry extract administration and on days 30 and 60.

**Preparation of urine samples**

Urine samples were collected in the morning and centrifuged at 1,000 X g for 5 minutes to precipitate particulate matter. Supernatant was removed with a sterile Pasteur pipette and vacuum-filtered by use of a commercial filtration unit with a 0.22-µm polyethersulfone filter; filtered urine was collected in a sterile 50-mL conical tube and frozen at −20°C for use in a bacteriostasis assay.

**Propagation of uropathogenic E coli strains and preparation of bacterial suspensions**

Three uropathogenic E coli strains (C1-50, C2-48, and C3-48) were isolated from dogs with UTI examined at the Veterinary Medical Teaching Hospital of the National Chung Hsing University. The E coli strains were grown on blood agar plates at 35°C for 24 to 48 hours. After distinct bacterial colonies appeared, the plates were sealed and stored at 4°C until used for the bacteriostasis assay.

An E coli colony was selected; it was then streaked onto trypticase soy agar and incubated overnight at 35°C. The next morning, E coli were suspended in 3 or 5 mL of saline (0.9% NaCl) solution. A standard bacterial concentration of 10⁶ CFUs/mL, as determined by use of a 0.5-McFarland standard, was used for the bacteriostasis assay.

**Bacteriostasis assay**

A swab specimen of the bacterial suspension was smeared onto plates containing Mueller-Hinton agar. Seven holes were punched in each agar plate; 1 hole was filled with 100 µL of sterile saline solution (negative control sample), and the remaining 6 holes were each filled with 100 µL of the urine sample of 1 dog at 1 time point. A disk containing enrofloxacin was used as the positive control sample. A positive result was considered to be an inhibition zone with a diameter > 21 mm. Plates were incubated at 35°C for 24 hours, and the inhibition zone around each hole was then assessed.

**Preparation of MDCK cells**

The MDCK cells were obtained from the Graduate Institute of Veterinary Pathobiology at National Chung Hsing University. They were maintained in DMEM that contained 4.5 g of glucose/L, sodium pyruvate, and 4 mM stable glutamine and 10% (vol/vol) heat-inactivated fetal bovine serum supplemented with 1 mM sodium pyruvate and 1% (vol/vol) antimicrobial (penicillin, streptomycin, and amphotericin B) solution. Stock cultures of cells were propagated in 75-cm² plastic flasks at 37°C in a humidified 95% O₂–5% CO₂ atmosphere and passaged as needed.

**Antiahesion assay**

The efficacy of cranberry extract for inhibiting bacterial adherence to MDCK cells was evaluated by use of an in vitro assay with modifications described elsewhere. Antiadhesion assays were performed as follows.
The MDCK cells that had grown to confluence at 37°C were placed in 96-well plastic plates (10^4 cells/well) for the antiadhesion assay. Culture media were discarded, and each well was washed with PBS solution (100 µL). The wash solution was discarded, and plates were tapped dry on absorbent paper. Immediately before the assay, MDCK cells were fixed with 5% methanol. An aliquot (100 µL) of methanol was added to each well, and plates were allowed to sit undisturbed for 2 minutes. The methanol was then discarded, and plates were tapped dry on absorbent paper. Plates were then further dried in a laminar flow hood for 10 minutes.

A test sample of urine plus bacteria was created by mixing an aliquot of the bacterial suspension (a standard bacterial concentration of 10^6 CFUs/mL, as determined by use of a 0.5-McFarland standard) with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration. The ratio was 1:10 (1 part bacterial suspension to 9 parts urine sample). Each well of a 96-well plastic plate was prepared by adding 50 µL of the test sample (urine plus bacteria) and 150 µL of DMEM (final volume, 200 µL/well) to the methanol-fixed MDCK cells. Plates were then incubated at 25°C for 30 minutes.

After incubation was complete, the plates were incubated for an additional 60 minutes at 35°C to permit bacterial attachment. After this 60-minute incubation was complete, nonadhered bacteria and media were removed by aspiration, and the wells were rinsed 3 times with PBS solution (200 µL/rinse). Then, 200 µL of DMEM plus 5% heat-inactivated fetal bovine serum was added to each well. Plates were incubated at 35°C for 18 hours to allow growth of attached bacteria. After the 18-hour incubation was complete, absorbance for each well was determined at 650 nm by use of a microplate reader and commercial software.

Microscopic examination

The MDCK cells that had grown to confluence at 37°C were placed in 24-well plastic plates (5 X 10^4 cells/well) for the antiadhesion assay. Culture media were discarded, and each well was washed with PBS solution (100 µL). The wash solution was discarded, and plates were tapped dry on absorbent paper. Immediately before the assay, MDCK cells were fixed with 5% methanol. An aliquot (200 µL) of methanol was added to each well, and plates were allowed to sit undisturbed for 2 minutes. The methanol was then discarded, and plates were tapped dry on absorbent paper. Plates were then further dried in a laminar flow hood for 10 minutes.

A test sample of urine plus bacteria was created by mixing an aliquot of the bacterial suspension (a standard bacterial concentration of 10^6 CFUs/mL, as determined by use of a 0.5-McFarland standard) with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration. The ratio was 1:10 (1 part bacterial suspension to 9 parts urine sample). Each well of a 24-well plastic plate was prepared by adding 200 µL of the test sample (urine plus bacteria) and 300 µL of DMEM (final volume, 200 µL/well) to the methanol-fixed MDCK cells. Plates were then incubated at 25°C for 30 minutes.

After the initial incubation was complete, the plates were incubated for an additional 3 hours. Slides were stained with crystal violet and examined microscopically (1,000X magnification).

Statistical analysis

All data were expressed as mean ± SEM. Differences between groups were tested by use of the Student t test. Values of P < 0.05 were considered significant. Linear regression analysis was used to evaluate results for the antiadhesion assay and microscopic examination.

Results

In vivo experiment

None of the 12 dogs developed a UTI during the experimental period.
Bacterial adhesion was reduced for urine samples obtained at 30 and 60 days from each of the 6 dogs, compared with results for the urine sample obtained before administration of cranberry extract. Mean ± SEM absorbance for C1-50 E. coli cultured in plates containing MDCK cells with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration was 0.81 ± 0.03, 0.24 ± 0.01, and 0.16 ± 0.02, respectively (Figure 2). Mean ± SEM absorbance for C2-48 E. coli cultured in plates containing MDCK cells with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration was 0.80 ± 0.03, 0.24 ± 0.01, and 0.14 ± 0.02, respectively. Mean ± SEM absorbance for C3-48 E. coli cultured in plates containing MDCK cells with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration was 0.81 ± 0.02, 0.24 ± 0.02, and 0.12 ± 0.01, respectively. Mean absorbance for the 3 E. coli strains cultured with MDCK cells and urine samples obtained at 30 and 60 days was significantly lower than the absorbance for culture with the urine sample obtained before onset of cranberry extract administration. Moreover, mean absorbance of the 3 E. coli strains cultured with MDCK cells and urine obtained at 60 days was also lower than that for urine samples obtained before and at 30 days after onset of administration of cranberry extract.

Adherence of the 3 E. coli strains was decreased from a mean of 101.84 adherent bacteria/MDCK cell after incubation with urine samples obtained before cranberry extract administration to 16.44 and 4.00 adherent bacteria/MDCK cell after incubation with urine samples obtained at 30 and 60 days, respectively. Mean ± SEM number of C1-50 E. coli adhering to MDCK cells was 95.17 ± 10.65, 12.67 ± 3.5, and 3.17 ± 2.04 for the urine samples obtained before and 30 and 60 days after onset of cranberry extract administration, respectively (Figure 3). Mean ± SEM number of C2-48 E. coli adhering to MDCK cells was 109.17 ± 10.61, 16.35 ± 3.5, and 4.17 ± 2.64 for the urine samples obtained before and 30 and 60 days after onset of cranberry extract administration, respectively. Mean ± SEM number of C3-48 E. coli adhering to MDCK cells was 101.17 ± 9.52, 20.33 ± 3.56, and 4.67 ± 1.86 for the urine samples obtained before and 30 and 60 days after onset of cranberry extract administration, respectively. Compared with the mean adherence for the urine sample obtained before onset of cranberry extract administration, the mean E. coli adherence to the MDCK cells for the urine samples obtained at 30 and 60 days was significantly lower. Moreover, the mean E. coli adherence to the MDCK cells was significantly lower for the urine sample obtained at 60 days than for the urine sample obtained at 30 days. Adherence of
Discussion

In the in vivo experiment reported here, an antimicrobial and powdered cranberry extract were administered to prevent UTIs in dogs. None of the dogs developed UTIs, as determined on the basis of clinical signs and laboratory results, which corresponded with results of another study.5 Some studies6,10,11 of humans indicate that the use of cranberries to prevent UTIs is better than the prophylactic use of low-dose antimicrobials because long-term use of antimicrobials increases the risk of antimicrobial resistance.

In the present study, the effect of cranberry extract on the prevention of bacterial adhesion was evaluated in vitro. Urine samples were collected from dogs receiving cranberry extract and used to determine antibacterial effects. Because *E coli* are the most common uropathogenic bacteria in dogs with UTIs,2–4 those bacteria were used in the present study. Three *E coli* strains were prepared for use in bacteriostasis and antiadhesion assays and microscopic examination. The bacteriostasis assay revealed no inhibition zone around the urine samples and negative control sample, whereas the positive control sample (enrofloxacin) had an antibacterial effect (diameter of inhibition zone > 30 mm). This indicated 2 possibilities: the concentration of bacteria was too high for the cranberry extract to inhibit growth, or the urine samples from dogs receiving cranberry extract had no bacteriostatic activity.

Results of previous studies12–15 as well as the present study suggest that cranberries do not have an effect on inhibition of bacterial growth. Instead, it is hypothesized that cranberries prevent UTIs by blocking adherence of bacteria to the uroepithelium.16–18 Evidence to support this hypothesis was obtained in an in vitro study11 of fimbriated *E coli* present in the urine 2 hours after ingestion of cranberry extract. In fact, the mean absorbance for the 3 *E coli* strains cultured with MDCK cells and urine samples obtained at 30 and 60 days was significantly lower than that after culture with the urine sample obtained before onset of cranberry extract administration, which indicated that the urine samples collected after administration of the cranberry extract had an antiadhesion effect. One possible mechanism of action may be that cran-

![Figure 4](image-url)

**Figure 4**—Photomicrographs of cultured MDCK cells indicating adherence of *E coli* strain C1-50, C2-48, and C3-48 to the cells after culture with urine samples obtained from a representative dog before (top row) and 30 (middle row) and 60 (bottom row) days after onset of cranberry extract administration. Notice the bacteria (arrowheads) that are adhered to the cells. Crystal violet stain; bar = 50 µm.
berry compounds act as receptor analogues and bind to the fimbrae of *E. coli*, which thus competitively inhibits their adhesion. It has been confirmed that *E. coli* isolated from dogs with UTIs most commonly express type-I fimbrae.\(^\text{19}\) Furthermore, the main mechanism of in vitro adherence to canine uroepithelial cells involves a mannose-sensitive mechanism.\(^\text{20}\) Components of the cranberry extract also might have altered P-fimbriated uropathogenic bacteria in other ways, such as by reducing adhesion capabilities, reducing fimbral length and density, or inducing other morphological changes.\(^\text{18,21,22}\)

Mean *E. coli* adherence to MDCK cells after incubation with urine samples obtained at 30 and 60 days was significantly lower, compared with adherence after incubation with the urine sample obtained before administration of the cranberry extract. Moreover, mean *E. coli* adherence was significantly lower after incubation with the urine sample obtained at 60 days, compared with results after incubation with the urine sample obtained at 30 days.

In the present study, MDCK cells were used because they are a good in vitro method of screening to detect bacteria virulence\(^\text{23}\) or determining the pathogenesis of various bacterial infections, including those attributable to uropathogenic *E. coli*.\(^\text{24,25}\) Adhesion of uropathogenic *E. coli* to epithelial cells can lead to ascending UTIs, which range from nonclinical bacteriuria to cystitis and acute pyelonephritis to more severe acute lobar nephronia.\(^\text{26}\) Bacterial adhesion to uroepithelial cells by fimbrial or nonfimbrial adhesins in bacterial renal infections is an important factor in the subsequent development of UTIs in the upper urinary tract (ie, calyx, renal pelvis, and ureter) via the ascending route.\(^\text{27}\) The effect of cranberry extract on *E. coli* adhesion to both kidney epithelial cells and uroepithelial cells derived from dogs has been described.\(^\text{28}\) Results for the microscopic examination of in vitro adherence to canine uroepithelial cells derived from dogs has been described.\(^\text{28}\) Moreover, the results from another study\(^\text{28}\) that also revealed antiaadhesion activity of cranberries or cranberry extract on *E. coli* adherence to specific primary-cultured uroepithelial cells.\(^\text{28}\) The antiaadhesion effect of cranberries is not restricted to a particular group of *E. coli* strains, which might otherwise be caused by interference with specific receptor-ligand modes of bacterial adhesion or by inhibition of expression of the bacterial fimbrae.\(^\text{21,29}\) The effect of cranberry intake might be synergistic, but the details remain unclear. In the present study, we minimized the possible bias associated with a noncontrolled trial by developing a bioassay to test adhesion of bacteria to MDCK cells that were cultured with urine obtained from dogs after they had received cranberry extract.

Antimicrobial resistance is an increasing concern. Therefore, alternative strategies such as consumption of cranberries or cranberry extract may be an option for prevention of UTIs in dogs. The present study revealed that the efficacy of cranberry extract for the prevention of UTIs was almost the same as that for an antimicrobial (cephalexin), with a lower risk of antimicrobial resistance or superinfection.\(^\text{30}\) Analysis of the results of the study reported here indicated that cranberry extract decreased *E. coli* adherence to MDCK cells but did not inhibit bacterial growth. This effect suggested that cranberry extract has a potential clinical benefit for the prevention of UTIs in dogs.

**Acknowledgments**

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**Footnotes**

a. Kellex, 250-mg capsule, Taiwan Biotech Co, Taoyuan City, Taiwan.

b. 120 g/pack, Cranimals, West Vancouver, BC, Canada.

c. Millex GV/Millipore Ireland BV Carrigtwohill Co, Cork, Ireland.

d. Difco, Becton Dickinson and Co, Franklin Lakes, NJ.

e. BBL, Becton Dickinson and Co, Franklin Lakes, NJ.


g. Mediotech Inc, Tewksbury, Mass.

h. Sunrise Infinite F200, Tecan, Männedorf, Switzerland.

i. Magellan, version 6.6, Tecan, Männedorf, Switzerland.

**References**


Topical Herbal Application in the Management of Atopic Dermatitis: A Review of Animal Studies

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Herbs are widely used in the treatment of atopic dermatitis (AD) in Eastern Asian countries, and certain herbs regarded have anti-inflammatory properties that can help with AD. With the goal of developing a topical herbal agent for AD, we conducted a systematic review of in vivo studies of AD-like skin models for screening potential herbs. Searches were conducted from PubMed and EMBASE. After all, 22 studies were included for this review. We judged most of the domains of all studies to be at unclear risk of bias. Among 22 included studies, 21 herbs have been reported to reduce AD-like skin lesions in mouse models by suppressing Th2 cell response. Our findings may offer potential herbs for the topical application treatment of AD.

1. Introduction

There are many chemical substances that have been derived from plants for use as drugs, and these include some of the most utilized drugs such as aspirin, atropine, digoxin, ephedrine, morphine, quinine, and taxol. The latest version of the Dictionary of Natural Products (DNP; http://dnp.chem-netbase.com/) has just over 260,000 entries. Over the past decades, natural sauces have only taken a secondary role in drug discovery and development after the advent of molecular biology and combinatorial chemistry. However, as a basis for drug development, a new interest in the role of natural sauces has been concentrated, because various “-omics” technologies now allow scientists to detail the exact biological effects of natural sauces [1].

Atopic dermatitis (AD) is a chronic inflammatory skin disease with an increasing prevalence in industrialized countries. AD is characterized by pruritus; eczematous lesions accompanied by excessive infiltration of inflammatory cells such as lymphocytes, macrophages, and granulated mast cells in the skin lesions; eosinophilia in the peripheral blood; and high levels of serum immunoglobulin IgE. Although the pathogenesis of AD has not yet been fully understood, genetic, environmental, pharmacological, psychological, immunological, and skin barrier dysfunction factors are believed to contribute to the underlying pathogenic mechanisms [2–4].

Topical steroids are commonly used to treat moderate-to-severe AD, but long-term use of steroids at high concentrations is associated with a number of side effects [5]. Among various natural sources such as plants, animals, or microorganisms, herbs are widely used in the treatment of atopic dermatitis (AD) in Eastern Asian countries, and certain herbs regarded have anti-inflammatory properties that can help with AD. Our interest is to develop a safe and curative herb derived agent for AD using medical knowledge and clinical experience of herbal medicine combining with molecular biology and combinatorial chemistry technologies.
Recently, since Sandercock and Roberts drew attention to the need for more animal studies before beginning studies in human patients [6], there has been an increasing interest in the systematic reviews of research involving animals. Systematic reviews can aid in the development of more effective therapeutic agents for AD by extrapolating the results of animal studies to humans [7]. We performed a systematic review with this goal in mind and our objectives were (i) to screen topically applicable herbs for AD, (ii) to suggest potential mechanisms of action of topical herbal application in animal models of AD, and (iii) to ascertain the conditions of animal experiments used in the studies.

2. Methods

2.1. Criteria for Considering Studies for This Review

2.1.1. Inclusion Criteria

(i) Studies on the use of topical herbs for AD in animal models
(ii) Published between 2009 and 2013
(iii) Full text available
(iv) Article in English.

2.1.2. Exclusion Criteria

(i) Not related to AD or allergic dermatitis
(ii) Not an animal study
(iii) Animal cell studies
(iv) Not an investigational study of herbs
(v) Not an investigational study of herbs alone
(vi) Studies investigating compounds isolated from herbs
(vii) Use of fermented herbs by *Lactobacillus plantarum* and so forth
(viii) Studies investigating oils from herbs
(ix) Pharmacocupuncture
(x) Preexisting herbal drugs
(xi) Anal, intraperitoneal, or oral administration of herbs
(xii) Herbal mixtures
(xiii) Biomarkers not used as outcome measurements.

2.2. Search Methods for Identification of Studies

2.2.1. Data Sources and Searches. Literature searches were performed using PubMed and EMBASE databases. Search terms contained three components: (A) intervention/exposure, (B) disease of interest, and (C) animal species, with adjustments made for the different databases. Herbs were defined as plants, part of plants, or plant extracts that are used for medical purposes. Since the administration method of the herbs, as well as the outcome measures, was commonly described in the main article and rarely indexed in many papers, we excluded the administration method and outcome measures in the search strategy. For identification of MeSH terms, we used the PubMed thesaurus and the MeSH database, while we used EMTREE terms for searches using EMBASE. To identify all animal studies in PubMed, we used the "Animal search filter" that Hooijmans et al. [8] designed, while we used the filters in EMBASE. The full lists of search terms are presented in Tables 1 and 2.

2.2.2. Selection of Studies. Two authors (Yun and Kim) independently conducted the database searches. Duplicate articles were removed by the first author (Yun). Moreover, the references lists of review articles on relevant topics were manually searched by the two authors. For identifying eligibility of each study, the two authors read all potentially relevant articles. Disagreements were resolved by discussions with the corresponding author (Choi and Ko).

3. Results

3.1. Identification of Studies. After adding the search results from PubMed (n = 165) and EMBASE (n = 33), duplicate articles (n = 24) were removed. References lists in review articles (n = 8) were searched but did not result in any
articles being retrieved. From the potentially relevant articles \( (n = 166) \), we excluded 144 articles based on the predefined exclusion criteria, resulting in a total of 22 studies being included in this review (Figure 1).

3.2. Risk of Bias. Figure 2 shows the study quality checklist items reported for each included study, including random allocation to treatment groups \( (n = 8, 36.4\%) \), compliance with animal welfare regulations \( (n = 22, 100\%) \), and statements of a potential conflict of interest \( (n = 16, 72.7\%) \). None of the studies reported allocation concealment, examiner blinding, sample size calculation, and if results were based on analysis of the intent-to-treat population.

3.3. Basic Characteristics and Investigated Herbs in the Included Studies. Twenty-one studies were conducted in Korea and one was conducted in Japan. In two studies, herbs of the genus *Chrysanthemum* were investigated. Otherwise, there were no studies investigating the same herb (Table 4). Herbal extracts were prepared using ethanol, water, methanol, butanol, chloroform, 1,3-butylene glycol, or indirect heat.

3.4. Animal Models Used in the Included Studies. All studies used mice to investigate topical herbal application in an *in vivo* setting. The NC/Nga mouse \( (n = 16) \) was the most frequently used mouse model in these studies, followed by BALB/c \( (n = 4) \), C57BL/6 \( (n = 1) \), and hairless mice \( (n = 1) \).

The methods used for induction of AD-like skin lesions varied depending on the study. Repeated cutaneous application of chemical allergens and house dust mite allergens was used in 15 and 10 studies, respectively. Skin injury by stripping using surgical tape was used in 2 studies. For barrier disruption, sodium dodecyl sulfate (SDS) was applied to the lesions in 8 studies. Dorsal skin, ears, or a combination of dorsal skin and ears was used in most of the studies (Table 3).

3.5. Main Outcomes Investigated, Results, and Suggested Mechanisms of Action. In most of the studies, clinical symptoms, serum IgE levels, and Th1- and/or Th2-related cytokines and/or chemokines were assessed as outcome measurements (Table 4). The clinical severity of dermatitis was scored, and severity was found to have decreased after topical herbal application in 15 studies. Epidermal, dermal, or ear thickness was measured and was found to be decreased in 13 studies, indicating a decrease in the severity of the inflammatory process. Frequency of scratching was measured by counting scratching episodes in 5 studies, either directly or by reviewing videos of the animal. After topical herbal application, the frequency of scratching was decreased in all 5 studies.

Elevated serum IgE levels are important characteristics of AD. Serum or plasma IgE levels were measured in 21 studies, and, in 20 of these, serum or plasma IgE levels were decreased after the topical herbal application. However, in one study [9], neither topical *Rehmannia glutinosa* extract nor tacrolimus reduced the increased serum IgE levels after
4 Mediators of Inflammation

Statement of a potential conflict of interest

Intention-to-treat concept respected

Sample size calculation

Examiner blinding

Allocation concealment

Compliance with animal welfare regulations

Percentage of studies (%)

Yes (low risk of bias)

Unclear

No (high risk of bias)

Percentage of studies (%)

Random allocation to group

Allocation concealment

Examiner blinding

Allocation concealment

Compliance with animal welfare regulations

Sample size calculation

Intention-to-treat concept respected

Figure 2: Risk of bias in the studies.

Table 3: Basic characteristics of the animal models used in the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sex/model species</th>
<th>Induction of AD-like skin</th>
<th>Barrier disruption</th>
<th>Investigation site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qi et al. [27]</td>
<td>F/haired mouse</td>
<td>DNBC</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Lee et al. [14]</td>
<td>M/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Choi et al. [15]</td>
<td>F/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Sohn et al. [28]</td>
<td>M/BALB/c mouse</td>
<td>DNBC, SDS</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Wu et al. [29]</td>
<td>M/NC/Nga mouse</td>
<td>DNBC</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Yang et al. [16]</td>
<td>F/NC/Nga mouse</td>
<td>DNBC</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Sung et al. [30]</td>
<td>M/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Nam et al. [31]</td>
<td>M/C57BL/6 mouse</td>
<td>DNBC, SDS</td>
<td>N/A</td>
<td>Abdominal skin, ears</td>
</tr>
<tr>
<td>Choi et al. [24]</td>
<td>M/BALB/c mouse</td>
<td>DNBC, DfE, and skin injury</td>
<td>N/A</td>
<td>Ears</td>
</tr>
<tr>
<td>Sung et al. [9]</td>
<td>M/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Lee et al. [22]</td>
<td>M/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Ngatu et al. [17]</td>
<td>M/NC/Nga mouse</td>
<td>DNCB, TNCB</td>
<td>N/A</td>
<td>Abdominal skin, ears</td>
</tr>
<tr>
<td>Yang et al. [18]</td>
<td>F/NC/Nga mouse</td>
<td>DNBC, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Hwang et al. [25]</td>
<td>F/BALB/c mouse</td>
<td>DNBC, DfE, and skin injury</td>
<td>N/A</td>
<td>Ears</td>
</tr>
<tr>
<td>Choi et al. [32]</td>
<td>M/NC/Nga mouse</td>
<td>DNBC</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Sung et al. [33]</td>
<td>M/NC/Nga mouse</td>
<td>DNBC and DfE</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Kang and Shin [34]</td>
<td>M/NC/Nga mouse</td>
<td>DNCB</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Sung et al. [35]</td>
<td>M/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Park et al. [10]</td>
<td>M/NC/Nga mouse</td>
<td>DNBC</td>
<td>N/A</td>
<td>Dorsal skin, right ear</td>
</tr>
<tr>
<td>Yang et al. [36]</td>
<td>M/NC/Nga mouse</td>
<td>DfE, SDS</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
<tr>
<td>Kim et al. [37]</td>
<td>F/BALB/c mouse</td>
<td>DNBC, SDS</td>
<td>N/A</td>
<td>Dorsal skin</td>
</tr>
<tr>
<td>Choi et al. [38]</td>
<td>M/NC/Nga mouse</td>
<td>DNBC</td>
<td>N/A</td>
<td>Dorsal skin, ears</td>
</tr>
</tbody>
</table>

DNCB: 1-chloro-2,4-dinitrobenzene, DfE: D. farinae extract, DNFB: 2,4-dinitrofluorobenzene, TNCB: 2,4,6-trinitrochlorobenzene, N/A: not applicable, SDS: sodium dodecyl sulfate, M: male, and F: female.

allergen sensitization, although they both suppressed the expression of interleukin-4 (IL-4) mRNA in the ear lesions. Antigen-specific IgE levels were measured in two studies, both of which used house dust mite allergen and DNCB to induce AD-like skin lesions.

Most of the included studies investigated the Th2-response suppressing effects and/or Th1-response modulating effects upon topical herbal application in the AD-like mouse models. In 14 studies, only Th2-related biomarkers were measured, while both Th1- and Th2-related biomarkers were measured in nine studies. In all 21 studies that measured Th2 responses, topical herbal application resulted in decrease of Th2-related cytokines, chemokines, proinflammatory factors, and adhesion molecules. Conversely, among the eight studies that measured interferon-γ (IFN-γ), topical herbal application resulted in increased IFN-γ levels in two studies, decreased levels in five studies, and no induced changes in one study. Interestingly, in one study [10], *Chrysanthemum indicum* L. decreased both Th1 (IFN-γ) and Th2 cytokines (IL-4 and IL-13); however, the ratio of Th1 to Th2 cytokines was increased by herbal application.

4. Discussion

Herbal medicine is the use of medicinal plants for prevention and treatment of disease. Herbs and their derivatives have been, and continue to be, rich sources for drug discovery. Recently, results from several studies have indicated that
### Table 4: Investigated herbs, results, and suggested mechanisms of action in the included studies.

<table>
<thead>
<tr>
<th>Ref. number</th>
<th>Herb</th>
<th>Outcomes and results</th>
<th>Suggested mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14]</td>
<td><em>Bambusae caulis</em></td>
<td>TEWL↓, serum IgE↓, eosinophil↓, spleen IFN-γ↑, TNF-α↓, IL-4↓, IL-13↓</td>
<td>Suppression of Th2 response and promotion of Th1 response</td>
</tr>
<tr>
<td>[15]</td>
<td><em>Broussonetia kazinoki</em></td>
<td>Plasma IgE↓, IL-4↓, skin mast cell↓</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[28]</td>
<td><em>Alnus japonica</em></td>
<td>Clinical score↑, serum IgE↑, histamine↓, skin IL-4↑, IL-5↑, IL-13↑, iNOS↑, COX-2↓</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[29]</td>
<td>Korean red ginseng</td>
<td>Scratching↑, serum IgE↑, IL-4↑, IL-10↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[16]</td>
<td><em>Cordyceps bassiana</em></td>
<td>Clinical score↑, epidermal thickness↑, serum IgE↑, skin IFN-γ↓, IL-4↓, mast cell↓</td>
<td>Suppression of both Th1 and Th2 responses</td>
</tr>
<tr>
<td>[30]</td>
<td><em>Chelidonium majus</em></td>
<td>Clinical score↑, ear thickness↑, scratching↑, serum IgE↑, IL-4↑, TNF-α↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[31]</td>
<td><em>Cinnamomum cassia</em></td>
<td>Serum IgE↑, histamine↑, TNF-α↑, skin IL-4↑, TNF-α↑, TARC↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[24]</td>
<td><em>Terminalia chebula Retzius</em></td>
<td>Ear thickness↓, skin inflammatory cells↓, eosinophils↓, ear IL-3↓, T-bet positive cell↑, MMP-9↑</td>
<td>Suppression of Th2 response and promotion of Th1 response</td>
</tr>
<tr>
<td>[9]</td>
<td><em>Lindera obtusiloba</em></td>
<td>Ear thickness↓, serum IgE↓, DfE specific IgE↑, histamine↑, ear mast cell↑, IL-4↑, IL-13↑, IL-3↑, TNF-α↓</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[22]</td>
<td><em>Rehmannia glutinosa</em></td>
<td>Clinical score↑, ear thickness↑, serum IgE↑, histamine↑, ear IL-4↑, TNF-α↑, TARC↑, MDC↑, RANTES↑, ICAM-1↑, VCAM-1↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[17]</td>
<td><em>Angelicae Dahuricae Radix</em></td>
<td>Clinical score↑, plasma IgE↑, histamine↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[18]</td>
<td><em>Vernonia amygdalina</em></td>
<td>Clinical score↑, ear thickness↑, scratching↑, serum IgE↑, IL-4↑, IL-5↑, MCP-1↑, eotaxin↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[25]</td>
<td><em>Chrysanthemum boreale Makino</em></td>
<td>Clinical score↑, ear thickness↑, scratching↑, serum IgE↑, TNF-α↑, IL-4↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[32]</td>
<td>Mycelium of <em>Phellinus linteus</em></td>
<td>Clinical score↑, ear thickness↑, serum IgE↑, DfE specific IgE↑, total IgG—ear IL-12↓, IFN-γ↓, IL-4↓, IL-5↑, IL-10↑, IL-13↑, TNF-α↓, CCL4↑, CCL22↑, CCL17—CCL20—eotaxin—IL-2↓</td>
<td>Suppression of both Th1 and Th2 responses</td>
</tr>
<tr>
<td>[33]</td>
<td><em>Psidium guajava</em></td>
<td>Clinical score↑, serum IgE↑, TARC↑, IL-10↑, ear IFN-γ↑, TNF-α↑, IL-4↑, IL-5↑, IL-13↑</td>
<td>Suppression of both Th1 and Th2 responses and upregulation of IL-10</td>
</tr>
<tr>
<td>[34]</td>
<td><em>Drynaria fortunei</em></td>
<td>Clinical score↑, ear thickness↑, serum IgE↑, IgG1↑, IgG2a—IL-4↑, IL-6↑, TNF-α↑, ear IFN-γ—IL-4↑, TNF-α↑, IL-6↑, IFN-γ—IL-4↑, TNF-α↑</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[35]</td>
<td><em>Schisandra chinensis</em></td>
<td>Clinical score↑, scratching↑, serum IgE↑, IgM↑, histamine↑, skin histamine receptors↑, spleen IL-4↑, IL-5↑, IL-13↑, FceRIβ↓</td>
<td>Suppression of Th2 response</td>
</tr>
<tr>
<td>[10]</td>
<td><em>Illicium verum</em></td>
<td>Clinical score↑, ear thickness↑, serum IgE↑, histamine↑, IL-6↑, IFN-γ↑, IL-10↑, IL-4↑, IL-5↑, IFN-γ↑, TNF-α↑, IFN-γ↑, IL-4↑, IL-13↑, TNF-α↑, IFN-γ↑, IL-4↑, IL-13↑, IL-4:IFN-γ ratio↑</td>
<td>Suppression of both Th1 and Th2 responses and promotion of Th1/Th2 cell responses</td>
</tr>
<tr>
<td>[36]</td>
<td><em>Chrysanthemum indicum L.</em></td>
<td>Clinical score↑, ear thickness↑, serum IgE↑, IgG1↑, IFN-γ↑, IL-4↑, skin eosinophil↑, mast cell↑, IFN-γ↓, IL-4↓, IL-13↓, IL-4:IFN-γ ratio↓</td>
<td>Suppression of both Th1 and Th2 responses and balancing of Th1/Th2 cell responses</td>
</tr>
</tbody>
</table>
patients with AD may benefit from herbal medicines [11–13]. Certain herbs are regarded to have anti-inflammatory properties that can reduce the symptoms of AD. In Asian herbal medicine, herbs are categorized according to their functions. One such group of categorized herbs is named the clear heat drug group (清熱藥), and these herbs can be used for treating fever, infectious disease, and inflammatory conditions. Among the included 22 studies, seven studies [9, 10, 14–18] investigated herbs belonging to the clear heat drug group. Among these seven studies, two studies investigated herbs of the genus Chrysanthemum.

Since multiple genetic and environmental factors may underlie AD, the notion of developing a single comprehensive animal model is unrealistic [19]. Since the description of the NC/Nga mouse as the first spontaneously occurring model of AD in 1997 [20], a number of mouse models have been developed. They can be classified into three groups: (1) models induced by epicutaneous application of sensitizers, (2) mice that spontaneously develop AD-like skin lesions, and (3) transgenic mice that either overexpress or lack selective molecules [21].

NC/Nga mice were used in 16 of the analyzed studies. These mice are free of dermatitis in pathogen-free conditions but develop a spontaneous AD-like eruption when conventionally housed, and they have historically been viewed as one of the best animal models for assessing this condition [19]. In other studies, models induced by epicutaneous application of sensitizers were used. However, none of the included studies used genetically engineered mouse models.

In most of the included studies, clinical symptoms, serum IgE levels, and Th1- and/or Th2-related cytokines and/or chemokines were measured as outcome measurements. Based on the decreased clinical scores, ear or epidermal thickness, scratching behaviors, and histological inflam-mations after herbal application, it can be hypothesized that topical herbal application has anti-inflammatory effects. However, we could not conduct a meta-analysis to integrate quantitative analyses, since the studies included in our study all investigated different types of herbs.

Elevated serum IgE levels are an important feature of AD. Several studies have demonstrated that serum IgE levels are elevated in patients with AD; furthermore, serum IgE levels have been shown to be elevated in NC/Nga mice with AD-like skin lesions [20]. Among the 22 included studies, serum or plasma IgE levels were measured in 21 studies, and, in 20 studies, serum or plasma IgE levels were decreased after herbal treatment. However, in one study [9], neither topical application of Rehmannia glutinosa extract nor tacrolimus reduced the increased serum IgE levels after allergen sensitization, although they both suppressed the expression of IL-4 mRNA in the ear lesions and serum. The authors discussed two possible reasons for this observation: (i) that locally expressed IL-4 in the ear lesions did not contribute to systemic IgE production or (ii) that, in a short-term study with topical herbal application, effects on serum IgE may not be observed [22]. However, we noted that the authors did not measure the levels of allergen-specific IgE, and, in general, total IgE concentrations are a relatively crude method of detecting allergic disorders, since normal values do not exclude the presence of allergic disease, particularly to a single allergen, and since elevated levels of total IgE can be found in many patients with no evidence of allergy [23].

Conversely, both total and allergen-specific IgE levels were measured and found to be decreased after topical application of water-soluble extract of Lindera obtusiloba and Phellinus linteus in two studies, which used both house dust mite allergens and DNCB to induce AD-like skin lesions [24, 25]. Furthermore, water-soluble extract of P. linteus did not affect the total IgG levels, and it was found to be more potent than ceramide in reducing mite-specific IgE levels. These data suggest that certain herbs can suppress allergic responses in an allergen-specific manner. Nevertheless, immunological and clinical parameters for the assessment of antigen-specific immune responses were not measured in most of the studies.

Both Th1- and Th2-type cytokines contribute to the pathogenesis of AD, and their expression patterns are not mutually exclusive [2]. Th2 cytokines such as IL-4, IL-5, and IL-13 play key roles in the hyperproduction of IgE, whereas Th1 cytokines, especially IFN-γ, are strong inhibitors of IgE synthesis, Th2 cell proliferation, and IL-4 receptor expression on T-cells [26]. Development of AD is induced by Th2-type responses, while the chronic inflammatory responses are dominantly mediated by Th1-type reactions.
Among the 22 included studies, 21 herbs were reported to reduce AD-like skin lesions in mouse models by suppressing Th2 cell response with or without balancing of the Th1/Th2 cell response. In eight studies, Th1 cytokines were measured and showed different results. Based on this review, it seems that investigators mainly assess Th1- and Th2-related mechanisms to explain the anti-inflammatory effects of herbs.

In the present study, out of 166 potential studies, we identified 22 studies that met all the selection criteria. It showed that there is room for methodological improvement in the studies. Most studies were at an unclear risk of bias; therefore, it was not possible to accurately determine the degree of bias of the described treatment effects. Further research should be conducted with well-designed methodological research protocols using random allocation, allocation concealment, assessor blindness, sample sizes calculation, and intention-to-treat-respected analyses.

Twenty-one studies were conducted in Korea and one was conducted in Japan. For identifying all potentially relevant researches, the search strategy in the present study included American (PubMed) and European (EMBASE) databases. No attempts were made to retrieve articles from Chinese, Korean, or Japanese databases. Also, non-English articles from PubMed and EMBASE were not included. Because of these, studies may have been excluded. However, when we made our search strategy, we did not expect different results among the countries (Korea, Japan, and China) which conduct a relatively large number of studies on herbs. We assume that the great interest in topical use of herbs and the large number of research and development (R&D) projects on herbs in Korea have been important factors of the results.

In summary, we have reviewed studies investigating topical herbal application in AD-like animal models. For all studies, we judged most domains to be at unclear risk of bias. Herbs of the genus Chrysanthemum were used in two studies, and seven studies investigated herbs of the clear heat drug group. Among the AD-like animal models, NC/Nga and BALB/c mice treated with chemical haptens, DNCB, DNFB, or TNCB were used in most of the studies. Clinical symptoms, serum IgE levels, and Th1- and/or Th2-related cytokines and/or chemokines were assessed as outcome measurements. Among the 22 included studies, 21 herbs were reported to reduce AD-like skin lesions in mouse models by suppressing Th2 cell responses. By summarizing the results from the published literature, we hope that this study might aid in finding a potential herbal therapeutic agent for the treatment of AD. The limitation of this study was that a meta-analysis was not conducted because of the variety of investigated herbs included in the studies. Nevertheless, this review may assist in identifying directions for further researches endeavors.

**Conflict of Interests**

The authors state that there is no conflict of interests. No financial support or benefits were received by the authors. The authors have no commercial associations or financial relationships to disclose.

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**References**


Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs after an *Escherichia coli* infection

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ABSTRACT: This study was conducted to characterize the effects of infection with a pathogenic F-18 *Escherichia coli* and 3 different plant extracts on gene expression of ileal mucosa in weaned pigs. Weaned pigs (total = 64, 6.3 ± 0.2 kg BW, and 21-d old) were housed in individual pens for 15 d, 4 d before and 11 d after the first inoculation (d 0). Treatments were in a 2 × 4 factorial arrangement: with or without an F-18 *E. coli* challenge and 4 diets (a nursery basal, control diet [CON], 10 ppm of capsicum oleoresin [CAP], garlic botanical [GAR], or turmeric oleoresin [TUR]). Results reported elsewhere showed that the plant extracts reduced diarrhea in challenged pigs. Total RNA (4 pigs/treatment) was extracted from ileal mucosa of pigs at d 5 post inoculation. Double-stranded cDNA was amplified, labeled, and further hybridized to the microarray, and data were analyzed in R. Differential gene expression was tested by fitting a mixed linear model in a 2 × 4 factorial ANOVA. Bioinformatics analysis was conducted by DAVID Bioinformatics Resources 6.7 (DAVID; National Institute of Allergy and Infectious Diseases [NIAID, NIH], http://david.abcc.ncifcrf.gov). The *E. coli* infection altered (*P* < 0.05) the expression of 240 genes in pigs fed the CON (148 up- and 92 down-regulated). Compared with the infected CON, feeding CAP, GAR, or TUR altered (*P* < 0.05) the expression of 52 genes (18 up, 34 down), 117 genes (34 up- and 83 down-regulated), or 84 genes (16 up- and 68 down-regulated), respectively, often counteracting the effects of *E. coli*. The *E. coli* infection up-regulated (*P* < 0.05) the expression of genes related to the activation of immune response and complement and coagulation cascades, but down-regulated (*P* < 0.05) the expression of genes involved in protein synthesis and accumulation. Compared with the CON, feeding CAP and GAR increased (*P* < 0.05) the expression of genes related to integrity of membranes in infected pigs, indicating enhanced gut mucosa health. Moreover, feeding all 3 plant extracts reduced (*P* < 0.05) the expression of genes associated with antigen presentation or other biological processes of immune responses, indicating they attenuated overstimulation of immune responses caused by *E. coli*. These findings may explain why diarrhea was reduced and clinical immune responses were ameliorated in infected pigs fed plant extracts. In conclusion, plant extracts altered the expression of genes in ileal mucosa of *E. coli*-infected pigs, perhaps leading to the reduction in diarrhea reported previously.

Key words: *Escherichia coli*, gene expression, immunomodulation, pigs, plant extracts

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INTRODUCTION

*Escherichia coli* postweaning diarrhea is one of the major causes of mortality in weaned pigs (NAHMS, USDA, 2008). The *E. coli* infection affects not only gut physiology but also immune responses of pigs. The toxins secreted by *E. coli* can stimulate the small intestine to increase water and electrolyte secretion and decrease fluid absorption, resulting in hypersecretory diarrhea (Gyles, 1993; Nataro and Kaper, 1998). In addition, lipopolysaccharide (LPS), the major component...
of the outer membrane of E. coli, can activate the innate immune system of pigs, triggering increased production of proinflammatory cytokines from antigen-presenting cells (Webel et al., 1997; Wright et al., 2000).

Feeding low levels of capsicum oleoresin (CAP), garlic botanical (GAR), or turmeric oleoresin (TUR) reduced diarrhea and mitigated disruption of intestinal morphology of weaned pigs caused by E. coli infection. Feeding these plant extracts also reduced serum TNF-α and haptoglobin concentrations and the number of white blood cells and lymphocytes in E. coli-infected pigs (Liu et al., 2013). These results indicated benefits of plant extracts in alleviating the negative effects of pathogenic infection and maintenance of normal intestinal integrity and function. The 3 plant extracts tested showed different effects, indicating they may work through different mechanisms.

The mucosal layer of the intestine is in direct contact with luminal contents, and contributes to the immune defense against pathogens (Schenk and Mueller, 2008). Therefore, investigating the alteration of ileal mucosa function is important to help understand the underlying mechanisms, by which the plant extracts protect against the consequences of the E. coli infection. The objective of this study was to characterize gene expression of ileal mucosa of pigs as affected by experimental infection with a pathogenic E. coli and by plant extracts using the porcine genome array followed by quantitative real-time PCR (qRT-PCR) validation.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. The experiment was conducted in the disease containment chambers of the Edward R. Madigan Laboratory at the University of Illinois at Urbana-Champaign.

Animals, Housing, Experimental Design, and Diet

A total of 64 weaned piglets with the same number of gilts and barrows (G-Performer boars × Fertilium 25 sows; Genetipore Inc., Alexandria, MN) averaging 6.3 kg initial BW were selected from 12 sows at the Swine Research Center of the University of Illinois at Urbana-Champaign. The sows and piglets used in this experiment did not receive vaccines against E. coli, antibiotic injections or antibiotics in creep feed. After weaning, all pigs were transferred to the disease containment chambers. They were blocked by weight within sex and randomly assigned to treatments with the restriction that litters were balanced across treatments to the extent possible. The pig was the experimental unit. Pigs were housed in individual pens for 9 d (4 d before and 5 d after the first E. coli challenge [d 0]). There were 2 suites of 8 chambers, and each suite was used for either E. coli-challenged or unchallenged pigs. There were a total of 64 individual pens, 4 in each of 16 chambers, and each diet was represented in each chamber. The piglets had ad libitum access to feed and water.

The treatments were in a 2 × 4 factorial arrangement (with or without E. coli challenge and 4 different dietary treatments) with 8 replicates per treatment. In the E. coli challenge group, all pigs were inoculated orally with 3 mL F-18 E. coli/d for 3 consecutive days from d 0 post infection (PI). The E. coli strain was derived from a field disease outbreak by University of Illinois Veterinary Diagnostic Lab (isolate number U.IL-VDL # 05–27242). The isolate expressed heat-labile (LT), heat-stable (STb) and Shiga-like (SLT-2) toxins. The inoculum provided at 10^10 cfu per 3 mL dose in PBS, a dose that previously caused mild diarrhea (Song et al., 2012). In the unchallenged group, pigs were inoculated with 3 mL PBS per day as the sham control (Sham) for the 3 consecutive days. The 4 dietary treatments were the complex nursery basal diet (CON), and the addition of 10 ppm CAP, 10 ppm GAR, or 10 ppm TUR (Pancosma S. A., Geneva, Switzerland) to the CON, respectively. Capsicum and turmeric are extracted oleoresins, which were standardized to 6% capsaicin and dihydrocapsaicin, and 98% curcuminoids, respectively. Garlic botanical is an extract from garlic, standardized to 40% propyl thiosulphonates. The CON diet was formulated to meet or exceed the NRC (1998) estimates of nutrient requirements of weaned pigs (Table 1). The same batches of ingredients were used for the preparation of all the diets. For mixing the 3 plant-extract diets, 1 of plant extracts was first premixed with 0.8 kg of soybean oil, and then this mixture was remixed with a small amount of corn (10 kg). Finally, the premix of plant extract, soybean oil, and corn was added to and mixed with the remaining ingredients. The same diets were fed throughout the experiment.

Sample Collection

After inoculation, all pigs in the E. coli challenged groups were verified to be successfully infected by F18 E. coli by analyzing β-hemolytic coliforms in feces (Song et al., 2012). As shown previously (Song et al., 2012) and duplicated in the current study in which the clinical signs of pigs used in this experiment were reported (Liu et al., 2013), d 5 PI is the peak of E. coli disease with the greatest diarrhea score and greatest proportion of β-hemolytic coliforms in the feces. Therefore, one-half of the pigs (4 pigs from each treatment, 2 males and 2 females) were euthanized on d 5 PI. Before being euthanized, pigs were anesthetized by intramuscular injection of a 1-mL combination of telazol, ketamine, and xylazine (2:1:1) per 23 kg BW. The final mixture contained...
100 mg telazol, 50 mg ketamine, and 50 mg xylazine in 1 mL (Fort Dodge Animal Health, Fort Dodge, IA). After anesthesia, pigs were euthanized by intracardiac injection with 78 mg sodium pentobarbital (Sleepaway, Vortech Pharmaceuticals, Ltd., Dearborn, MI) per kilogram of BW. The 5-cm ileal samples (collected within 10 cm of the ileocecal junction) were cut longitudinally in 10 cm of the ileocecal junction) were cut longitudinally and washed with ice-cold PBS. The specific location for ileal sample collection were based on the presence of a continuous aggregate of lymphoid tissue as a bulky area of granular appearance, which is involved in the gastrointestinal mucosal immunity (Solano-Aguilar et al., 2000). The mucosal layer of the entire ileal sample was carefully removed by scraping with a surgical scalpel and immediately stored in liquid N for further analysis.

**Total RNA Extraction and Gene Expression by Microarrays**

Total RNA (4 pigs/treatment) from ileal mucosa isolated at d 5 PI was extracted using a kit according to the manufacturer’s instructions (PureLink RNA Mini Kit; Invitrogen, Carlsbad, CA) and a spectrophotometer (ND-1000 Nanodrop; Thermo Scientific, Wilmington, DE), respectively. All samples used for further analysis had a ratio of optical density read at 260 and 280 nm from 1.9 to 2.1, a ratio of optical density read at 260 and 230 nm of >1.8, and an RNA integrity number of ≥7.7. Double-stranded cDNA was first synthesized and employed as a template for in vitro amplification and labeling (GeneChip Expression 3'-Amplification IVT Labelling Kit; Affymetrix Inc., Santa Clara, CA). Then, cDNA was used to synthesize cRNA, which was hydrolyzed to produce fragmented cRNA in the 35 to 200 nucleotide size range for proper hybridization. The fragmented cRNA was labeled and further hybridized to the porcine genome array (Affymetrix GeneChip Porcine Genome Array; Affymetrix Inc.). Each array consisted of 23,937 probe sets to interrogate 23,256 transcripts in the porcine genome, which represents 20,201 genes. Thirty-two microarrays were probed at d 5 PI was used to evaluate the effects of infection and its interaction with diet and the treatment interventions. The limma model was fit and summarizes the multiple probes into a single probe set value using a median polish algorithm (Wu and Irizarry, 2005). Testing for differential gene expression was done by fitting a mixed linear model equivalent to a 2 × 4 factorial ANOVA using the limma package (Che et al., 2011), which uses an empirical Bayes correction that helps to improve detection power by borrowing information across genes (Smyth, 2004). The statistical model included effects of E. coli challenge, diet, and their interaction as fixed effects. Block was a random effect. The appropriate pairwise comparisons were fit as contrasts from the model. The following 4 comparisons were of interest: infected control (ICON) vs. infected CAP (ICAP) vs. ICON, infected GAR (IGAR) vs. ICON, and infected TUR (ITUR) vs. ICON. A total of 23,937 gene probe sets were included in the porcine array, but only 16,363 probe sets were detected in the ileal mucosa samples. The limma model was fit and P-values were calculated using all 16,363 probe sets on the array. The modulated genes were defined by 1.5-fold difference and a cutoff of P < 0.05 by parameter tests.

### Table 1. Ingredient composition of basal diet (as-fed basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground</td>
<td>41.54</td>
</tr>
<tr>
<td>Whey, dried</td>
<td>15.00</td>
</tr>
<tr>
<td>Soybean meal, dehulled</td>
<td>10.82</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>10.00</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.00</td>
</tr>
<tr>
<td>Soy protein concentrate</td>
<td>5.00</td>
</tr>
<tr>
<td>Poultry byproduct meal</td>
<td>4.27</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2.67</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>0.20</td>
</tr>
<tr>
<td>L-Lysine-HCl</td>
<td>0.05</td>
</tr>
<tr>
<td>DL-Met</td>
<td>0.05</td>
</tr>
<tr>
<td>L-Thr</td>
<td>0.03</td>
</tr>
<tr>
<td>L-Trp</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated energy and nutrients

| ME, kcal/kg                   | 3,480     |
| CP, %                         | 22.67     |
| Fat, %                        | 6.34      |
| Ca, %                         | 0.80      |
| P, %                          | 0.72      |
| Available P, %                | 0.49      |
| Lys, %                        | 1.50      |
| Lactose, %                    | 21.00     |

1Provided per kg of diet: 3,000 mg of NaCl; 100 mg of Zn from zinc oxide; 90 mg of Fe from ferrous sulfate; 20 mg of Mn from manganese oxide; 8 mg of Cu from copper sulfate; 0.35 mg of I from calcium iodide and 0.3 mg of Se from sodium selenite.

2Provided per kg of diet: 2,273 μg of retinyl acetate; 17 μg of cholecalciferol; 88 μg of DL-α-tocopheryl acetate; 4 mg of menadione from menadione sodium bisulfite complex; 33 mg of niacin; 24 mg of D-Ca-pantothenate; 9 mg of riboflavin; 35 μg of vitamin B₁₂; 324 mg of choline chloride.
Bioinformatics Analysis

A bioinformatics resources (DAVID Bioinformatics Resources 6.7; National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD; http://david.abcc.ncifcrf.gov) consists of an integrated biological knowledge base with analytic tools used to systemati-
cally extract biological meaning from large gene lists (Huang et al., 2009). In brief, the analysis of selected
genes in DAVID was as follows. First, all 16,363 probe
sets in the porcine genome array (Affymetrix GeneChip
Porcine Genome Array, Affymetrix Inc.) were submit-
ted to DAVID and 5,168 of them were mapped with
identified functions for 3,715 porcine genes. These
5,168 genes’ Entrez [National Center for Biotechnology
Information (NCBI), USHHS, Washington, DC] identi-
fications were uploaded as background for the analysis.
Second, the modulated genes with 1.5-fold difference
and a cutoff of $P < 0.05$ in each comparison were up-
loaded as the tested gene list. Third, the parameters and
subparameters of interest in this experiment were estab-
lished. In the present analysis, the main parameters in
DAVID included gene ontology and pathways. The sub-
parameter under gene ontology was gene ontology for
biological process, and the sub-parameter under path-
ways was Kyoto Encyclopedia of Genes and Genomes
(KEGG) pathways. Finally, the functional annotation
chart, which provided typical gene-term enrichment
analysis, was run. The expression analysis systematic
explorer (EASE) score, a modified Fisher Exact $P$-value,
was used to examine the significance of gene-term en-
richment with a modified Fisher’s exact test. The EASE
score $< 0.05$ was considered as significantly affected.

Quantitative Real-Time PCR

The same total RNA (4 pigs/treatment) from ileal mu-
cosa used to run the microarray was also employed for qRT-
PCR. First-strand cDNA was produced from 1 μg of total
RNA per sample (SuperScript III First-Strand Synthesis
SuperMix for qRT-PCR; Invitrogen) in a total volume of
20 μL. Total RNA was denatured at 65°C for 5 min and
immediately annealed on ice for at least 1 min. Then,
the reverse transcription reaction was performed at 50°C for 5 min,
followed by heat inactivation at 85°C for 5 min.

To verify the results from the microarray, quantitative
analysis of IL-1 β (IL1b), tumor necrosis factor α (TNFa),
toll-like receptor 4 (TLR4), lipopolysaccharide binding
protein (LBP), and myeloid differentiation primary re-
sponse gene 88 (MyD88) were assayed by qRT-PCR. In
addition, the expression levels of mucin 2 (MUC2), p65
nuclear factor kappa-light-chain-enhancer of activated B
cells (p65 NFkB), p38 mitogen-activated protein kinases
(p38 MAPK), cystic fibrosis transmembrane conductance
regulator (CFTR), and cyclooxygenase 2 (COX2) in ileal
mucosa were analyzed by qRT-PCR. Data normalization
was accomplished using β-actin as a housekeeping gene,
which has been validated as an effective internal control
for studying gene expression in porcine ileal mucosa (data
are not shown). Primers (Supplementary Table 1) were
designed based on published sequences in pigs using
the NCBI (USHHS) online primer design tool and pub-
lished literature, and synthesized commercially (Applied
Biosystems, Foster, CA). One hundred nanograms of total
RNA were assayed for each sample in triplicate. Each PCR
reaction consisted of 5 μL of mixture (SYBR Green PCR
Master Mix; Applied Biosystems, Foster, CA), 0.4 μL of
10 μM forward primer, 0.4 μL of 10 μM reverse primer, 0.2
μL of DNase/RNase free water, and 4 μL of diluted cDNA.
The qRT-PCR analysis was done (ABI PRISM 7900
Sequence Detection System; Applied Biosystems, Foster,
CA). Thermal cycling conditions were 50°C for 2 min and
95°C for 10 min, followed by 40 cycles with 15 sec at 95°C
and 1 min at 60°C. The dissociation cycle was 95°C for 15
s plus 65°C for 15 s. Standard curves were generated using
serial dilutions of pooled cDNA from all samples. The ar-
britary values were calculated based on the standard curve
and normalized using the housekeeping genes.

RESULTS

Gene Expression Profiles Induced by E. coli and Plant Extracts

The E. coli infection of pigs fed the CON diet al-
tered ($P < 0.05$) the expression of 418 genes in ileal
mucosa of pigs compared with the uninfected CON
(Table 2). The supplementation of the 3 plant extracts
displayed different effects on the gene expression in
ileal mucosa of E. coli-infected pigs. Compared with

| Table 2. Gene expression profiles induced by Escherichia coli infection and dietary supplementation of plant extracts to E. coli-infected pigs1 |
|---------------------------------|-----------------|-----------------|-----------------|
| Dietary supplement              | Comparison2     | Up-regulated    | Down-regulated  | Total |
| Escherichia coli                | ICON vs. CON    | 252             | 166             | 418   |
| Capsicum oleoresin, 10 mg/kg    | ICAP vs. ICON   | 35              | 39              | 74    |
| Garlic botanical, 10 mg/kg      | IGAR vs. ICON   | 53              | 150             | 203   |
| Turmeric oleoresin, 10 mg/kg    | ITUR vs. ICON   | 37              | 146             | 183   |

1The gene expression changed by fold-change cutoff of 1.5 and a $P$-value cutoff of 0.05. All data were analyzed by DAVID Bioinformatics Resources 6.7 (National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD).
2ICON = uninfected control diet–fed pigs; ICAP = infected capsicum oleo-
resin–fed pigs; ICON = infected control diet–fed pigs; IGAR = infected garlic
botanical–fed pigs; ITUR = infected turmeric oleoresin–fed pigs.
Differential Immune Gene Expression in Ileal Mucosa of Pigs

The modulated immune gene expression indicated that *E. coli* infection induced gut immune responses of pigs fed the CON diet (Fig. 1). The *E. coli* infection altered the expression level of genes related to LPS activation (increase in LBP and TLR2), and MyD88, cytokines (increase in IL16, IL7, IL15, and TNFA), decrease in IL10 and TGBF1), chemokines (increase in CCL28, CCL5 and IL8; decrease in CCL4 and CCL3L1), complement cascades (increased C7, A2M, CFB, and IL10, IL16, IL7R, IL7R, LBP, MHCII, TGBF1, and TNFA; Turmeric oleoresin: C5, C7, CASP3, CASP8, CCL3L1, CCL4, CFB, HSP90AA1, IFIT1, IFIT2, IFNGR1, IFN7, MHCII, and TNFA; Garlic botanical: C7, CASP3, CASP8, CCL3L1, CCL4, CFB, HSP90AA1, IFIT1, IFIT2, IL7, IL7R, LBP, TGBF1, and TNFA; Turmeric oleoresin: C5, C7, CASP3, CASP8, CCL3L1, CCL4, CFB, HSP90AA1, IFIT1, IFIT2, IL7, IL7R, LBP, TGBF1, and TNFA. See online version for figure in color.

![Diagram of immune gene expression](image-url)
CASP1, CASP3, and CASP8), and endoplasmic reticulum stress (increased CREB3L4).

Feeding each of the plant extracts had effects on expression of several genes that were counter to the effects of *E. coli*. Feeding CAP counteracted (*P* < 0.05) effects of *E. coli* on C7, CCL5, CCR3, IFIT1, IFIT2, IFNγR1, IRF7, MHCII, and TNFα. Feeding GAR counteracted (*P* < 0.05) the disease effects on C7, CASP3, CASP8, CCL3L1, CCL4, CFB, HSP90AA1, IFIT1, IFIT2, IL7, IRF7, LBP, TGFB1, and TNFα. Finally, feeding TUR reversed (*P* < 0.05) the effects of *E. coli* on expression of 24 genes, including C5, C7, CASP3, CASP8, CCL5, CCL28, CCR9, CXCR4, CFB, HSP90AA1, HSP90B1, IFNAR1, IFNGR1, IFIT1, IFIT2, IL8, IL10, IL16, IL17R, IRF7, LBP, MHCII, TGFB1, and TNFα, compared with *E. coli*-infected control.

### Biological Process Analysis

The altered genes were analyzed by the gene ontology biological process using DAVID and presented in Fig. 2 and Supplementary Tables 2 to 5. The *E. coli* infection up-regulated (EASE score < 0.05) the expression of genes related to the biological processes of immune response and glycosylation, but down-regulated (EASE score < 0.05) metabolism and intracellular signaling cascade biological processes (Fig. 2A). Compared with the infected CON, the supplementation of CAP up-regulated (EASE score < 0.05) gene expression integral to membranes and Na ion transport, but down-regulated (EASE score < 0.05) the expression of genes associated with 3 different biological processes: response to stimulus, immune response, and antigen processing and presentation (Fig. 2B). Feeding GAR up-regulated (EASE score < 0.05) the expression of genes related to metal ion transport, chemokine binding, and integral to membrane biological processes, but down-regulated (EASE score < 0.05) regulation of immune response in the ileal mucosa of *E. coli*-infected pigs (Fig. 2C). In addition, the addition of TUR up-regulated (EASE score < 0.05) gene expression related to carbohydrate catabolic process, but down-regulated (EASE score < 0.05) the expression levels of genes associated with plasma membrane component and antigen processing and presentation (Fig. 2D).

### The Kegg Pathway Analysis

The altered genes were analyzed by the Kegg Pathway using DAVID and presented in Fig. 3 and Supplementary Tables 6 to 9. Compared with the sham CON, *E. coli* infection up-regulated (EASE score < 0.05) genes involved in the following pathways, complement and coagulation cascades, amino sugar and nucleotide sugar metabolism, and metabolism of xenobiotics by cytochrome p450, but down-regulated (EASE score < 0.05) the expression of genes involved in the pathway of spliceosome (Fig. 3A). Compared with the infected CON, CAP up-regulated
(EASE score < 0.05) the expression of genes related to steroid hormone biosynthesis and metabolism of xenobiotics by the cytochrome p450 pathway, but down-regulated (EASE score < 0.05) the gene expression associated with the antigen processing and presentation pathway and peroxisome proliferator-activated receptor (PPAR) signaling pathway (Fig. 3B). The supplementation of GAR up-regulated (EASE score < 0.05) gene expression related to 4 different pathways: cytokine–cytokine receptor interaction, chemokine signaling pathway, natural killer cell mediated pathway, and T cell receptor signaling pathway; but down-regulated (EASE score < 0.05) gene expression related to the expression of genes involved in pyruvate metabolism (Fig. 3C). Moreover, the supplementation of TUR down-regulated (EASE score < 0.05) the expression of genes related to metabolism of xenobiotics by cytochrome p450, vial myocarditis, and focal adhesion pathways (Fig. 3D).

Quantitative Real-Time PCR

Five genes, IL1b, LBP, MyD88, TLR4, and TNFa were tested by qRT-PCR to verify the expression of genes detected by microarray and as shown in Table 3, the transcriptional changes in these genes as assessed by qRT-PCR showed similar patterns when compared with the original microarray data, although the magnitude of the responses of those genes varied from one method to another with the exception to the MyD88 responses.

Another 5 genes not included in the microarray, CFTR, COX2, MUC2, p38 MAPK, and p65 NFkB, were tested by qRT-PCR. The E. coli infection increased (P < 0.05) the expression of COX2 and p38 MAPK in the ileal mucosa of pigs, but feeding the plant extracts counteracted (P < 0.05) this effect. In addition, E. coli infection reduced (P < 0.05) the expression of p65 NFkB in the ileal mucosa of pigs. No effect of plant extracts on p65 NFkB was detected. Otherwise, feeding CAP increased (P < 0.05) the expression of CFTR and MUC2, whereas feeding TUR enhanced (P < 0.05) MUC2 gene expression in the ileal mucosa compared with the infected control.

DISCUSSION

General

The present results describe the impacts of infection with a pathogenic E. coli on gene expression in the ileal mucosa of the weaned pigs and indicate that feeding specific plant extracts to pigs challenged with E. coli changed the expression levels of genes related to the biological processes of membrane integrity and immune responses. Several of the changes in expression...
of key genes caused by plant extracts were counter to the changes caused by \textit{E. coli}, and may help explain the benefits of reduced diarrhea and improved gut morphology previously observed (Liu et al., 2013).

**Host Gene Response to \textit{E. coli} Infection**

The mucosal surface in the intestine has dual functions. The surface allows exchange of nutrients and ions across the intestinal epithelium and supports an immune defense against potentially harmful luminal antigens and microbes (Schenk and Mueller, 2008). Therefore, the delicate balance of intestinal immunity is important for animal health and growth. Pathogenic \textit{E. coli} and other pathogenic bacterial infections can induce a defect of intestinal mucosal immunity and enhance inflammation in animals and humans (Savkovic et al., 2003; Sansonetti, 1996; 2003; Zhou et al., 2003). Cyclooxygenase-2 is the inducible form of the prostaglandin synthetase enzymes that catalyzes the committed step in the prostaglandin production pathway (Dubois et al., 1998). The expression of COX-2 is increased by several different stimuli under inflammatory conditions, including proinflammatory cytokines such as TNFα (Akarasereenont et al., 1995; Cohen, 2001). In the present study, \textit{E. coli} infection increased the expression level of some genes related to the Kegg Pathway of complement and coagulation cascades, indicating increased immune responses in the intestinal mucosa of infected pigs. Otherwise, the increased IL-8 expression in the ileal mucosa indicated that another potent pro-inflammatory mediator from the flagella of \textit{E. coli}, flagellin, might be involved in the activation of intestinal immune responses.

Zhou et al. (2003) and Im et al. (2009) have reported flagellin induced the production of IL-8 in the epithelial cells. Interleukin-8 can facilitate the recruitment of inflammatory associated immune cells, such as polymorphonuclear leucocytes (Savkovic et al., 1996; 2003; Zhou et al., 2003). Cyclooxygenase-2 is the inducible form of the prostaglandin synthetase enzymes that catalyzes the committed step in the prostaglandin production pathway (Dubois et al., 1998). The expression of COX-2 is increased by several different stimuli under inflammatory conditions, including proinflammatory cytokines such as TNFα (Akarasereenont et al., 1995; Abdalla et al., 2005). The qRT-PCR revealed upregulation

### Table 3. Verification of gene expression in ileal mucosa by quantitative real-time PCR (qRT-PCR)$^{1,2}$

<table>
<thead>
<tr>
<th>Gene</th>
<th>ICON vs. CON</th>
<th>ICAP vs. ICON</th>
<th>IGAR vs. ICON</th>
<th>ITUR vs. ICON</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microarray qRT-PCR</td>
<td>Microarray qRT-PCR</td>
<td>Microarray qRT-PCR</td>
<td>Microarray qRT-PCR</td>
</tr>
<tr>
<td>$IL1B$</td>
<td>$-1.13$</td>
<td>$-3.64^*$</td>
<td>$1.03$</td>
<td>$2.69^*$</td>
</tr>
<tr>
<td>$LBP$</td>
<td>$4.39$</td>
<td>$1.68^*$</td>
<td>$-1.24$</td>
<td>$-1.63^*$</td>
</tr>
<tr>
<td>MyD88</td>
<td>$1.54$</td>
<td>$1.02$</td>
<td>$-1.97$</td>
<td>$-1.28^*$</td>
</tr>
<tr>
<td>TLR4</td>
<td>$1.23$</td>
<td>$1.21$</td>
<td>$-1.62$</td>
<td>$-1.52^*$</td>
</tr>
<tr>
<td>TNFa</td>
<td>$2.21$</td>
<td>$1.04$</td>
<td>$-1.89$</td>
<td>$-1.34$</td>
</tr>
<tr>
<td>CFTR</td>
<td>$-1.31$</td>
<td>$1.72^*$</td>
<td>$-1.24$</td>
<td>$-1.07$</td>
</tr>
<tr>
<td>COX2</td>
<td>$-5.37^*$</td>
<td>$-1.73^*$</td>
<td>$-1.62$</td>
<td>$-1.07$</td>
</tr>
<tr>
<td>MUC2</td>
<td>$-1.19$</td>
<td>$1.36^*$</td>
<td>$-1.62$</td>
<td>$-1.07$</td>
</tr>
<tr>
<td>p38 MAPK</td>
<td>$-1.47^*$</td>
<td>$-1.55^*$</td>
<td>$-1.78^*$</td>
<td>$-1.40^*$</td>
</tr>
<tr>
<td>p65 NFkB</td>
<td>$-1.19^*$</td>
<td>$1.07$</td>
<td>$-1.78^*$</td>
<td>$-1.40^*$</td>
</tr>
</tbody>
</table>

$^*$Different (P < 0.05) in qRT-PCR studies.

1 The total RNA samples (4 pigs/treatment) that were used to run the porcine microarray (Affymetrix Inc., Santa Clara, CA) were employed for qRT-PCR.

2 Negative value indicates reduction in gene expression. CON = uninfected control diet–fed pigs; ICAP = infected capsicum oleoresin–fed pigs; ICON = infected garlic botanical–fed pigs; IGAR = infected garlic botanical–fed pigs; ITUR = infected turmeric oleoresin–fed pigs.
of COX-2 genes, which is directly related to the intensity of organ inflammation (Poljakovic et al., 2001; Jana et al., 2009), providing further evidence of increased gut mucosa inflammation caused by E. coli. Therefore, the present results support the clinical responses of E. coli-infected pigs in our previous study, indicated by increased white blood cells and serum proinflammatory cytokines (Liu et al., 2013).

The present study indicated that E. coli infection reduced the expression levels of genes linked to several metabolic processes of pigs, including protein synthesis (Rps23, rps20, and rpl14), unwanted protein degradation (Uch11 and UBC1), fatty acid metabolism (Cpt1b and PLCD4), carbohydrate metabolism (PGAM2 and GAPDH), DNA repair (RAP80), and intracellular transcription factors (NOR-1 and OCT2). However, the infection increased the expression level of several genes related to amino sugar and nucleotide sugar metabolism. In addition, previous studies reported that the reduced growth caused by E. coli infection was related to suppressed protein synthesis and accumulation in the muscle tissues (Jepson et al., 1986; Tian and Baracos, 1989). Similarly, our clinical data showed E. coli infection reduced growth rate and feed efficiency (Liu et al., 2013), which may be related to the increased immune responses of pigs, decreased protein synthesis, or both.

**Capsicum oleoresin on E. coli Infection**

Capsicum oleoresin, obtained from peppers, contains capsaicin as the major active component involved in maintenance of mucosal integrity and protection against gastric mucosal injury induced by noxious agents (Abdel Salam et al., 1997, 1999, 2005). In the present study, the supplementation of CAP up-regulated the expression level of genes related to the integrity of membranes, such as Ocln, SLC5A1, ST6GALNAC-V, SLC5A4, SLC4A4, Slc5a2, Abcg2, Lpar2, SLC7A7, Slc15a1, and ITGB8. Among these genes, Ocln encodes an integral membrane protein, occludin, that is required for formation and maintenance of tight junctions (McCarthy et al., 1996). The ITGB8 encodes integrin β8, a receptor, which plays an important role in mediating cell–cell and cell–matrix interaction and communication (Benoit et al., 2009). The genes SLC5A1, SLC5A4, SLC4A4, Slc5a2, Abcg2, Lpar2, SLC7A7, and Slc15a1 are related to the cell membrane transporters and channels, which facilitate the transport of glucose and different ions, and help to maintain ion homeostasis in the small intestine (Sterling and Casey, 2002; Robey et al., 2009; Choi et al., 2010). Diarrhea may occur when tight junctions of epithelial cells that cover the small intestine become disordered (Hecht, 1995; El Asmar et al., 2002; Sawada et al., 2003). The microarray results indicate that feeding dietary CAP enhances the integrity of membranes, especially several proteins involved in the tight junctions, which may support the reduced diarrhea of pigs infected with E. coli compared with the control diet in our clinical data. Mucins play a central role in maintenance of the gut barrier function through interfacing with food, water, and luminal microorganisms. Mucin 2 is the major core polypeptide of membrane-associated and secretory gel-forming mucins in the intestine, and it plays an important role in defense against inflammation (Hollingsworth and Swanson, 2004; Nishida et al., 2009; Hansson, 2012). Therefore, qRT-PCR results show that feeding CAP up-regulated the expression of MUC2 and supported a beneficial effect of CAP on the gut barrier.

Capsicum oleoresin fed to pigs affected the gut mucosal immune responses by downregulating the expression of genes in the categories of responses to stimulus and antigen processing and presentation. These genes include DDX58, C7, SLA-DRB1, Hsp27, IL1RN, IRG6, SLA-2, C2, mxi1, TNFRSF1A, SLA-1, CD163, and B2M. The downregulation of B2M and DDX58 genes and genes of the major histocompatibility complex, including both MHCII (SLA-1 and SLA-2) and MHCII (SLA-DRB1), may indicate moderation of the increase of antigen processing and presentation caused by E. coli infection. The primary function of MHCII is to continuously present intracellular antigen-derived peptides to cytotoxic T lymphocytes, ensuring a rapid immune response against infectious pathogens and initiating the adaptive immune response (Yang, 2003). Similarly, MHCII presents processed peptides from exogenous antigen to helper T lymphocytes, driving both the humoral and cell-mediated immune responses (Radosevich and Ono, 2003). The DDX58 encodes a pattern-recognition receptor, called retinoic-inducible gene-1 protein that has antiviral responses and regulation of immune response (Takeuchi and Akira, 2008). Perhaps the reduced antigen presentation contributes to the attenuation of the expression of components in the complement system, such as C2 and C7 (Sarma and Ward, 2011), as well as the reduced mRNA expression of other inflammatory mediators, including IRG6, TNFα and TNFRSF1A, IL1RN, mxi1, and Hsp27. Among these genes, IRG6 encodes an interferon-induced antiviral protein, viperin, which is induced in lymphoid cells and dendritic cells during acute infection and is highly induced in neutrophils and macrophages (Hinson et al., 2010). The TNFRSF1A expresses a major receptor for TNF-α, activates the transcription factor NF-κB, and regulates inflammation (Micheau and Tschopp, 2003). The Hsp27 encodes an ATP-independent chaperone that can regulate cell apoptosis and enhance NF-κB activity (Parcellier et al., 2003). Moreover, the qRT-PCR results showed that feeding CAP down-regulated COX-2 gene expression in the ileal mucosa. Consistent with our clinical data, these gene expression results support the observation that feeding CAP attenuated the increased...
total white blood cell numbers and serum inflammatory mediators, TNF-α and haptoglobin, caused by *E. coli* infection (Liu et al., 2013). Thus, CAP may reduce the immune responses of pigs infected with *E. coli*.

**Garlic Botanical on *E. coli* Infection**

Garlic botanical is extracted from garlic, standardized to 40% propyl thiosulfonates. Feeding GAR to *E. coli*-infected pigs down-regulated the mRNA expression of several immune genes, such as *C7, CASP3, CASP8, CFB, HSP90AA1, IFIT1, IFIT2, IL7, IL10RB, IRF7, LBP, MHCI*, and *TNFA*, related to the regulation of immune responses. The reduced LBP indicated that GAR might decrease the impact of LPS, a cell-wall component of *E. coli*. The IRF7 is a transcription factor and potentially induced by TNF-α and LPS. It is involved in regulation of a variety of cellular functions, such as IFN-mediated immune responses, cell growth, and apoptosis (Ning et al., 2011). Therefore, at the transcriptional level, the IRF7 expression was consistent with the reduced LBP, indicating that the failure of LPS activation may block the transcriptional signaling pathway mediated by IRF7. The *CASP3* and *CASP8*, encoding death proteases involved in cell apoptosis, were also in agreement with the possibly reduced LPS activation by GAR (Porter and Jänicke, 1999). The reduced mRNA expression of *CFB, IFIT1, IFIT2, and MHCI* indicated feeding GAR may decrease antigen presentation (de Veer et al., 1998; Jensen, 2007; Pichlmair et al., 2011).

On the other hand, the supplementation of GAR up-regulated other genes related to cytokine–cytokine receptor interaction, chemokine signaling pathway, natural killer cell mediated pathway, and T cell receptor signaling pathway. Among these pathways, several immune genes, including *CXCR6, Ccr3, IFNG, Cxcl9, CCL4, CCL3L1, CCR9, CCR5, FYN, FCGR3B, CD8B, Cd3 g, Cda8a, and others* were involved. The *cxcl9, CCL4, CCL3L1, CXCR6, Ccr3, CCR9, and CCR5* encode several chemokines and chemokine receptors, which play an important role in host defense because of their abilities to trigger leukocyte mobilization to sites of injury (Bennett et al., 2011). The *CD8B, Cd3 g, Cda8a* and IFNG code the molecules involved in the cytotoxic T cell signaling pathway, which recognizes peptides bound to the MHCI and helps to remove these pathogenic peptides from the host (Frankenberger et al., 2005). However, the CD8-positive T cells require various stimuli, such as cytokines, costimulatory molecules, and other immune cells, to become fully activated and to induce differentiation and proliferation (Ito and Seishima, 2010), and these other stimuli were not increased by GAR. Therefore, the increase in molecules related to cytotoxic T cells does not indicate increased antigen presentation and immune responses.

In the present study, GAR increased the expression of some genes of the immune system and reduced expression of others. Overall, the reduction in the transcriptional signaling pathway and antigen presentation indicates the net effect of GAR was to attenuate the increased immune responses caused by *E. coli* infection. These results were also in agreement with the qRT-PCR result, indicated by the reduced COX-2 gene expression, and also by our clinical data, indicated by the reduced serum haptoglobin and total white blood cell numbers when GAR was fed to *E. coli*-infected pigs (Liu et al., 2013). Further evidence for the modulation of immune responses in response to GAR comes from the study of Kim et al. (2013) showing that feeding GAR to *Escherichia acevulina*-infected chickens inhibited the expression of *TLR3, TLR5, TNFRSF8*, and other genes involved in the inflammatory response.

**Turmeric Oleoresin on *E. coli* Infection**

Turmeric oleoresin is specifically known for wound-healing and anti-inflammatory properties (Aggarwal et al., 2007; Krishnaswamy, 2008). In the present study, feeding TUR reduced the mRNA expression of several immune genes in the ileal mucosa of *E. coli*-infected pigs. These genes were involved in LPS recognition and subsequently transcriptional signaling (*LBP* and *MyD88*), interferon effects (*IFNAR1* and *IFNGR1*), antigen presentation (*IFIT1, IFIT2*, *MHCI*, and *MHCII*), cytokines and receptors (*IL16, IL10RB*, and *TNFA*), chemokines (*CCL5, CCL28, CCR9*, and *IL8*), complement cascades (*C5, C7, and CFB*), heat stress (*HSP90A1* and *HSP90B1*), and cell apoptosis (*CASP3* and *CASP8*; Porter and Jänicke, 1999; Jensen, 2007; Ning et al., 2011). Curcuminoids are the vital constituents of turmeric that were beneficial in treatment of various chronic inflammatory conditions such as cancer and arthritis (Shishodia et al., 2005; Aggarwal et al., 2007; Goel et al., 2008). Ahmed and Gilani (2011) reported that a curcuminoid mixture effectively decreased caspase-3 (encoded by *CASP3* gene) level in the hippocampus after 20 d of treatment. The current microarray results indicated that feeding TUR reduced the gut inflammation of *E. coli*-infected pigs, especially the impact of LPS released by *E. coli*. In addition, the qRT-PCR results showed that feeding TUR down-regulated the expression of COX-2, which suggests a reduction of gut inflammation by TUR.

Feeding TUR down-regulated the mRNA expression of plasma membrane components, including the genes involved in the tight junctions (*CLDN3, cdh5, CLDN4*, and *Ocln*). The *CLDN3* and *CLDN4* encode proteins claudin 3 and claudin 4, which are considered as major proteins in the structural backbone of tight junctions (Van Itallie et al., 2001; Elkouby-Naor and
Ben-Yosef, 2010). The Ocln codes another transmembrane protein, occludin, associated with tight junctions. Occludin interacts with claudin and is involved in the regulation of intermembrane diffusion and paracellular diffusion of small molecules (Balda et al., 1996; Tsukita and Furuse, 1999). The cdh5 encodes cadherin, a transmembrane protein that function as adherens junctions (Oda and Takeichi, 2011). Previous studies reported that dysfunctional tight junctions may induce diarrhea of humans and animals during a pathogenic bacterial infection (Hecht, 1995; Sears and Kaper, 1996; Sawada et al., 2003). However, the qRT-PCR measurement of the MUC-2 gene indicates that feeding TUR increases the secretion of mucins, which may enhance the gut barrier function and contribute to the reduced diarrhea of E. coli-infected pigs. Therefore, the information about the delicate interactions between tight junction proteins and diarrhea of pigs is still fragmentary, and more research may need to be conducted.

Conclusions

Overall, the analysis of gene expression patterns shows that dietary plant extracts affected the expression of genes in ileal mucosa of pigs at d 5 post infection with E. coli. The increased expression of genes related to membrane structure and function in the ileal mucosa of E. coli-infected pigs consuming CAP and GAR may enhance the gut mucosa health. Feeding CAP and TUR increased the MUC2 gene expression. These findings support earlier observations that feeding these plant extracts are associated with reduced diarrhea of pigs infected with E. coli (Liu et al., 2013). Moreover, feeding the 3 plant extracts each reduced the mRNA expression of several immune genes involved in antigen presentation and other immune responses–related pathways, indicating that these plant extracts may attenuate the increased immune responses caused by E. coli infection. These results also support observations that feeding each of the 3 plant extracts reduce total white blood cell numbers and serum inflammatory mediators compared with the control diet (Liu et al., 2013). In conclusion, the current findings provide new insights into the immunomodulatory and physiologomodulatory properties of plant extracts. Feeding plant extracts provide benefits by reducing the over stimulation of the immune system by E. coli and enhancing gut physiological defenses.

LITERATURE CITED


Phytotherapy as an alternative for treating fish disease

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Abstract

Intensification of livestock rearing often promotes an increase in inappropriate practices that disregard care for the environment, animal health, and workers’ health. Intensive fish farming systems are often associated with higher stocking density and massive use of artificial feed. Currently, outbreaks of parasitic, bacterial, and fungal diseases act as major limiting factors for fish farming, meaning that producers have to make use of massive amounts of antibiotics, disinfectants, and pesticides in order to control mortality and avoid huge economic losses. Because of adverse effects on the aquatic environment, terrestrial organisms, and human health (both fish handlers and consumers), this therapy has been criticized. Use of herbal medicines within animal production has shown promise, in that it is natural and biodegradable and has antimicrobial activity against various pathogens, including those relating to fish. Recently, researchers have reported promising effects from many herbal medicines for treating parasitic diseases caused by protozoa and metazoa, and broad activity against bacteria and fungi. This review addresses the current issues regarding indiscriminate use of chemicals and antibiotics in aquaculture and discusses the main findings and methodologies of the latest research on herbal medicines to stimulate and accelerate research in this field, especially in developing countries.

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INTRODUCTION

Over recent years, farming of aquatic organisms has been encouraged throughout the world. It received special emphasis in 2012, when this form of farming overtook beef production (Earth Policy Institute, 2013). In the production chain, the demand for maximum productivity over a short period of time and at lower cost leads to some barriers that directly or indirectly affect the health status of the product. High stocking densities (Garcia et al., 2013), poor nutritional quality, poor quality of the aquatic environment (Longo et al., 2013), and lack of biosafety measures are among the major problems in aquaculture, especially in rearing fish, because these conditions favor the spread of pathogen and/or immunosuppression of host.

Researchers have been stimulated to find solutions to health problems in aquaculture. The solutions for controlling and preventing fish diseases include development of prophylactic agents as vaccines (Pridgeon & Klesius, 2013) and development of immunostimulant diets (Skalli et al., 2013). In this regard, several natural products have shown promising prophylactic results and improvement of immune response in fish (Samad et al., 2014; Vaseeharan & Thaya, 2014). Nonetheless, to control outbreaks of mortality caused by pathogens, it is essential to use a therapeutic product at some stage of production, with the aim of at least reducing the pressure of pathogens on the host.

There is a big gap in discussing treatments used to control fish diseases. The lack of veterinary products registered for use in aquaculture is a current problem in most countries worldwide. Until the present day, much of the information about treatments has been extrapolated from cold water fish (especially salmonids) to freshwater fish (Athanassopoulos et al., 2004). The drugs used in aquaculture are antimicrobials, disinfectants, pesticides, and other chemicals, which have an uncertain future because of the various negative effects that they present toward the aquatic environment (Rico & Van den Brink, 2014), animals, and humans (Sapkota et al., 2008). In a review on the use of chemotherapy in salmon, Burr ridge et al. (2010) reported that there was a great need for research to develop appropriate interventions with lower risks.
Phytotherapeutics with activity for treating bacterial infections and parasitic infestations of humans and animals have been described for centuries (Silva & Fernandes-Júnior, 2010). Thus, such agents can be considered to have promise with regard to therapeutic control over pathogens of fish. In vitro tests have recently been conducted to search for parasiticides (Qi et al., 2012; Yi et al., 2012), bactericides (Ostrand et al., 2012; Albert & Ransangan, 2013), and fungicides (Xue-Gang et al., 2013) against major pathogens in aquaculture. Moreover, some in vivo tests have shown positive effects relating to treatment of and recovery from various fish diseases (Hari-krishnan et al., 2005; Muniruzzaman & Chowdhury, 2008; Abd El-Galil & Aboelhadid, 2012; Schelkle et al., 2013).

The present review clarifies the prospects in relation to current and future treatments of diseases in fish farming and discusses the main herbal medicines surveyed with a view to use as parasitic, bacterial, and fungal agents.

**REVIEW OF THE LITERATURE**

**Intensification of aquaculture and pathogens**

For decades, one of the main health barriers caused by intensification of fish farming has been occurrences of parasitic infestations (Kugel et al., 1990). Several parasitic agents affect fish production, with effects ranging from reduced productivity (Evans et al., 2007) to outbreaks of mortality (Khan, 2009), which consequently generate significant economic losses. Among the major parasitic diseases in aquaculture, those caused by parasites that are visible to the naked eye, that is, lernaeids (Raissy et al., 2013) and branchiurans (Pekmezci et al., 2011), can be highlighted, along with microscopic parasites such as myxosporeans (Müller et al., 2013), trichodinids (Valladão et al., 2014b), monogeneans (Akoll et al., 2012), and Ichthyophthirius multifiliis (Wei et al., 2013). Each of these types is responsible for causing huge losses in fish production.

Opportunistic infections caused by bacterial agents are common in aquaculture and may have primary or secondary origin. However, this usually occurs when the host is exposed to adverse environmental or farming conditions that lead diminished effectiveness of the immune system, which may be due to poor nutrition, stress, or recurrent challenges by parasite infestations (Xu et al., 2012a,b).

Among the main bacterial diseases in fish from continental waters are those notoriously caused by bacteria of the genera Streptococcus (Zhang et al., 2013a), Aeromonas (Griffin et al., 2013), Flavobacterium (Sebastião et al., 2013; Evenhuis et al., 2014), Edwardsiella (Park et al., 2012; Hawke et al., 2013), and Francisella (Soto et al., 2013).

**Treatment in aquaculture**

**Conventional treatments for fish diseases.** The treatment of diseases in farm animals is necessary regardless of the species farmed or type of rearing. The pathogen pressure on hosts is high in intensive farming systems, and sometimes it is essential to use therapeutic drugs. The situation is no different in aquaculture. The most common substances used in aquaculture are disinfectants, pesticides (Rico & Van den Brink, 2014), and antibiotics (Cabello, 2006).

The main criticisms surrounding the use of these drugs is the presence of residues in water, sediment, and fish; toxicity in nontarget organisms such as plants, crustaceans, and even wild fish; carcinogenic potential for handlers and consumers; and bacterial resistance (Sapkota et al., 2008; Tavecchio et al., 2009; Burridge et al., 2010; Rico et al., 2013; Rico & Van den Brink, 2014). Nonetheless, producers have made use of highly toxic substances, including molecules that have not been registered for treating fish diseases, which is worrying.

In aquaculture, disinfectants are used to reduce the load of pathogenic micro-organisms on the biological surface of aquatic organisms, and their use on fish is associated with broad-spectrum, fast-acting, and low-cost characteristics (Burka et al., 1997). The best-known substances are hydrogen peroxide, quaternary ammonia, formalin, and malachite green. These compounds are mainly used for treating parasitic (Picón-Camacho et al., 2012) and fungal diseases (Sudova et al., 2007) in fish.

Among the disinfectants, formalin is one of the main products used in treating diseases caused by Saprolegnia parasitica (Gieseker et al., 2006), monogeneans (Pahor-Filho et al., 2012), trichodinids (Noga, 2010), and I. multifiliis (Heinecke & Buchmann, 2009). However, fish that survive the treatment may present compromised health (Tieman & Goodwin, 2001). Although disinfectants have low potential for bioaccumulation in the environment and in aquatic organisms, their deleterious effects on fish and on workers through direct contact with this product present risks, thus making it uncertain whether there is a future for their use in aquaculture (Picón-Camacho et al., 2012).

Malachite green is widely used for controlling various fish parasites and especially for combating I. multifiliis. It has been shown to be effective against this important pathogen of fish (Srivastava et al., 2004). However, this product has been banned in several countries because of its mutagenic and carcinogenic effects (Picón-Camacho et al., 2012) and its high persistence in the environment, given that it bioaccumulates in the ecosystem and in fish tissue (Henderson et al., 1997).

Use of antibiotics and veterinary drugs in aquaculture can pose risks to food safety and consumers (Heuer et al., 2009; Love et al., 2011), as well as causing toxic injuries to fish and deleterious effects on the aquatic ecosystem (Tavecchio et al., 2009). Most parasiticides and disinfectants currently used in aquaculture are highly toxic and are able to affect nontarget organisms. These factors contribute to environmental degradation (Rico et al., 2012). In addition to affecting human health by containing toxic and mutagenic effects, parasiticides present higher environmental risk than antibiotics (Rico & Van den Brink, 2014). In study of most important aquaculture region (Asia), disinfectants showed acute toxicity for all three evaluated taxonomic groups (primary producers, invertebrates, and fish). The highest ecological risks were calculated for the par-
Phytotherapeutics for fish

Phytotherapy: alternative treatment. Phytotherapy, by definition, consists of treatment using products obtained from medicinal plants, or derivatives thereof, with prophylactic, curative, or palliative purposes (Brazil, 2011). Medicinal plants have broad antiparasitic, antibacterial, and antifungal activity proven by scientific studies on animals and humans (Silva & Fernandes-Júnior, 2010). Although herbal medicines have been used as therapy for human diseases for centuries, their potential for use in aquaculture has only recently been discussed. This has come about because of the demand for products to replace the current drugs, which pose great risks to the aquatic environment.

Regarding the antimicrobial properties of substances derived from plants, studies on controlling parasitic infections in humans and animals, including fish, have been stimulated (Abd El-Galil & Aboelhadid, 2012). Their use in fish farming can contribute toward reducing the use of chemotherapeutic parasiticides, thereby providing greater sustainability for fish production, with reduced environmental risks, while having the capacity to treat fish diseases and consequently avoid mortality and therefore economic losses. A few years ago, phytotherapeutics were studied in relation to treatments for some fish parasites (Ekanem et al., 2004a,b; Chitmanat et al., 2005; Steverding et al., 2005). They have gained prominence over more recent years through the publication of numerous papers in important aquaculture journals.

An extensive search for studies in which herbal medicines were used to treat fish parasites was conducted, and the data from this search have been presented in Table 2. These data may help future researchers in selecting herbal medicines to deepen the research and development studies on new products. It can be seen that herbal medicines against I. multifiliis parasites and various species of monogeneans have been widely studied. This is mainly because of the great importance of these fish parasites in aquaculture worldwide and the lack of safe treatments, and this information is discussed in this review.

The main pathway for treating parasitic infections consists of bathing the fish in water containing the phytotherapeutic agent. However, use of herbal medicines in water for large-scale treatments is difficult (i.e., in commercial fish farming), which may explain the lack of commercial products. In contrast, these substances are already in use in fishkeeping, where the treatment pathway of bathing in water is facilitated. Some products made from medicinal plants are already found in aquarium shops around the world.

In view of the great diversity of types of herbal products, methodologies, and target pathogens, comparisons between studies are complicated. The present review revealed that few studies have been conducted regarding the effects of phytotherapeutics for treating parasitic diseases when these are added to the diet. The effectiveness of these molecules when added to the diet can increase the feasibility of their use in aquaculture. Therefore, research on this administration route should be encouraged, especially in relation to endoparasites or ectoparasites that have part of their cycle in the host tissue, like I. multifiliis. This type of treatment is a challenge, as it requires technical knowledge, so that the treatment starts at the first signs of the disease, when the fish are still feeding.
<table>
<thead>
<tr>
<th>Parasite</th>
<th>Host</th>
<th>Phyttherapic</th>
<th>Most effective treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Argulus</em> spp.</td>
<td><em>Carassius auratus</em></td>
<td>Piperine <em>Piper longum</em></td>
<td>Water 48 h 9.0 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Osthol and isopimpellin <em>Fructus cnidii</em></td>
<td>Water 48 h Osthol 1.6 mg/L and isopimpellin 9.5 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Methanol extract <em>Semen aesculi</em></td>
<td>Water 48 h 10 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Dioscin and polyphyllin D <em>Paris polyphylla</em></td>
<td>Water 48 h Dioscin EC50* = 0.44 mg/L and polyphyllin D EC50 = 0.70 mg/L 1.5–3.0 times more effective than the positive control, mebendazole</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Gracillin <em>Dioscorea zingiberensis</em></td>
<td>Water 48 h 0.9 mg/L High antiparasitic activity; almost 10 times more effective than the positive control, mebendazole</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Sanguinarine <em>Macleaya micrantha</em></td>
<td>Water 48 h 0.7 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Ctenopharyngodon idella</em></td>
<td>Chelerythrine <em>Chelidonium majus</em></td>
<td>Water 48 h 1.60 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Osthol <em>Radix anglicae pubescens</em></td>
<td>Water 48 h 1.6 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Bruceine A and bruceine D <em>Brucia javanaica</em></td>
<td>Water 48 h 1 mg/L Bruceine A = 97% efficacy and bruceine D = 91.2% efficacy; 2–2.5 times more effective than the positive control, mebendazole</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Methanol extract <em>Radix Braurei chinensis</em></td>
<td>Water 48 h 10 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Aqueous extract <em>Cinnamomum cassia</em></td>
<td>Water 48 h 30 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td><em>Dactylogyrus intermedius</em></td>
<td><em>Carassius auratus</em></td>
<td>Methanol extract <em>Dryopteris crassirhizoma</em></td>
<td>Water 48 h 40 mg/L 100% efficacy compared to control</td>
</tr>
<tr>
<td>Parasite</td>
<td>Host</td>
<td>Type/Active compound</td>
<td>Plant</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------</td>
<td>----------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><em>Gyrodactylus spp.</em></td>
<td><em>Gasterosteus aculeatus</em></td>
<td>Essential oil</td>
<td><em>Melaleuca alternifolia</em></td>
</tr>
<tr>
<td><em>Gyrodactylus elegans</em> and <em>Dactylogyrus extensus</em></td>
<td><em>Carassius auratus</em></td>
<td>Methanol extract</td>
<td><em>Piper guineense</em></td>
</tr>
<tr>
<td><em>Gyrodactylus turnbulli</em></td>
<td><em>Pecilia reticulata</em></td>
<td>Freeze-dried</td>
<td><em>Allium sativum</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius nectator</em></td>
<td><em>Oncorhynchus keta</em> and <em>Oncorhynchus masou</em></td>
<td>Epigallocatechin gallate</td>
<td><em>Camellia sinensis</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifilis</em></td>
<td><em>Carassius auratus</em></td>
<td>Methanol extract</td>
<td><em>Mucuna pruriens</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Carica papaya</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifilis</em></td>
<td><em>Ctenopharyngodon idella</em></td>
<td>Sanguinarine</td>
<td><em>Macleaya cordata</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifilis</em></td>
<td><em>Squaliobarbus curriculus</em></td>
<td>Dihydrosanguinarine and dihydrochelerythine</td>
<td><em>Macleaya microcarpa</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifilis</em></td>
<td><em>Carassius auratus</em></td>
<td>Aqueous extract</td>
<td><em>Capsicum frutescens</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifilis</em></td>
<td><em>Carassius auratus</em></td>
<td>Methanol extract</td>
<td><em>Magnolia officinalis</em> and <em>Sophora alopecuroides</em></td>
</tr>
<tr>
<td>Parasite</td>
<td>Host</td>
<td>Type/Active compound</td>
<td>Plant</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------</td>
<td>-------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td><em>Ichthyophthirius multifiliis</em></td>
<td><em>Ictalurus punctatus</em></td>
<td>Pentagalloylglucose</td>
<td><em>Galla chinensis</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifiliis</em></td>
<td><em>Carassius auratus</em></td>
<td>Chelerythrine and chloroxylonine</td>
<td><em>Todalia asiatica</em></td>
</tr>
<tr>
<td><em>Ichthyophthirius multifiliis</em></td>
<td><em>Piaractus mesopotamicus</em></td>
<td>Essential oil</td>
<td><em>Melaleuca alternifolia</em></td>
</tr>
<tr>
<td>Monogenea</td>
<td><em>Heterobranchus longifilis</em></td>
<td>Ethanol extract</td>
<td><em>Artemisia annua</em></td>
</tr>
<tr>
<td><em>Myxobolus</em> sp.</td>
<td><em>Diplodus puntazzo</em></td>
<td>Essential oil</td>
<td><em>Origanum minutiflorum</em></td>
</tr>
<tr>
<td><em>Neobenedenia</em> sp.</td>
<td><em>Lates calcarifer</em></td>
<td>Alllicin</td>
<td><em>Allium sativum</em></td>
</tr>
<tr>
<td><em>Pseudodactylogyrus</em></td>
<td><em>Anguilla anguilla</em></td>
<td>Ginkgolic acid C13:0 and C15:1</td>
<td><em>Ginkgo biloba</em></td>
</tr>
<tr>
<td><em>Trichodina</em> sp.</td>
<td><em>Oreochromis niloticus</em></td>
<td>Crude extract</td>
<td><em>Allium sativum and Terminalia catappa</em></td>
</tr>
<tr>
<td><em>Trichodina</em> sp.</td>
<td><em>Oreochromis niloticus</em></td>
<td>Aqueous extract</td>
<td><em>Camellia sinensis</em></td>
</tr>
<tr>
<td><em>Trichodina</em> sp.</td>
<td><em>Parabramis pekinensis</em></td>
<td>Chelidionine, chelerythrine, and sanguinarine</td>
<td><em>Chelidonium majus</em></td>
</tr>
</tbody>
</table>
A wide range of phytotherapeutics is also known to have antibacterial activity against human pathogens (Ushimaru et al., 2012) and animal pathogens (Dal-Pozzo et al., 2011). Replacement of the current antimicrobials used in aquaculture, with herbal products, is not a utopian concept given that several medicinal plants have shown activity against important pathogenic bacteria of fish, such as Aeromonas hydrophila (Muniruzzaman & Chowdhury, 2008; Harikrishnan et al., 2009, 2010c), Streptococcus iniae (Abutbul et al., 2004; Zilberg et al., 2010), Streptococcus agalactiae (Zilberg et al., 2010), Flavobacterium columnare (Rattanachaikunsopon & Phumkhachorn, 2010), Pseudomonas fluorescens and Edwardsiella tarda (Muniruzzaman & Chowdhury, 2008). There is great concern with the emergence of bacterial strains of the aquatic environment that are resistant to antibiotics, and this tends to increase with the wrong use in aquaculture, mainly with its use as a prophylactic (Cabello, 2006). On the other hand, phytotherapeutics are less prone to development of bacterial resistance (Kulkarni et al., 2013) because of their mode of action affecting several targets at the same time (Bakkali et al., 2008). The main phytotherapeutics used in treating bacterial fish diseases have been reviewed in Table 3.

The most common administration routes for herbal medicines used in treating bacterial diseases are by immersion or orally. Because of the large number of studies, many types of phytotherapeutics, and different forms of treatment, comparison between the data in the literature is difficult. This review table may foster development of future research in the field of alternative treatments against bacterial fish diseases.

Oral treatment has been showing excellent survival results in fish with bacterial disease. Batches of sick fish need to be treated even if some of them are not eating, because within the same fish cages or ponds, there are many subclinically infected fish and/or newly infected fish, which have a high chance of cure. Therefore, use of herbal medicines in the diet is promising for treating large batches of fish that are suffering from bacterial diseases, so as to be able to avoid large losses.

Studies on the effects of herbal medicines in treating fungal diseases in aquaculture were also reviewed here. Use of eucalyptus extract at 100 mg/L showed significant fungus growth inhibition and a high hatching rate for Rutilus frisii eggs (Najafi & Zamini, 2013). An extract of Radix sanguisorbae was considered to be a promising phytotherapeutic for treating eggs that were experimentally infected with Saprolegnia australis (Cao et al., 2013). Use of herbal medicines in water for treating fungi in eggs is facilitated in incubators or small tanks, compared with their use in ponds or cages. This is because it is possible to have high density and large numbers of eggs in incubators or small tanks, with easy management. In these cases, the efficacy of the substance is not affected by the massive presence of debris and organic matter that is seen in the cultivation system and thus it is certain that the target organism (in this case, the eggs) will be exposed to the product. The major studies involving treatment of fungal diseases within aquaculture are shown in Table 4.
### Table 3. Phytotherapics with greatest potential for use in aquaculture to treat bacterial diseases

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Host</th>
<th>Type/Active compound</th>
<th>Plant</th>
<th>Route</th>
<th>Period</th>
<th>Concentration</th>
<th>Results</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td><em>Channa punctatus</em></td>
<td>Ethyl acetate extract</td>
<td><em>Solanum nigrum</em></td>
<td>Water</td>
<td>10 min/day for 30 days</td>
<td>1 g/L</td>
<td>Recovery of the injuries; potential treatment of ulcer</td>
<td>Rajendiran et al. (2008)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td><em>Carassius auratus</em></td>
<td>Ethanol extract</td>
<td><em>Azadirachta indica</em> + <em>Curcuma longa</em> + <em>Ocimum sanctum</em></td>
<td>Oral (Feed)</td>
<td>30 days</td>
<td>2.5 g/kg</td>
<td>100% survival compared to 5% of untreated group</td>
<td>Harikrishnan et al. (2009)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td><em>Cyprinus carpio</em></td>
<td>Aqueous extract</td>
<td><em>Azadirachta indica</em> + <em>Curcuma longa</em> + <em>Ocimum sanctum</em></td>
<td>Oral (Feed)</td>
<td>30 days</td>
<td>0.1% of diet</td>
<td>35% lower mortality rate than in control group</td>
<td>Harikrishnan et al. (2010a)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td><em>Carassius auratus</em></td>
<td>Concoction*</td>
<td><em>Azadirachta indica</em> + <em>Ocimum sanctum</em> + <em>Curcuma longa</em></td>
<td>Water</td>
<td>5 min/day for 45 days</td>
<td>1%</td>
<td>26.6% lower mortality rate than in control group</td>
<td>Harikrishnan et al. (2010c)</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td><em>Barbodes gonionotus</em></td>
<td>Bulb extract</td>
<td><em>Allium sativum</em></td>
<td>Oral (Feed)</td>
<td>10 days s.i.d.**</td>
<td>3% LW* of feed mix</td>
<td>100% and 90% recovery (A. hydrophila and P. fluorescens, respectively) compared to 0% in control group</td>
<td>Muniruzzaman and Chowdhury (2008)</td>
</tr>
<tr>
<td><em>Edwardsiella tarda</em></td>
<td><em>Pangasius hypophthalmus</em></td>
<td>Decoction‡</td>
<td><em>Calotropis gigantea</em></td>
<td>Oral (Feed)</td>
<td>10 days s.i.d.**</td>
<td>3% LW* of herbal mix</td>
<td>96.66% recovery compared to 0% in control group</td>
<td>Muniruzzaman and Chowdhury (2008)</td>
</tr>
<tr>
<td><em>Flavobacterium columnare</em></td>
<td><em>Oreochromis niloticus</em></td>
<td>Aqueous extract</td>
<td><em>Centella asiatica</em></td>
<td>Water</td>
<td>Single bath two days postinfection</td>
<td>100 mg/L</td>
<td>100% survival compared to 50% in control group</td>
<td>Rattanachaikunsopon and Phumkhachorn (2010)</td>
</tr>
</tbody>
</table>

*LW, liveweight; **s.i.d., once a day; *Combination of various phytotherapics macerated into a liquid; ‡Phytoterapic extracted by boiling.
Negative effects of using phytotherapeutics. Deleterious effects and contraindications cannot be ignored when discussing the exposure of living organisms to chemical molecules, whether these are natural or not. The toxic effect of herbal medicines on aquatic organisms has been poorly studied. These effects vary significantly according to the type of phytotherapeutic and the species of the exposed organism. Some authors have described cases of acute and chronic toxicity of phytotherapeutics in fish. For acetone extract of *Morus alba*, the 96-h LC50 for grass carp was approximately eight times higher than the 4-h EC50 of nonencysted tomonts of *I. multifiliis*. For ethyl acetate extract, the 96-h LC50 for grass carp was 29 times higher than the 4-h EC50 of nonencysted tomonts of the same parasite (Fu *et al.*, 2014), which represents a wide safety margin for its use for fish. These studies are essential, because the lack of such information can be an obstacle when developing new products.

Nonetheless, studies on the toxicity of herbal medicines in nontarget organisms are highly encouraged by the authors of the present review. Although these substances are natural and biodegradable, their use in water can expose nontarget organisms that are more sensitive than fish, such as microcrustaceans and molluscs. Among the few studies that have been conducted, Conti *et al.* (2014) reported that the essential oil of *Melaleuca alternifolia* presented acute toxicity against arthropod nontarget *Daphnia magna*, with a 24-h LC50 of 80.64 ppm, while the LC50 for killing the target *Aedes albopictus* was about three times higher. In contrast to that study, Valladão *et al.* (2015) were successful in treating severe cases of ichthyophthiriasis in *Piaractus mesopotamicus* fish using two-hour daily baths of the essential oil of *M. alternifolia* for five days, using a concentration of 50 ppm, which shows that this has great potential for use in aquaculture.

CONCLUSION

Medicinal plants have broad antimicrobial activity against important fish pathogens. Further studies on chronic and acute toxicity and on the deleterious effects of herbal medicines on treated organisms, nontarget organisms, and the environment are encouraged. Most studies on effectiveness have been based on in vitro testing or have even been conducted under laboratory conditions. Therefore, further practical and economic studies are needed to enable replacement of the current treatments. Therefore also, joint work between the supply chain, industry, and researchers is paramount.

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intermedius (Monogenea) in goldfish (Carassius auratus). Parasitology Research, 111, 97–104.


A medicinal herb-based natural health product improves the condition of a canine natural osteoarthritis model: A randomized placebo-controlled trial

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A R T I C L E   I N F O

Keywords:
Force platform
Locomotor activity
Phytochemical compounds
Naturally-occurring osteoarthritis
Canine model

A B S T R A C T

An oral herb-based natural health product (NHP) was evaluated in the canine natural osteoarthritis model. At baseline, the peak vertical force (PVF, primary endpoint) and case-specific outcome measure of disability (CSOM) were recorded in privately-owned dogs. Dogs (16/group) were randomized to receive NHP formulations or a negative control. The PVF was measured at week (W) 4 and W8. Daily locomotor activity was recorded using accelerometer. The CSOMs were assessed bi-weekly by the owner. The NHP-treated dogs had higher locomotor activity at W8 (p = 0.020) and W8 (p < 0.001) when compared to baseline. The changes at W8 were higher than control dogs (n = 14, p < 0.027) and consistent with Cohen’s d effect size of 0.7 (95% confidence interval: 0.0–1.5). The NHP-treated dogs had higher locomotor activity at W8 (p = 0.025) when compared to baseline. No significant change was observed for the CSOM. The NHP improved the clinical signs of osteoarthritis in this model.

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1. Introduction

Osteoarthritis (OA) is by far the most common human musculoskeletal disease, affecting millions worldwide (Lawrence et al., 2008). The prevalence of OA in dogs is also high, particularly in geriatric animals, being estimated to be five times that observed in mature adults (Shearer, 2011). In dogs, OA results mainly from traumatic insults to the cranial cruciate ligament (CCL), and hip or elbow dysplasia (McLaughlin, 2001; Roush, 2001). Cascades of biological and biomechanical events then merge to induce and perpetuate structural changes at the level of the entire joint, which, as in humans, lead to crippling pain, disability and poor quality of life (Cook, 2010; Johnston, 2001; Madsen and Svalastoga, 1994; Martinez, 1997; Martinez and Coronado, 1997).

Naturally-occurring models of OA have been proposed to accelerate the development of human therapeutics (Pelletier et al., 2010), and a recent review of experimental data underlined the high translatability of outcomes obtained from canine OA models, in particular the response to treatment (Moreau et al., 2013). Undertaking a trial in privately-owned dogs afflicted by natural OA would provide preclinical data and additional evidence on the therapeutic potential of new compounds under development. Of note, the potential of several therapeutic approaches has been tested in different randomized controlled trials (RCTs) in the canine natural OA model using force platform gait analysis as an outcome measure of pain-related functional impairment. These tested compounds include non-steroidal anti-inflammatory drugs (NSAIDs) (Budsberg et al., 1999; Moreau et al., 2003, 2007), therapeutic diets (Moreau et al., 2012b; Rialland et al., 2013; Roush et al., 2010) as well as natural substances (naturaceuticals) used to restore or maintain good health status (Hielm-Bjorkman et al., 2009; Moreau et al., 2004, 2012a). The latter therapeutic class is considered by the authors as natural health products (NHPs) which originate from plants, fruits and vegetables, animals, microorganisms and marine sources.
Currently, no effective therapy seems able to alleviate the clinical signs of OA in humans or dogs. As relief of pain and the preservation of joint structure cannot be claimed with certainty for currently approved treatments, there is a need for effective strategies to improve the condition of afflicted patients.

Medicinal herbs have long been used in traditional medicine and there is considerable evidence that such NHP and their derivatives may play beneficial roles in OA (Mobasher, 2012). Harpagophytum procumbens, also known as devil’s claw, is a South African plant which includes harpagoside as one of its major biologically active phytochemical compounds. A large body of evidence supports the efficacy of harpagoside and related extracts in alleviating symptoms of OA in humans (Gagnier et al., 2004). Resin extracts from the Boswellia serrata tree have been demonstrated to be effective in alleviating the clinical signs of OA in humans (Kimmatarak et al., 2003) and dogs (Reichling et al., 2004). Active phytochemical compounds isolated from Ribes nigrum leaves showed anti-inflammatory properties in vivo in chondrocyte assays (Garbacki et al., 2002), while its seed oil was an effective treatment for active rheumatoid arthritis (Leventhal et al., 1994). Salk alba extracts have recently been reported to have in-vitro chondroprotective properties in primary canine articular chondrocyte culture (Shakibaei et al., 2012). These extracts seem also to be potent in countering low back pain in humans (Gagnier et al., 2007). In rodent models of inflammation, an extract from Tanacetum parthenium demonstrated antinociceptive and anti-inflammatory effects (Jain and Kulkarni, 1999). Classified as a herb, bromelain is a digestive enzyme found in the stem and the fruit of Ananas comosus. This herb has been shown to have anti-inflammatory properties mediated through prostaglandin synthesis (Lotz-Winter, 1990). Finally, curcumin, which is the main biologically active phytochemical compound of Curcuma longa, showed inhibitory actions against major inflammatory mediators (Aggarwal et al., 2013; Henrotin et al., 2013; Mathy-Hartert et al., 2009; Mobasher et al., 2012) while being effective in reducing pain in OA knee patients (Kuptmiratsaikul et al., 2009; Madhu et al., 2013). In agreement with those findings, a recent Cochrane systematic review concluded to potential benefits of oral herbal medicines, being more effective than placebo (Cameron and Chrubasik, 2014). However, as also highlighted, further high quality, fully powered studies are required to gain insight in the therapeutic potential of medicinal plants as well for other NHPs (Vandeweerd et al., 2012).

These studies suggest that NHP formulations containing the aforementioned medicinal herbs as principal ingredients might be useful in the management of OA. Whether or not such formulations are effective against the functional impairment that prevails in a model of natural OA needs to be scrutinized rigorously. With the scope of providing strong evidence-based findings, the aim of this RCT was to assess NHP formulations in the canine natural OA model when compared with dogs receiving a placebo over an 8-week duration.

2. Materials and methods

2.1. Design and subject selection

This study was a randomized, double-blind, parallel-group, placebo-controlled trial. Dogs were evaluated over either 56 or 61 days depending on the balanced attribution of locomotor activity recording (see Section 2.3). The trial was conducted under the approval of the Institutional Animal Care and Use Committee (#Rech-1437) in accordance with the guidelines of the Canadian Council on Animal Care. All owners provided written informed consent.

Adult dogs weighed more than 20 kg and had radiographic evidence of OA exclusively at the hip or stifles joints. Radiographs (hips, stifles, and elbows) were obtained under sedation as previously described (Moreau et al., 2010). Hind limb lameness in association with the presence of OA was confirmed by veterinary surgeons.

At the time of screening, all dogs were free of any compound purported to relieve the clinical signs of OA according to washout periods ranging between 4 and 12 weeks. Hence, a 4-week washout period was respected for oral NSAIDs and a 6-week period for NHPs including fatty acid supplements, OA therapeutic diets or treats. Dogs having received injectable pentosan polysulfate sodium or corticosteroid 1 year before the screening visit were not eligible. A 12-week washout period was requested for injectable polysulfated glycosaminoglycan and hyaluronom, and for oral or topical corticosteroid. During the study, dogs were exempted from the administration of any type of medication except those prescribed for exo- and endoparasite control. Additional exclusion criteria were as follows: dogs with surgical repair of the cranial cruciate ligament within 1 year prior to study initiation, dogs suffering from neurologic or other musculoskeletal lesions, dogs that underwent orthopedic surgery within the past year and dogs with CCL disease having gross instability (positive drawer motion upon orthopedic examination).

2.2. Complete blood count and biochemistry panel

To ensure that some parameters were within normal limits during the study, each dog underwent routine blood hematological and biochemical analyses in order to evaluate health status at study initiation (baseline, day 0) as well as at week 4 (day 28) and week 8 (day 56). A veterinary clinical pathologist examined all blood counts and biochemistry panels.

Many herbs can increase the risk of bleeding through anti-platelet properties (Samuels, 2005). The buccal mucosal bleeding time is a simple test commonly used in the clinical setting to detect platelet dysfunction in dogs (Callan and Giger, 2001). Each dog underwent a buccal mucosal bleeding time procedure at baseline and at week 8. Mucosal punctures were performed on the upper labial mucosa, using a disposable, fully automated incision device (Surgicut® Bleeding Time device, International Technidyne Corporation, USA). This device provided a controlled incision of 1.0 mm (depth) per 3.5 mm (length). The time of incision was noted, and circular filter paper (Whatman®, USA) was held 1–2 mm away from the incision to blot the blood, taking care not to disrupt the clot, or to allow blood to drip into the dog’s mouth. The end point was when the incision stopped bleeding. Normal buccal mucosal bleeds time is defined to be less than 3 minutes.

2.3. Randomization, blinding and therapy regimen

Thirty-two privately-owned dogs were randomly allocated in two equal groups (placebo or NHP) according to a permuted-block randomization procedure, which included six blocks of four treatment possibilities (A or B) distributed in a 1 to 1 ratio (i.e. AABB, ABAB, ABBA, BABA, BABA and BABB). Among those blocks, eight were randomly selected using random integers to define the treatment allocation sequence. Also, seven blocks were randomly selected using random integers to allocate seven motor activity recordings to treatment A and seven others to treatment B. The 32 treatment allocations (with or without locomotor activity recording) were transcribed on individual cards in sequentially numbered, sealed, opaque envelopes to ensure concealment. A third party was responsible for the randomization process and for the treatment preparation. At the trial site, both treatments were labeled exclusively as treatment A or treatment B and were encapsulated identically. The trialists, the animal health technicians and all dog owners were blinded to which treatment (A or B) was given to each dog. The key code revealing what referred to treatments A and B remained confidential with the third party and was revealed only after study completion and preliminary analyses.
Table 1

<table>
<thead>
<tr>
<th>Ingredients (mg/capsule)</th>
<th>Formulations</th>
<th>Minimal contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>Beta</td>
<td></td>
</tr>
<tr>
<td>Medicinal herbs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harpagophyton procumbens</td>
<td>240</td>
<td>60</td>
</tr>
<tr>
<td>Boswellia serrata</td>
<td>240</td>
<td>180</td>
</tr>
<tr>
<td>Ribes nigrum</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Salix alba</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Tanacetum parthenium</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Ananas comosus</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>-</td>
<td>15.0</td>
</tr>
<tr>
<td>Omega-3 PUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>40.0</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucosamine sulfate</td>
<td>-</td>
<td>300.0</td>
</tr>
<tr>
<td>Methylsulfonyl methane</td>
<td>-</td>
<td>90.0</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>-</td>
<td>60.0</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>-</td>
<td>30.0</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>-</td>
<td>15.0</td>
</tr>
<tr>
<td>Excipient</td>
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<td>280.0</td>
</tr>
<tr>
<td>Total weight/capsule</td>
<td>908.0</td>
<td>1190.0</td>
</tr>
</tbody>
</table>

GDUs, GELatin digesting unit; PUFA, Polynsaturated fatty acids.

The ingredients of the NHP formulations are described in Table 1. Dogs allocated to the NHP formulations received the Alpha formulation from day 1 to day 29, and then received the Beta formulation from day 29 to day 56. The dosage regimen was as follows: one capsule for dogs <25.0 kg; two capsules for dogs 25.0–39.9 kg; three capsules for dogs 40.0–49.9 kg; four capsules for dogs 50.0–59.9 kg and five capsules for dogs >59.9 kg. Dogs allocated to the negative (placebo) control received capsules filled of excipient to match the amount of the NHP formulations. The negative control (placebo) was given under the same dosing regimen as for the NHP formulations.

2.4. Force platform measurement

Peak of the vertically-oriented ground reaction force (PVF) was measured at baseline (day 0), week 4 (day 28) and week 8 (day 56) at the trot (1.9–2.2 m/s) using a force platform, as previously described (Moreau et al., 2010). The PVF was defined as the primary endpoint of the study. Normalized PVF values in percentage of body weight (%BW) from the first five valid trials were used for statistical purposes. To be eligible, dogs must have at least one-hind limb with PVF value lower than 66.0 %BW. This value was consistent to full incapacity (four). Each activity was selected by the owner according to his/her own perception of what characterized the disability of the dog. Assessments were conducted twice weekly using a specific form that remained in the possession of the owner. For each dog, median of the activities scores was determined for each assessment, giving a total of 17 median CSOM scores over the study. Among all the assessments, three periods were predefined: baseline (assessment on day 0), week 4 (assessments on days 24, 28 and 31) and week 8 (assessments on days 49, 52 and 56).

2.6. Case-specific outcome measure of disability (CSOM)

Assessment of at-home functional disability was accomplished using CSOM as previously described (Moreau et al., 2012a; Rialland et al., 2012, 2013). Owners assessed the ability of their dogs to perform two to five activities, and scored on a five-point Likert-type scale for each activity that ranged from no problem (zero) to full incapacity (four). Each activity was selected by the owner according to his/her own perception of what characterized the disability of the dog. Assessments were conducted twice weekly using a specific form that remained in the possession of the owner. For each dog, median of the activities scores was determined for each assessment, giving a total of 17 median CSOM scores over the study. Among all the assessments, three periods were predefined: baseline (assessment on day 0), week 4 (assessments on days 24, 28 and 31) and week 8 (assessments on days 49, 52 and 56).

2.7. Statistical analysis

All statistical tests were two-tailed with significance determined by reference to a 5% threshold. Normality of the data was tested using Shapiro–Wilk test. Data were log-transformed when requested to assure transformed data Gaussian distribution. Equality of efficacy was the null hypothesis based on the PVF (primary endpoint) as measured for the hind limb having the lowest value. Per trial log-transformed PVF values were analyzed with a repeated-measures general linear mixed model that included two fixed factors (time and group) and their interaction (time × group interaction), with trials and dogs nested in treatment group as random effects. The change in log-transformed PVF values (week 8 – baseline) were analyzed with a repeated–measures general linear mixed model that included group as fixed factor with trials as random effect. Log-transformed DDAP were analyzed similarly to PVF (period and group as fixed factors) and their interaction (period × group interaction) with days and dogs nested in treatment group as random effects. A repeated–measures generalized linear model was used to analyze median CSOM data under Poisson distribution function using independent working matrix. Fixed factors were period and group and their interaction (period × group interaction) with assessments and dogs nested in treatment group as random effects. Scale factor was estimated by Pearson’s chi-square. Covariance structures were defined as recommended (Littell et al., 2000). All post hoc analyses were conducted with appropriate Bonferroni adjustments. Data are presented as mean (SD).

2.8. Sample size calculation

According to previous works conducted under similar conditions (Moreau et al., 2007), a sample size of 16 dogs/treatment group ensured that a difference of 4.25 %BW in the primary endpoint (PVF) between groups could be detected assuming 75% power, a SD of 4.5 and a 5% significance threshold.
3. Results

3.1. Animal description

No clinically relevant changes were obtained from hematological and biochemical analyses in the entire study cohort. In addition, abnormal buccal mucosal bleeding times were not observed during the study. The numbers of dogs screened, randomly assigned, and analyzed in each group are detailed in Fig. 1. The NHP dog with persistent diarrhea was diagnosed to have gastrointestinal intolerance. Complete CCL rupture ($n = 2$) and humeral bone inflammation resulted in acute lameness and consequently, to the withdrawal of these dogs.

Baseline characteristics of the dogs stratified per group are presented in Table 2. Groups were well balanced according to the outcomes of interest, as significant difference was not observed for the level of PVF, DDAP and CSOM recorded at baseline. It should be noted that in each group, the dogs did not experience significant change in BW over time.

3.2. Peak vertical force measurement

The PVF generated by the disabled hind limb was increased in the overall study cohort (time effect; $p = 0.016$), without significant group effect ($p = 0.299$) (Fig. 2). Increment in PVF was mostly attributed to the changes observed in the NHP-treated dogs. Hence, a significant time $\times$ group interaction ($p < 0.001$) was observed which indicates that groups evolved distinctively from baseline to the end of the study. More specifically, analyses revealed that the PVF of NHP-treated dogs ($n = 13$) was significantly increased at week 4 [58.9 (5.4)%BW, $p = 0.020$] and at week 8 [59.8 (6.3)%BW, $p < 0.001$], when compared to baseline 57.3 (4.9)%BW. Placebo dogs ($n = 14$) did not have significantly different values at week 4 [56.4 (5.8)%BW] or week 8 [56.9 (6.8)%BW] than baseline [57.2 (4.5)%BW]. Both groups did not differ significantly at week 8. Fig. 3 presents the respective individual changes in PVF recorded over the study (i.e., week 8 − baseline) as well as the mean change denoted in each group. The mean changes in PVF values were significantly different between groups ($p = 0.027$).

3.3. Locomotor activity recording

The analysis of DDAP indicated no significant period ($p = 0.862$), or group ($p = 0.414$) effect, but a significant period $\times$ group interaction ($p < 0.001$). Analyses revealed that the week 4 period [7.3 (1.9) h/day] in NHP-treated dogs ($n = 7$) was not significantly different
to the baseline, reaching significant increase for the week 8 period [8.2 (3.4) h/day, \(p = 0.025\)] (Fig. 4). The DDAP values of placebo dogs (\(n = 7\)) at the week 4 [6.7 (2.1) h/day] and week 8 [6.0 (2.3) h/day] periods were not significantly different than the baseline (Fig. 4). A statistical trend (\(p = 0.064\)) was observed for a difference in DDAP values between-groups over the study (i.e., week 8 – baseline).

### 3.4. Case-specific outcome measure

The CSOM analysis revealed no significant period (\(p = 0.053\)), group (\(p = 0.960\)) and period \(\times\) group (\(p = 0.524\)) effect. Fig. 5 presents the evolution of the CSOM recorded over the entire study duration.
4. Discussion and conclusions

Current therapeutic approaches used to manage OA-afflicted patients remain largely palliative, NSAIDs being the first line of treatment (Bennell et al., 2012). The effect sizes reported for therapeutic modalities range from small to moderate (Bjordal et al., 2004; Zhang et al., 2007). Therefore, there is an opportunity for novel and effective therapeutics to alleviate pain for the OA-afflicted patient. As naturally-occurring models of OA have recently been proposed to accelerate the development of human therapeutics (Pelletier et al., 2010), and since canine OA models have a high translational value to human OA (Moreau et al., 2013), this randomized, double-blind, placebo-controlled trial was undertaken in the canine natural OA model to assess the efficacy of novel phytotherapeutics for human use. A recent systematic review concluded that NHPs had poor therapeutic potential for the treatment of companion animals affected by OA (Vandevenued et al., 2012). This disappointing conclusion was largely based on the limited number of rigorous RCTs developed to challenge the proposed therapeutic efficacy of NHP. The quality and quantity of current research studies were also criticized for oral herbal medicines purported to alleviate the clinical signs of human OA (Cameron and Chrubasik, 2014). The present trial was undertaken with the second intention to provide rigorous evidence regarding the therapeutic potential of medicinal herb-based NHP formulations to alleviate the clinical signs of canine OA, and to identify the occurrence of adverse effects with multi-NHP preparations.

According to the present trial, medicinal herb-based NHP formulations improved the functional ability in dogs afflicted by naturally-occurring OA to a higher degree than placebo-control animals. When given once daily, improvements were noted as early as 4 weeks after the initiation of the alpha formulation administration, and were even better when the beta formulation was given for an additional 4-week duration. It has to be noted that the NHP dosing regimen in this trial was not constant across the entire dog’s body mass observed (i.e. alpha formulation 58 (10) mg/kg, beta formulation 76 (13) mg/kg). The manufacturer’s limitations in producing capsules with variable content in multi-NHP preparations support the necessary use of dosing by intervals.

The study primary endpoint was selected as the PVF measured using a force platform. Such an objective evaluation tool was previously used to measure the disability that characterized human OA patients as well as their response to treatment (Detrembleur et al., 2005; Gok et al., 2002; Messier et al., 1992; Schnitzer et al., 1993). Similarly, alterations from normality were detected in OA dogs based on the measurement of the PVF (Madore et al., 2007) while strong improvements in the pain-related limb disuse were reported for several therapeutic approaches including NSAIDs (Budsberg et al., 1999; Moreau et al., 2003), a dual inhibitor of cyclooxygenase and 5-lipoxgenase enzymes (Moreau et al., 2007), therapeutic diets (Moreau et al., 2012b; Rialland et al., 2013; Roush et al., 2010) and NHPs (Helm-Bjorkman et al., 2009; Moreau et al., 2004, 2012a).

The change over the initial condition [i.e., 2.6 (2.1)%BW provided by the medicinal herb-based NHP formulations is similar to common therapeutic approaches as recently reviewed (Moreau et al., 2012a). It outweighs the 95% minimal detectable change (MDC95), calculated as 2.0%BW for PVF in canine OA (Moreau et al., 2013). The MDC95 can be interpreted as the change magnitude, below which there are more than 95% chances that the change has occurred as a result of measurement error (Kovacs et al., 2008). Outside this cut-off point (i.e., lower than –2.0 or higher than 2.0 %BW), the change does reflect a real difference in the functional impairment toward worsening or improvement, in the canine natural OA model. Establishing such a cut-off point fulfills the requirement to define the magnitude of the measurement that corresponds to a clinically recognizable improvement in the individual animals, as previously criticized in a recent review (Sharkey, 2013).

The MDC95 can also serve as a responder criteria, similar to that developed for humans by the OARSI Standing Committee for Clinical Trials Response Criteria Initiative (Pham et al., 2004). According to Fig. 3, 46% (6/13) of the medicinal herb-based NHPs treated dogs were positive responders while negative responders were absent. At the opposite, 36% (5/14) of placebo-control dogs had more severe clinical signs while 36% (5/14) had improved.

In the present study, statistical analyses revealed a significant difference between groups according to the changes in PVF values with a statistical power of 60%. The magnitude of the therapeutic benefits was consistent with a moderate Cohen’s $d$ effect size of 0.7 (95% confidence interval: 0.0–1.5). The effect size is recognized as a simple and straightforward index to quantify the effects of an intervention relative to a comparator (Coe, 2012). However, effect sizes are not commonly reported in canine models of OA, which compromise comparisons among studies. Nevertheless, the effect size reported herein was similar to other therapeutic approaches including a therapeutic diet rich in omega-3 fatty acids of fish origin (Moreau et al., 2012b) as well as a plant extract from Brachystemma calycinum D don (Moreau et al., 2012a).

As previously demonstrated in this model of natural OA (Brown et al., 2010; Moreau et al., 2012a; Rialland et al., 2012, 2013), the usefulness of the continuous monitoring of daily locomotor activity was sustained in the present study. After an 8-week period of treatment with the NHP formulations, the DDAP was increased, reaching more than 1.5 h/day of additional time spent on daily life activities. This finding is consistent with a recent review of experimental data aimed to determine the relationship between the limb function (as reflected by the measurement of the PVF) and the locomotor activity recording (Moreau et al., 2013). Hence, the effect of an additional 54 minutes/day of activity is expected to be mirrored confidently by an increase in PVF measurement exceeding the MDC95 (Moreau et al., 2013). As reported herein, the effects of the medicinal herb-based NHP formulations might have been translated into more active dogs, being able to rehabilitate their pain-related limb disuse toward a better muscular strength. This increase in limb use led to dogs more willing to accentuate their limb support by an average of 1.0 kg. These findings sustain the beneficial role of activity in OA dogs. Nevertheless, the level of activity has to be low to moderate to avoid an exacerbation of lameness as reported after intense running (Beraud et al., 2010).

Unlike the objective measures of function, the CSOM did not document an improvement in NHP-treated dogs. The CSOM is a validated proxy method of assessment, which was shown to complement the information provided by the measurement of the PVF (Rialland et al., 2012, 2013). Hence, the CSOM reflects the behavioral aspects of the OA disease affliction as perceived by the owner based on day-to-day environment and situation. The CSOM was used in the present study as an attempt to mirror the dog’s quality of life over the 8 weeks. This was done however without knowing the level of functional improvement required to be translated into a better quality of life. The present results suggest at first glance a need for more effective therapy based on owner perception, recognized as less sensitive and more prone to placebo response bias (Conzemius and Evans, 2012; Moreau et al., 2013) or to changes in behavior or perception when being utilized as a proxy assessor. On the other hand, as OA is a lifelong disease, the limb impairment which occurs over several years may have compromised the sensitivity of the owner to detect an improvement in their dog. This is also supported by the relatively low value of CSOM at baseline, compared to other similar population samples (Rialland et al., 2012, 2013), inducing a risk of floor effect for CSOM masking the responsiveness to NHP treatment. Therefore, much time may be required by the owner to appreciate a better quality of life concomitantly to a functional improvement, as previously denoted in OA dogs after a 13-week treatment duration (Moreau et al., 2012b).
Our results indicate that treating with the medicinal herb-based NHPs did not result in a significant buccal mucosal bleeding time prolongation. This indicates that the platelet function was not affected by the treatment. Moreover, the NHP-treated dogs did not demonstrate clinically significant hematological or biochemical alterations when administered for 8 weeks. This result is encouraging for promoting the clinical use of multi-NHP preparations, but would require further confirmation on larger sample size.

Several limitations to this clinical trial study need to be acknowledged. First, the study duration was 8 weeks despite the chronic nature of OA. Second, the content and strength of the NHP capsules were based on empirical evidences (internal data files) suggesting anti-inflammatory and anti-nociceptive potential in rodent models of inflammation and pain. Whether or not the content and strength of the NHP capsule were optimal for dogs afflicted by naturally-occurring OA was unknown. Third, the design of the study did not allow conclusions about the respective potential of each NHP formulation (i.e., alpha versus beta formulation). Therefore the efficacy of the medicinal herb-based NHP formulations should be considered as a whole therapeutic regimen involving alpha followed by the beta formulations. Finally, whether or not the improvement denoted in OA dogs is consistent with disease modifying effects is unknown and should be addressed. Of note licofelone, a dual inhibitor of cyclooxygenase and 5-lipooxygenase enzymes, demonstrated similar functional improvement than the one observed with the NHP formulations in addition to a reduction in the progression of structural changes in experimental dog OA model induced by CCL sectioning (Boileau et al., 2002; Moreau et al., 2006).

This RCT provided evidence of the efficacy of a medicinal herb-based NHP in alleviating the clinical signs of canine OA. The present findings provide relevant and new information about the potential of medicinal phytochemical compounds as a therapeutic modality for human OA. Such NHP appears also interesting for the management of canine OA as not only clear benefits were demonstrated on the function, but also this NHP mixture (with low grade dosage of each component) was not associated with any clinical toxicity.

Acknowledgments

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References


An open-field study comparing an essential oil-based shampoo with miconazole/chlorhexidine for hair coat disinfection in cats with spontaneous microspororiasis

Simona Nardoni¹, Angela Grazia Costanzo¹, Linda Mugnaini¹, Francesca Pisseri², Guido Rocchigiani¹, Roberto Papini¹ and Francesca Mancianti¹

Abstract

Objectives The goal of the present study was to compare the antifungal efficacy of an essential oil (EO) shampoo proven to be effective against Microsporum canis with miconazole/chlorhexidine for topical hair coat disinfection in cats treated concurrently with oral itraconazole.

Methods Cats received treatment with oral itraconazole (Itrafungol) at a dose of 5 mg/kg/day pulse administration for 1 week, every 2 weeks for at least 6 weeks and were washed twice a week with a neutral shampoo with added EOs of Thymus serpyllum (2%), Origanum vulgare and Rosmarinus officinalis (5% each) for the period of systemic treatment. This protocol was compared with a conventional treatment (oral itraconazole + 2% miconazole/2% chlorhexidine shampoo).

Results The treatment was well tolerated and adverse effects were not recorded. All cats were clinically negative at week 11. With respect to animals with extensive lesions, the speed of resolution was higher in cats with focal lesions. The animals showing diffuse lesions required more than a course of treatment to achieve a mycological cure. There was no significant difference between the number of weeks to obtain mycological cure for cats treated with EOs and animals treated conventionally.

Conclusions and relevance The treatment appeared to be effective and well appreciated by the owners. The use of shampoo with the added EOs of T serpyllum, O vulgare and R officinalis would seem an interesting, natural alternative to conventional topical treatment.

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Introduction

Microsporum canis, a zoophilic and zoonotic dermatophyte that is highly infectious and has a broad host range, is the main responsible agent for dermatophytosis in cats worldwide. The infection, even if not life threatening, is highly contagious and spontaneous healing can require several months.¹

Therapeutic measures of feline microsporiosis should include the combination of systemic and topical treatment.² The main goal of local drug administration is to minimise the spreading of infective arthrospores, which are the source of both reinfection and new infections. Topical therapy is needed to disinfect the hairs, as systemic therapy combined with the host immune response eradicates the infection from the coat. Repeated disinfection is needed as the hair coat is reseeded with infective arthrospores until this occurs.

The most recent systemic treatment protocol licensed for use in cats in Europe is based on oral itraconazole 5 mg/kg/day pulse administration for 1 week, every 2 weeks,³ with a total treatment period of 6 weeks, while most commonly recommended topical options include rinses and shampoos. Many commercial rinse formulations containing enilconazole, lime sulfur, accelerated hydrogen peroxide and miconazole/chlorhexidine are available. These products have been tested and reviewed

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both in vitro and in vivo, with excellent results, and as no rinsing is required, their administration is advisable in multiple cat situations. Some (enilconazole, lime sulfur) are indicated as first-choice options. However, shampoo combines the antifungal effect and the physical act of shampooing, helping to remove fungal propagules, and is recommended in animals kept as pets. A combined 2% miconazole/chlorhexidine shampoo is largely available on the market and has been proven to be effective.

In recent years, the interest in selecting sustainable products from landscape plants has increased and some data are available for M canis, indicating that a number of chemically defined essential oils (EOs) from several plants can yield antifungal activity both in vitro and in vivo. In particular, EOs derived from Thymus serpyllum, Origanum vulgare and Litsea cubeba have shown strong efficacy against several dermatophyte species. Such plant-derived compounds are of interest as they have not yet been manipulated by pharmaceutical industry. Herbal remedies are usually well accepted by pets and owners, and many owners are interested in alternative treatments.

The goal of the present study was to compare the antifungal efficacy of an EO-based shampoo with miconazole/chlorhexidine for topical hair coat disinfection in cats treated with oral itraconazole.

Materials and methods
Cats
Fourteen symptomatic cats affected by spontaneous dermatophytosis caused by M canis were included in the study after obtaining informed consent from the owners. The animals were of both sexes, of different breeds (11 domestic shorthairs and three Persians), with ages ranging from 3 months to 8 years.

Fungal infection was confirmed by direct hair examination, Wood lamp examination when possible and culture. Mycotic load was evaluated by counting colony-forming units (CFUs) as previously described, indicating each infection as heavy (≥50 CFUs/plate), mild (49–5 CFUs) and low (<5 CFUs).

Inclusion/exclusion criteria
Inclusion criteria were the presence of local or generalised lesions due to M canis, associated with positive culture (heavy or mild) of hair drawn with the brush technique, achieved on Sabouraud CAF agar + actidione (Liofilchem). Animals treated from less than 6 months before the inclusion day and/or with concomitant dermatoses were not admitted to the study.

Scoring and monitoring
Skin lesions were evaluated at day 0 and scored. In detail were considered ease of epilation (1 = within normal limits; 2 = mild but excessive; 3 = moderate; 4 = severe and extensive), degree of seborrhea (1 = none; 2 = mild; 3 = moderate; 4 = severe) and extent of the primary lesions (1 = none; 2 = single, small area; 3 = more than one small area; 4 = extensive lesions). The three scores were then added to give a total lesion score. The number of skin lesions ranged from four to 12 for the two groups. The occurrence of human infection (n = 7) was also recorded. The owners of Persian cats were advised to clip their cats. Detailed data are reported in Tables 1 and 2.

Study design
Diagnosis was achieved in different private clinics. Once diagnosed the cats enrolled in the present open study were assigned to two different groups at one time. The treatment took place at home. Cats in group 1 received oral itraconazole (Itrafungol; Eli Lilly Italia) at a dose of 5 mg/kg/day pulse administration for 1 week, every 2 weeks, with a total treatment of at least 6 weeks and they were washed twice a week with about 5 ml of a neutral shampoo with the added EOs of T serpyllum (2%), O vulgare and R officinalis (5% each). The EOs were provided by Flora; their chemical composition and proven antymycotic activities have been reported elsewhere. The shampoo was immediately removed by rinsing after the application.

Cats in group 2 received oral itraconazole at the same dose plus 2% miconazole/2% chlorhexidine shampoo (Malaseb; Eli Lilly Italia) twice a week for the period of systemic treatment. The shampoo was left on the hair coat for 10 mins before rinsing with warm water. All treatments were administered by one of the authors (AGC). Cats were examined weekly to evaluate an improvement of their clinical status and a fungal culture was achieved by brush technique. The animals were treated until they had two negative consecutive weekly cultures, and the protocol treatment was stopped at week 44.

Statistical analysis between the groups was performed by means of Wilcoxon, and Mann–Whitney tests to evaluate significant differences in weeks needed to obtain mycological cure. Statistical significance was defined as P <0.01. When not all study subjects reach mycological cure, the adjusted Kaplan–Meier method was applied to evaluate the probability of healing.

To avoid passive contamination of hair coat, environmental mycotic pollution was monitored by the use of both an air sampler (Sas super-100 Air Sampler; PBI) and contact plates, as previously reported. The owners were advised to clean the environment thoroughly by vacuuming following by a deep clean with disinfectants available commercially in Italy. Cultural controls were repeated weekly.
Table 1 Anamnestic and clinical data in treated cats, and lasting outcome of therapies

<table>
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<td>5</td>
<td>7</td>
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(++) 5-49 CFUs per plate; (+++) ≥50 CFUs per plate
CFU = colony-forming unit; DSH = domestic shorthair; F = female; P = Persian; NE = not executed; M = male
Results

All enrolled cats had never been treated with antifungal drugs, except for cat 3 (group 1), who had been treated with griseofulvin and then with ketoconazole, without any improvement of clinical and mycological features. Antimycotic treatments had been stopped about 1 year before the beginning of the present study.

Both treatments were well tolerated and adverse effects were not recorded. In group 1, two animals were clinically healthy at week 3 post-treatment, while all other cats were clinically healthy at week 11. All cats were culturally negative at the end of the trial. In group 1 cats, mean time to clinical and mycological cure was 6 weeks (median 4 weeks, range 3–11 weeks) and 15 weeks (median 14 weeks, range 7–42 weeks), respectively.

One cat in group 2 was dermatologically normal by week 3, while all cats were clinically cured at week 10. By the end of the study 6/7 animals were negative on fungal culture. For cats in group 2, the mean time to clinical and mycological cure was 5.9 weeks (median 6 weeks, range 3–10 weeks) and 12.8 weeks (median 6 weeks, range 7–21 weeks), respectively.

With respect to cats with extensive lesions, as expected, the speed of resolution was higher in cats with focal lesions, ranging from 3 to 4 weeks in group 1 cats and from 3 to 5 weeks in group 2 cats. Cats with diffuse lesions required more than one course of treatment (requiring 2–6 treatments for both groups) to achieve a mycological cure. A reduction in CFUs was observed by week 3 post-treatment in all examined cats. All cats with focal lesions had healed culturally at week 7. After the start of treatment no cases of new human infection or reinfection were reported. Two Persian cats out of three were randomly assigned to group 1; only one was clipped (the other Persian cat from group 2 was clipped as recommended).

With regard to aetiological cure, a significant difference between treatments was not observed ($z$ score $-0.1429$; $U = 19.5$), even if the probability of healing at week 42 was 46% more for cats in group 1.

Detailed data on treatment outcome are reported in Tables 1 and 2.

Discussion

Treatment with shampoo with added EOs yielded results comparable with conventional therapy. Owing to the lack of contact time required, it was particularly appreciated by the owners; the miconazole/chlorhexidine shampoo had to be left on the cats’ coats for 10 mins, and some cats exhibited nervous behaviour during this time.

Studies on the use of antifungal rinses to control dermatophytosis have been conducted on cats, both naturally and experimentally infected, living in catteries and other communities. To the best of our knowledge, our trial is the first study carried out on owned cats. We are aware of our small sample size, but this was due to the difficulty in the simultaneous recruitment of pet cats that met the inclusion criteria. Although the epidemiology and clinical situation in shelters is more controlled and homogeneous, such studies can not be fully applicable to indoor cats, living in very close contact with people, including children, in an environment with furniture, curtains, cushions and other household items. The best treatment protocol is difficult to identify, depending on the number of cats involved, the owner’s resources and global health

Table 2 Clinical score data before and after treatment in cats from groups 1 and 2

<table>
<thead>
<tr>
<th>Cat</th>
<th>group</th>
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<th>Degree of seborrhoea</th>
<th>Extent of the primary lesions</th>
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of cats, so the use of shampooing in such animals is advisable, while in catteries the rinse is more useful.

In the present study the determination of CFUs was applied to evaluate the efficacy of local treatment and the capacity of active compounds to limit the spreading of arthrospores. Both local treatments were able to decrease heavy and mild mycotic loads until elimination of arthrospores on the hair coat. An effective topical treatment together with correct management of environmental disinfection are of primary importance to cure dermatophytoses and to avoid reinfection and/or new infections. Therefore, in our study no relapses or new cases of human infections were recorded during the observation period.

Considering their potential toxicity, EOs should be carefully administered in animals, especially in cats. Oils from *Thymus* species are toxic when administered orally, and carvacrol and thymol, the main components of both *Thymus* and *Origanum* oils are skin sensitisers and antigens, so the use of these oils undiluted should be avoided. Rosemary oil is considered safe for mammals, although chronic exposure to rosemary oil at high concentrations has rarely been reported to cause contact dermatitis; acute toxicity of rosemary oil has not been reported. In general, toxicity testing is concerned with pure single oils rather than mixtures. In the present study, EOs as a mixture were administered to optimise their efficacy and to minimise toxic effects.

Nevertheless, even if EOs, properly diluted, are generally safe, attention must be paid to use chemically defined compounds under the supervision of a skilled phytotherapist.

**Conclusions**

On the basis of our observations the use of shampoo with the added EOs of *T serpyllum*, *O vulgare* and *R officinalis* would seem a natural and interesting alternative to conventional topical treatment.

**Conflict of interest** The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**References**

**In vitro effects of Yunnan Baiyao on canine hemangiosarcoma cell lines**

K. A. Wirth, K. Kow, M. E. Salute, N. J. Bacon and R. J. Milner

Department of Clinical Sciences, University of Florida, Gainesville, FL, USA

**Abstract**

*Yunnan Baiyao* is a Chinese herbal medicine that has been utilized for its anti-inflammatory, haemostatic, wound healing and pain relieving properties in people. It has been utilized in the veterinary profession to control bleeding in dogs with hemangiosarcoma (HSA) and has been anecdotally reported to prolong survival times in dogs with this neoplasm. This study evaluated the *in vitro* activity of *Yunnan Baiyao* against three canine HSA cell lines after treatment with increasing concentrations of *Yunnan Baiyao* (50, 100, 200, 400, 600 and 800 μg mL⁻¹) at 24, 48 and 72 h. Mean half maximum inhibitory concentration (IC₅₀) at 72 h for DEN, Fitz, SB was 369.9, 275.9 and 325.3 μg mL⁻¹, respectively. Caspase-3/7 activity increased in correlation with the IC₅₀ in each cell line which was confirmed by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL, APO-BRDU Kit; BD Biosciences, San Jose, CA, USA) assay. VEGF in cells supernatant was also quantified. Overall, the study found that *Yunnan Baiyao* causes dose and time dependent HSA cell death through initiation of caspase-mediated apoptosis, which supports future studies involving *Yunnan Baiyao*.

**Keywords**
canine hemangiosarcoma, Chinese herbal medicine, *Yunnan Baiyao*

**Introduction**

Hemangiosarcoma (HSA) is a highly malignant neoplasm of vascular endothelial cell origin. HSA is a relatively common neoplasm in the dog, accounting for up to 21% of all soft tissue sarcomas and 0.3–2% of all malignant tumours in this species.¹⁻⁴ The incidence of disease is significantly higher in large breed dogs such as German Shepherds, Golden and Labrador Retrievers.⁴⁻⁷ HSA can affect any tissue in the body; however, the spleen is the most common site of tumour development, accounting for 50–65% of all canine HSAs.² HSA is also the most common primary cardiac tumour and tumours of the right atrium account for 3–25% of all HSAs in the dog.⁸ Other common sites include the subcutaneous tissues (13–17%) and the liver (5–6%).⁹ Canine HSA is an aggressive malignancy, characterized by pathologic angiogenesis and early, aggressive metastasis that is poorly chemo-sensitive.³⁻⁹⁻¹⁹ Previously reported prognostic factors for canine HSA include location (cutaneous versus viscera), histological grading and stage.⁵⁻¹⁹ Despite available multi-modal therapies to address local and systemic disease, few patients survive beyond 6 months with most succumbing to symptoms associated with metastatic disease.

Malignant tumours of the vascular endothelium are rare in humans; however, this type of cancer is extremely aggressive when it does occur. HSA, also called angiosarcoma, accounts for approximately 2% of soft tissue sarcomas in humans and most commonly occurs in liver, spleen, breast and scalp. As in dogs, this tumour frequently metastasizes and despite multimodal treatment, 5-year survival rates remain between 10 and 35%.²⁰⁻²⁴

The lack of effective adjuvant therapies warrants the investigation of novel treatment options and in recent years, traditional Chinese medicine (TCM) has been receiving increased attention for the treatment of malignant neoplasia. *Yunnan Baiyao* is an herbal TCM that has been used frequently by veterinarians and their clients as an adjunctive treatment
for canine HSA. It has been anecdotally reported to prolong survival times and control bleeding in dogs with this aggressive neoplasm.

*Yunnan Baiyao* is a well-known Chinese herbal patent formula that has been utilized for its anti-inflammatory, haemostatic, wound healing and pain relieving properties in people for over 100 years. It was developed in the Yunnan Province of China around 1902 and gained popularity among Chinese soldiers during World War II for use as a haemostatic agent on the battlefield.\(^{25,26}\) *Yunnan Baiyao* has been shown to improve clotting and enhance platelet function.\(^{26-30}\) This may benefit canine patients with HSA due to the frequency of clotting abnormalities and potential for fatal haemorrhage although this was not evaluated in this study.

*Yunnan Baiyao* is a class-I protected TCM and the exact herbal formula is a trade secret. Due to this protected status, component analysis and quality control measures for *Yunnan Baiyao* have been slow to develop; however, due to international demand for quality assurance and the development of Good Manufacturing Practice (GMP), the product is now labelled to identify its major components per 0.5 g serving.\(^{31}\) The following ingredients are listed based on 2011 manufacturer’s label: 200 mg Tienchi ginseng root (*Panax notoginseng*), 85 mg Ajuga forrestii Diels plant, 66.5 mg Chinese yam root, 57.5 mg Dioscoreae nipponica Makino root, 36 mg Erodium stephanianum and Geranium wilfordii plant, 30 mg Dioscoreae parvilora ting root and 25 mg Inula cappa plant (*Yunnan Baiyao; Yunnan Baiyao Group, Kunming, China*).

There is a vast body of scientific literature showing that components of *Yunnan Baiyao* have various anti-cancer properties; however, studies on *Yunnan Baiyao* itself as an anti-cancer therapy have not been previously performed.\(^{32-37}\)

*Panax notoginseng root extract* (NGRE), which is a major component of *Yunnan Baiyao*, showed significant growth inhibition and increased apoptosis of SW480 human colorectal cancer cells *in vitro*. NGRE also enhanced cell growth inhibition when combined with either 5-fluorouracil or irinotecan.\(^{32}\) The saponin ginsenoside Rd, isolated from *P. notoginseng*, was shown to inhibit proliferation of human cervical cancer (HeLa) cells in *vitro* and induce apoptosis by upregulation of Bax, downregulation of Bcl-2 and activation of the caspase-3 pathway.\(^{33}\) Additionally, *P. notoginseng* has been documented to inhibit DNA synthesis and cell proliferation in human umbilical vein endothelial cells (HUVEC) *in vitro*.\(^{34,35}\)

Wild yam root (*Dioscoreae spp.*), another major component of *Yunnan Baiyao*, was shown to have the most potent effects on cell viability and induction of apoptosis in a murine malignant neuroblastoma cell line when compared with 373 other naturally derived herb, seed, root, plankton and fungi extracts.\(^{36}\) Wild yam root has also been shown to induce anti-proliferative and pro-apoptotic effects in a range of tumour cells by G2/M arrest, downregulation of NF-κB, Akt, cyclin D, c-myc and initiating PARP cleavage/DNA fragmentation.\(^{36}\) *Dioscoreae nipponica* extract exerted dose dependent inhibition on the invasion, motility, secretion of MMPs and u-PA in murine melanoma (B16F10) and human melanoma (A2058) cells *in vitro*.\(^{37}\) It was also shown to inhibit activation of NF-κB and increase expression of I-κB in the B16F10 cells *in vitro*. Additionally, lung metastasis formation was significantly reduced in mice treated with the extract versus the control group *in vivo* in the same study.\(^{37}\)

Novel therapeutic options are needed if we hope to improve outcomes associated with canine HSA. Studies on the anti-cancer properties of *Yunnan Baiyao* components combined with anecdotal evidence to its efficacy suggest that it may enhance the traditional medical approach to treatment of canine HSA. This study aims to take the first step in evaluating the biological activity of *Yunnan Baiyao* against canine HSA cells *in vitro*. We studied *Yunnan Baiyao*’s ability to inhibit growth of canine HSA cells and to induce apoptosis. Cell survival assays were performed for HSA cell lines exposed to *Yunnan Baiyao*. Apoptosis was investigated by measuring caspase-3/7 activity and the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). Changes in cell cycle kinetics were evaluated using flow cytometry. Due to the association of increased VEGF levels in dogs with HSA,\(^{38}\) we also investigated levels of VEGF found in supernatant from untreated and *Yunnan Baiyao* treated HSA cells. The information gained from this...
study will be used to establish a proof-of-concept for clinical use as well as support for further in vitro investigation of the use of Yunnan Baiyao as a novel anti-cancer agent.

Materials and methods

Cell cultures

Three established canine HSA cell lines were evaluated: DEN-HSA, Fitz-HSA (provided by Dr Ilene Kurzman, University of Wisconsin, Madison, WI, USA) and SB-HSA (provided by Dr Stuart Helfand, Oregon State University, Corvallis, OR, USA). DEN was established from a renal HSA from a Golden Retriever, Fitz was from a splenic HSA of a Golden Retriever and SB was obtained from a subcutaneous HSA of a German Shepherd dog. It has recently been shown that DEN and Fitz were derived from the same source. This does not mean that DEN and Fitz might not show differences in drug sensitivity as they have been cultured as separate cell lines for several years now and may have ‘drifted’ apart. All cell lines were cultured under standard conditions (37°C, 5% CO2, humidified air). DEN and Fitz were maintained in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (Cellgro, Mediatech, Manassas, VA, USA). SB was maintained in Roswell Park Memorial Institute (RPMI, Buffalo, NY, USA) medium supplemented with 10% heat-inactivated FBS, sodium pyruvate, L-glutamine, HEPES, penicillin AND streptomycin.

Yunnan Baiyao preparation

Yunnan Baiyao (Yunnan Baiyao Group) was generously provided as a stock powder (4 g per vial) by Dr Shen Huisheng Xie (University of Florida, Gainesville, FL, USA). A 200 mg mL⁻¹ stock solution was prepared in 0.1% dimethylsulfoxide (DMSO) at room temperature, vortexed for 5 min and filtered with a 0.22 μm filter. Aliquots of the stock solution were stored at −20°C and protected from light. Dilutions of the stock solution were prepared immediately prior to use in cell culture medium such that the DMSO concentration did not exceed 1%.

Evaluation of cell viability

The DEN and Fitz cells were plated at 5000 per well and SB cells were plated at 10 000 per well in 100 μL media in 96-well flat-bottom plates (Falcon, Becton Dickinson Bedford, MA, USA). The plates were incubated under standard conditions for 24 h. After 24 h, Yunnan Baiyao was added to the wells at increasing concentrations (50, 100, 200, 400, 600 and 800 μg mL⁻¹) in 100 μL media solution. Control wells were prepared for each assay containing media with 1% DMSO only or 800 μg mL⁻¹ Yunnan Baiyao in 100 μL media solution. After incubation times of 24, 48 or 72 h, the relative viable cell number was assessed using a one-step tetrazolium-based (MTS) colorimetric assay (CellTiter-Blue Cell Viability Assay, Promega, Madison, WI, USA) in accordance with the manufacturer’s specifications. Fluorescence was quantified with a fluorescence plate reader at an excitation wavelength of 530 nm and emission wavelength of 590 nm. Relative viable cell number was assessed by means of triplicate wells for each drug concentration and triplicate wells for each control, and each experiment was repeated three times.

Effect of Yunnan Baiyao on apoptosis

To measure and characterize cell death, the effects on caspase-3/7 activity were assessed as an important signalling and effector step in the apoptotic cascade. The DEN and Fitz cells were plated at 5000 per well and SB cells were plated at 10 000 per well in 100 μL media in 96-well flat-bottom plates (Falcon, Becton Dickinson). The plates were incubated under standard conditions for 24 h. After 24 h, Yunnan Baiyao was added to the wells at increasing concentrations (50, 100, 200, 400, 600 and 800 μg mL⁻¹) in 100 μL media solution. Control wells were prepared for each assay containing cells and media with 1% DMSO only or 800 μg mL⁻¹ Yunnan Baiyao in 100 μL media solution. After incubation for 24, 48 or 72 h, caspase-3/7 activity was measured using a commercial assay (Apo-ONE Homogeneous Caspase-3/7 Assay; Promega) performed in accordance with the manufacturer’s specifications. Fluorescence was quantified with a fluorescence plate reader at an excitation wavelength of 485 nm and emission...
wavelength of 528 nm. All samples were analysed in triplicate, and each experiment was repeated three times with each of the cell lines.

TUNEL assay

Detection of fragmented DNA, one of the later steps in apoptosis, was performed using a TUNEL assay (APO-BRDU Kit; BD Biosciences, San Jose, CA, USA). Cells were plated into six-well plates (50 000 per well DEN, 75 000 per well Fitz and 100 000 per well SB) and placed in the incubator under standard conditions for 24 h. After 24 h, *Yunnan Baiyao* was then added to the wells (50, 100, 200, 400, 600 and 800 μg mL\(^{-1}\)). Control wells were prepared for each assay containing cells and media with 1% DMSO. After incubation, the cells were fixed with 1% (w/v) paraformaldehyde in phosphate buffered saline (PBS) and kept at −20 °C until assayed. The commercial assay was performed in accordance with the manufacturer's specifications. The APO-BRDU kit is a two-colour staining method for labelling DNA breaks and total cellular DNA in order to detect apoptotic cells by flow cytometry. Apoptotic cells with exposed 3′-hydroxyl DNA ends were labelled with brominated deoxyuridine triphosphate nucleotides (BR-dUTP). FITC labelled anti-BrdU mAb provided by the commercial kit was then used to stain apoptotic cells. Propidium iodide (PI) was used as a counterstain to label total cellular DNA for cell cycle analysis. Flow cytometry was performed using a flow cytometer (FACSort; BD Biosciences) with a green fluorescence (520 nm) and a red fluorescence (623 nm) detection. Data were processed by use of Cell Quest software (Cell Quest software, version 3.3; BD Biosciences). The samples were pooled and this assay was performed as a single run for all three cell lines at 24, 48 and 72 h. The percentage of apoptotic cells and cell cycle kinetics were evaluated.

VEGF enzyme linked-immunosorbent assay

A commercial enzyme linked-immunosorbent assay (ELISA) kit (Quantikine Canine VEGF ELISA Kit; R&D systems, Minneapolis, MN, USA) was used to measure VEGF levels in the cell culture supernatants before and after treatment with *Yunnan Baiyao*. The kit contains Sf21-expressed, recombinant VEGF and antibodies raised against the recombinant protein. Results obtained for naturally occurring canine VEGF show linear curves that are parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural canine VEGF.39 In brief, HSA cell lines were plated into six-well plates (50 000 per well DEN, 75 000 per well Fitz and 100 000 per well SB) and placed into the incubator under standard conditions for 24 h. Then *Yunnan Baiyao* (50, 100, 200, 400, 600 and 800 μg mL\(^{-1}\)) was added to the wells. Untreated (control) wells containing cells only were also plated. After incubation for an additional 24, 48 or 72 h the supernatant was removed, centrifuged and stored at −20 °C until assayed. The samples were added in duplicate to a 96-well plate and the VEGF immunoassay was performed in accordance with the manufacturer's specifications. All samples were run in duplicate and calibration on the microtitre plate included a standard series of dilutions of recombinant human VEGF. The optical density of the standard solutions was plotted against their corresponding concentrations to generate a standard curve and allow determination of all VEGF concentrations. All samples were analysed at the same time. This assay has been previously validated for measurement of canine VEGF.39

Statistical analysis

Statistical analyses were performed with Sigma-Plot software (SigmaPlot for Windows, version 12.5; Systat Software, Erkrath, Germany). Cell survival data were fitted to a four-equation regression model to determine the mean half maximum inhibitory concentration (IC\(_{50}\)) for each cell line. The IC\(_{50}\) was defined as the drug concentration that caused 50% cell death compared with the control. For the cell viability assay and caspase-3/7 assay, a two-way analysis of variance (ANOVA, two-factor repetition) was used to determine whether time and concentration had an effect on cell viability and caspase-3/7 activity, and pair-wise multiple comparisons procedures (Hom-Sidak method) were performed for post hoc analysis. To account for changes in cell number which may influence...
levels of apoptosis, the reading was normalized to the cell viability of non-untreated cells at the same time-point under investigation. For the VEGF assay, a one-way ANOVA was used to determine if time had an effect on median VEGF concentrations of controls incubated for 24, 48 and 72 h. A two-way ANOVA was then used to analyse if *Yunnan Baiyao* concentration had an effect on mean VEGF levels for all three cell lines treated at 72 h. To account for changes in cell number which may influence VEGF levels, the reading was normalized to the cell viability of non-treated cells at the same time-point under investigation. Overall significance was set at *P* = 0.05.

**Results**

**Effects of *Yunnan Baiyao* on cell viability**

For all three canine HSA cell lines, cell viability decreased after incubation with higher concentrations of *Yunnan Baiyao* at 24, 48 and 72 h. (see Fig. 1A–C). For the DEN cell line, a significant decrease in cell viability was found at ≥400 μg mL⁻¹ concentrations at 24 h, and at ≥200 μg mL⁻¹ concentrations at 48 and 72 h (*P* < 0.001). For the Fitz cell line, a significant decrease in cell viability was found at ≥400 μg mL⁻¹ concentrations at 24 and 48 h, and at ≥200 μg mL⁻¹ concentrations at 72 h (*P* < 0.001). For the SB cell line, a significant decrease in cell viability was found at ≥400 μg mL⁻¹ concentrations at 24 and 48 h, and at ≥200 μg mL⁻¹ concentrations at 72 h (*P* < 0.001).

Cell viability data were fitted to a four-equation regression model in order to determine the IC₅₀ for each cell line (see Table 1). The IC₅₀ values at 72 h were 275.9 and 325.3 μg mL⁻¹ for the Fitz and SB cell lines, respectively. The IC₅₀ was slightly higher at 369.3 μg mL⁻¹ for the DEN cell line at 72 h. The correlation coefficient or *R*² value was evaluated to determine the goodness of fit of the derived values for each dose response curve. The mean *R*² value for DEN, Fitz and SB was 0.98 at 72 h where unity is considered a perfect correlation.

The duration of *Yunnan Baiyao* incubation time (24, 48 and 72 h) was found to be a significant factor (*P* < 0.001) in mean cell viability for all three cell lines, with the proportion of cell viability of *Yunnan Baiyao* treated cells to cell viability of the control samples decreasing with time. Time was found to be a significant factor for concentrations ≤200 μg mL⁻¹ for the DEN and Fitz cell lines and at ≤100 μg mL⁻¹ for the SB cell line. Time was no longer a factor at concentrations ≥400 μg mL⁻¹ for all three cell lines.

**Effects of *Yunnan Baiyao* on apoptosis**

**Caspase-3/7**

Overall, the duration of *Yunnan Baiyao* incubation time and concentration were significant (*P* < 0.001) factors in the mean caspase-3/7 activity (apoptosis) for all cell lines (see Fig. 2A–C). For the DEN cell line, significant increases in caspase-3/7 were found at ≥400 μg mL⁻¹ concentrations (*P* < 0.001) at 24, 48 and 72 h. For the Fitz cell line, significant increases in caspase-3/7 activity were found at ≥400 μg mL⁻¹ for 24 and 48 h, and at ≥200 μg mL⁻¹ at 72 h (*P* < 0.001). For the SB cell line, significant increases in caspase-3/7 activity were found at ≥600 μg mL⁻¹ for 24 h, ≥400 μg mL⁻¹ at 48 h, and at ≥200 μg mL⁻¹ at 72 h (*P* < 0.001). This suggests that the SB cell line may be more sensitive to the effects of *Yunnan Baiyao* than the other two cell lines.

Of note is that the caspase-3/7 activity relative to the number of viable cells increased significantly compared with the control sample at close approximation with the IC₅₀ of each cell line (see Table 1 and Fig. 2A–C). The duration of incubation time with *Yunnan Baiyao* was also a significant factor in caspase-3/7 activity in all three cell lines (*P* < 0.001).

**TUNEL assay**

Flow cytometry was used to detect the number of TUNEL-positive cells as a measure of percentage of apoptotic cells in the population (see Table 2 and Fig. 3). Samples for all three cell lines at 24, 48 and 72 h were pooled and the TUNEL assay was performed as a single experiment in order to minimize cost and sample processing time. No appreciable levels of apoptosis were noted in the control samples or in cells treated at *Yunnan Baiyao* concentrations ≤100 μg mL⁻¹. However, there was a statistically significant (*P* < 0.001) increase in the percentage of apoptotic cells at concentrations ≥200 μg mL⁻¹ for all cell lines incubated for 72 h (see Table 2). Specifically, at the 200 μg mL⁻¹
Figure 1. *Yunnan Baiyao* causes a concentration dependant decrease in HSA cell viability over time as measured by the CellTitre-Blue Cell Viability Assay. An increase in fluorescent signal is correlated with an increase in viable cells. Control samples are designated as 24 h (*), 48 h (**) and 72 h (***) . Error bars represent standard deviation (SD). A statistically significant decrease in cell viability compared with untreated control sample at the corresponding time point is represented on the graph by *, ** and *** for 24, 48 and 72 h, respectively. (A) DEN cell line treated with increasing concentrations of *Yunnan Baiyao*. (B) Fitz cell line treated with increasing concentrations of *Yunnan Baiyao*. (C) SB cell line treated with increasing concentrations of *Yunnan Baiyao*.

concentration the percentage of apoptotic cells (TUNEL-positive) was 19.63, 56.34 and 86.47% for DEN, Fitz and SB, respectively. The percentage increased to 90.63, 99.69 and 98.98% for DEN, Fitz and SB (respectively) at the 400 μg mL⁻¹ concentration; then remained high at 600 and 800 μg mL⁻¹ (see Table 2). Curiously, there was also a statistically significant (*P* < 0.001) increase in percentage of apoptotic cells at the 50 and 100 μg mL⁻¹ concentrations at 24 h for the SB cell line which was not noted in the other cell lines (data not shown).

Overall, the greatest percentage change in apoptosis occurred between 200 and 400 μg mL⁻¹ for DEN, Fitz and SB which correlates with the calculated IC₅₀ for all three cell lines (see Table 1 and Fig. 3).

**Cell cycle analysis**

Cell cycle analysis was performed on data recorded for all three cell lines at 24, 48 and 72 h (see Fig. 4A–C). The DEN cell line (see Fig. 4A) when
Table 1. Cell viability data were fitted to a four-equation regression model in order to determine the IC₅₀ for each cell line.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN (µg mL⁻¹)</td>
<td>313.4</td>
<td>313.4</td>
<td>369.3</td>
</tr>
<tr>
<td>Fitz (µg mL⁻¹)</td>
<td>356.5</td>
<td>285.8</td>
<td>275.9</td>
</tr>
<tr>
<td>SB (µg mL⁻¹)</td>
<td>497.6</td>
<td>414.4</td>
<td>325.3</td>
</tr>
</tbody>
</table>

The IC₅₀ for the Fitz and SB cell lines decreased with increasing exposure time to Yunnan Baiyao. The IC₅₀ for the DEN cell line slightly increased at 72 h when compared with the 24 and 48 h time points.

In cell viability with increasing concentrations of Yunnan Baiyao, the IC₅₀ of the Fitz cell line lacked statistical significance at 100 (±0.1) × 10⁻¹, was 3170 (±1.9) × 10⁻¹, which was significant considering the control cell concentration was only 58.1 (±2.2) pg mL⁻¹ (see Table 3). The Fitz cell line also showed a statistically (P < 0.001) significant fold increase from baseline in VEGF levels when compared with control cells at 100 (±3.7 × 10⁻¹), 200 (±4.5 × 10⁻¹), 600 (±3.8 × 10⁻¹) and 800 µg mL⁻¹ (±4.0 ± 0.3) of Yunnan Baiyao. Nevertheless, the fold increase for Fitz was lower when compared with the SB cell line. Both SB and the Fitz cell line lacked statistical significance at 400 µg mL⁻¹ of Yunnan Baiyao. The SB cell line also lacked significance at 800 µg mL⁻¹ which was due to the wide standard deviation from the mean (see Fig. 5).

Discussion

This study showed that Yunnan Baiyao causes time and concentration dependant death of canine HSA cells. The Cell Titer Blue results showed a decrease in cell viability with increasing concentrations of Yunnan Baiyao. Results from the APO-ONE caspase-3/7 and TUNEL assays suggested that this decrease in cell viability occurred due to apoptosis. Caspase-3 activation occurs downstream of both the extrinsic and intrinsic apoptotic pathways; thus, should reflect the amount of apoptosis occurring regardless of the pathway. In this study, caspase-3/7 activity was shown to increase in correlation with the IC₅₀ consistently in each cell line which was confirmed by the TUNEL assay. These results suggest that caspase-mediated apoptosis is a mechanism of cell death in all three cell lines. The TUNEL assay showed an increase in the percentage of cells undergoing apoptosis as...
Figure 2. Yunnan Baiyao causes a concentration dependant increase in caspase-3/7 activity in HSA cells over time as measured by the Apo-ONE Homogenous Caspase-3/7 Assay. An increase in fluorescent signal is correlated with an increase in caspase-3/7 activity which is an important signalling and effector step in the apoptotic cascade. The results are expressed as a ratio of change compared with the baseline apoptosis measured in the control at 24, 48 and 72 h (dotted line represents baseline of 1). Control samples are designated as 24 h (′), 48 h ( ′′) and 72 h ( ′′′). Error bars represent standard deviation (SD). A statistically significant increase in caspase-3/7 activity compared with the untreated control sample at the corresponding time point is represented on the graph by ′, ′′ and ′′′ for 24, 48 and 72 h, respectively. (A) Level of apoptosis measured in the DEN cells treated with increasing concentrations of Yunnan Baiyao. (B) Level of apoptosis measured in the Fitz cells treated with increasing concentrations of Yunnan Baiyao. (C) Level of apoptosis measured in the SB cells treated with increasing concentrations of Yunnan Baiyao.

the concentration increases in correlation with the APO-ONE caspase-3/7 results. This suggests that later mechanisms in the apoptotic cascade, such as DNA fragmentation are also involved in inhibition of HSA cell growth by Yunnan Baiyao. The mechanism by which Yunnan Baiyao causes apoptosis has not been elucidated. It is possible that Yunnan Baiyao could cause blockage of a receptor that triggers initiation of apoptotic pathways through downregulation of anti-apoptotic factors or upregulation of apoptotic factors which has been previously shown with P. notoginseng. Another possibility is that it may directly alter downstream signalling proteins in the apoptotic pathway.

Cell cycle analysis did show some minor change in cell cycle kinetics. The changes were not inconsistent with normal cell cycling. No evidence was found to indicate cell cycle arrest was present in
Table 2. This table demonstrates the percentage of apoptotic cells as detected by the APO-BRDU Kit for cells incubated for 72 h at increasing Yunnan Baiyao concentrations.

<table>
<thead>
<tr>
<th>Yunnan Baiyao concentration (mg mL⁻¹)</th>
<th>Den (%)</th>
<th>Fitz (%)</th>
<th>SB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.29</td>
<td>0.20</td>
<td>0.08</td>
</tr>
<tr>
<td>50</td>
<td>1.91</td>
<td>2.76</td>
<td>0.41</td>
</tr>
<tr>
<td>100</td>
<td>4.68</td>
<td>7.01</td>
<td>6.47</td>
</tr>
<tr>
<td>200</td>
<td>19.63*</td>
<td>56.34*</td>
<td>86.47*</td>
</tr>
<tr>
<td>400</td>
<td>90.63*</td>
<td>99.69*</td>
<td>98.98*</td>
</tr>
<tr>
<td>600</td>
<td>100.00*</td>
<td>99.81*</td>
<td>98.78*</td>
</tr>
<tr>
<td>800</td>
<td>100.00*</td>
<td>99.78*</td>
<td>99.84*</td>
</tr>
</tbody>
</table>

A significant increase in apoptosis occurred at Yunnan Baiyao concentrations ≥200 μg mL⁻¹ in each cell line (DEN, Fitz and SB).

*Represents a statistically significant increase in apoptotic cells compared with the untreated control sample.

Figure 3. This graph demonstrates the percentage of apoptotic cells as detected by the APO-BRDU Kit for cells incubated for 72 h at increasing Yunnan Baiyao concentrations. A significant increase in apoptosis occurred at Yunnan Baiyao concentrations ≥200 μg mL⁻¹ in each cell line (DEN, Fitz and SB).

VEGF levels in cell supernatant were measured in untreated (control) cells and found to increase over time for all three cell lines. Although the VEGF levels for SB were negligible at 24 and 48 h, it was significant at 72 h (see Table 3). We have previously reported in vitro VEGF concentrations for these cell lines at 24, 48 and 72 h and the findings from this study are consistent with the variation found in our previous report. We then evaluated the ability of Yunnan Baiyao to modulate VEGF levels in the cell supernatant for all three cell lines at 72 h. Yunnan Baiyao did cause some significant increases in VEGF levels in Fitz and SB cell lines, but not in the DEN cell line (see Fig. 5). The increases in VEGF occurred at concentrations of Yunnan Baiyao that were approaching the IC₅₀ for Fitz and SB, namely 275.9 and 325.3 μg mL⁻¹, respectively. Moreover, these concentrations of Yunnan Baiyao were also consistent with the induction of apoptosis in the cell lines. These findings are not unlike our previous findings from a report that showed the induction of VEGF when mastinib concentrations approached the IC₅₀ for Fitz and SB. However, in the report by Lyles et al. the DEN cell line was marginally affected, but similar to this study the SB cell showed the greatest fold increase of VEGF. This needs to be investigated further by examining the effects of these drugs on cellular pathways involved in VEGF signalling and production, e.g. hypoxia inducible factor 1α (HIF1α). Interestingly, human cancer patients treated with anti-angiogenic tyrosine kinase inhibitors show increased plasma levels of VEGF and placental growth factor in the face of clinical efficacy. The relationship between cell supernatant concentration and in vivo plasma concentration of VEGF is not clear. In the study by Clifford C et al., median VEGF concentrations actually
Figure 4. Cell cycle analysis was performed using flow cytometry and propidium iodide counter staining and data were recorded for all three cell lines at 24, 48 and 72 h. Remarkably, all cell lines at concentrations >200–400 μg mL\(^{-1}\) and at all time points the cell phases disappeared and were replaced by DNA debris. This DNA debris is considered a sign of late apoptosis due to endonuclease cleaving of DNA which correlates with the noted increase in caspase-3/7 activity. (A) Cell cycle kinetics measured in the DEN cells treated with increasing concentrations of Yunnan Baiyao. (B) Cell cycle kinetics measured in the Fitz cells treated with increasing concentrations of Yunnan Baiyao. (C) Cell cycle kinetics measured in the SB cells treated with increasing concentrations of Yunnan Baiyao.

decreased with increasing stage of disease and 4 of 17 dogs with HSA did not have detectable VEGF levels in the plasma.\(^{38}\) This may be due to the fact that VEGF can differ within the tumour versus in circulation or this may not be the primary factor involved with progression of HSA in dogs.

This is the first documentation of Yunnan Baiyao’s ability to cause a decrease in cell viability via apoptosis in canine HSA cells. It lends evidence to the anecdotally reported improvement in survival times in canine patients with HSA receiving this medication.

Pharmacokinetic studies on Yunnan Baiyao itself have not been performed; however, studies of the major component,\(^{38}\) P. notoginseng, have been performed. A pharmacokinetic study of intravenous panaxatrol disuccinate sodium, a ginsenoside derivative, was performed in healthy human volunteers and human patients with advanced solid tumours. The steady-state peak concentration,
Effects of Yunnan Baiyao on canine hemangiosarcoma

Figure 5. VEGF levels were measured in canine HSA cell supernatant after treatment with increasing concentrations of Yunnan Baiyao at 72 h. A logarithmic scale has been used due to the wide variation in VEGF levels among cell lines (dotted line represents baseline of one). The DEN cell line had no significant increases or decreases ($P < 0.001$) in VEGF levels. Significant increases ($P < 0.001$) in VEGF levels were found in the Fitz ($\ast$) cells treated with 100, 200, 600 and 800 μg mL$^{-1}$ Yunnan Baiyao, and for the SB ($\ast\ast$) cells with 50, 100, 200 and 600 μg mL$^{-1}$ Yunnan Baiyao.

average concentration and mean steady state AUC in plasma were 13.96±15.48, 0.15±0.29 and 148.00±117.18 mg L$^{-1}$, respectively. An intravenous injection at a dose of 100 mg m$^{-2}$ has been suggested for further phase II clinical trials.$^{43}$ We can not necessarily correlate what is achievable in vitro to in vivo availability based upon our study as we are not examining an individual component of Yunnan Baiyao. However; the average IC$_{50}$ for the three cell lines across the three time points (24, 48 and 72 h) was 350.17 μg mL$^{-1}$ (equal to 350.17 mg L$^{-1}$). This value does exceed the above noted steady state peak concentration and average concentration in plasma but is in a similar range with the mean steady state concentration achieved in plasma.$^{43}$ On the basis of dosing of other chemotherapeutic medications in veterinary medicine and the results of this in vitro study, this would appear to be a clinically attainable dose in the canine patient. It should also be noted that the IC$_{50}$ data presented here is based on the entire Yunnan Baiyao compound and separation of the individual ingredients is more likely to result in even more comparable data. Pharmacokinetic studies in canine patients on the individual components as well as whole compound Yunnan Baiyao are needed to have a better understanding of clinically achievable levels. Ginsenosides have also been identified as pharmacokinetic markers in the serum of rats after oral administration of P. notoginseng.$^{44}$ Panax notoginseng may serve as a marker of Yunnan Baiyao plasma concentration in the future.

Novel medications for the treatment of canine HSA are needed and Chinese herbal medications are being studied at an increasing rate for the purposes of cancer treatment in people. Increased demand for herbal medications worldwide as well as voluntary use of Good Agricultural Practice (GAP) has advanced knowledge as well as safety of these medications.$^{31}$ A nutrient and metal analysis on various marketed herbal products showed that contaminants such as Ni, Pb and Cd were equal to or lower than previously reported. Concentrations of these minerals were also below National Research Council proposed tolerances at recommended dosing.$^{45}$ Another study performed HPLC specifically on different Yunnan Baiyao batch preparations and showed that the total content of 13 saponins varied insignificantly (<4.78%) for different batches of powder and capsule forms when purchased from the Yunnan Baiyao Group.$^{46}$ On the basis of these studies, Yunnan Baiyao also appears to be a safe medication for further study in the canine and human patient.

In conclusion, Yunnan Baiyao induces both time-dependant and concentration-dependant cell death through apoptosis in canine HSA cells in vitro. This is the first study to document Yunnan Baiyao’s ability to induce apoptosis in canine HSA cells and the associated IC$_{50}$ values. VEGF expression was also documented in untreated (control) and treated HSA cells. The information gained from this study supports the further investigation of Yunnan Baiyao in treatment of canine HSA in the laboratory and clinical settings.

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Conflicts of interest

The authors have declared no conflicting interests.

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The effect of a spot-on formulation containing polyunsaturated fatty acids and essential oils on dogs with atopic dermatitis

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ABSTRACT

Recent studies have shown that immunological aberrations and epidermal barrier defects could be important in the pathogenesis of canine atopic dermatitis (CAD) and that oral polyunsaturated fatty acids (PUFAs) might influence the epidermal barrier. The aim of this study was to evaluate the effects of a spot-on formulation containing PUFAs and essential oils on pruritus and lesions caused by CAD. Forty-eight privately owned dogs of different breeds, ages and genders diagnosed with atopic dermatitis were included in a randomized, double-blinded, placebo-controlled, multicentre clinical trial. Dogs were treated with a spot-on formulation containing PUFAs and essential oils or placebo on the dorsal neck once weekly for 8 weeks. Before and after the study, CAD extent and severity index-03 (CADESI-03) and pruritus scores were determined by veterinarians and owners, respectively.

There was significantly more improvement in CADESI-03 and pruritus scores in the treatment group than in the placebo group (P = 0.011 and P = 0.036, respectively). Additionally, more dogs improved by at least 50% in CADESI-03 and pruritus scores in the treatment group than in the placebo group (P = 0.008 and P = 0.070, respectively). No adverse reactions were observed. The topical preparation containing PUFAs and essential oils was a safe treatment and beneficial in ameliorating the clinical signs of CAD.

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Introduction

Canine atopic dermatitis (CAD) is a commonly presented disease in veterinary practice (Scott and Paradis, 1990) and is associated with pruritus (Saridomichelakis et al., 1999; Griffin and DeBoer, 2001) and skin lesions (Griffin and DeBoer, 2001; Favrot et al., 2010). It is diagnosed by history, clinical signs and the exclusion of differential diagnoses, and clinical diagnostic criteria have been recently introduced (Favrot et al., 2010). In CAD, a hypersensitivity response against environmental or food allergens develops due to a genetic predisposition and could be associated with disturbances in the skin barrier function (Merryman-Simpson et al., 2008; Sandilands et al., 2009; Wood et al., 2009). Allergens involved in the pathogenesis of non-food-induced CAD include house dust mites, pollens, moulds and insect antigens (Hill and DeBoer, 2001). Allergens can be inhaled or percutaneously absorbed (Olivry and Hill, 2001; Marsella et al., 2006).

Symptomatic treatment for CAD includes antihistamines, glucocorticoids, cyclosporin, topical therapy, and polyunsaturated fatty acids (PUFAs), while specific treatment employs allergen-specific immunotherapy (Olivry et al., 2010). PUFAs cannot be synthesized de novo and need to be ingested pre-formed in the diet. They contain one or more double bonds, and are classified as omega-3 and omega-6 fatty acids, depending on the position of the first double bond relative to the carboxy end of the chain. Important omega-3 fatty acids are α-linolenic acid (in linseed oil), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; in fish oils). Omega-6 fatty acids are linoleic acid (in sunflower or safflower oil), γ-linoleic acid (in evening primrose oil) and dihomo-γ-linoleic acid.

In vitro, PUFAs are reported to have anti-inflammatory (Ziboh and Chapkin, 1988; Ziboh et al., 2000) and immunomodulating (Stehle et al., 2010) effects. A further possible mechanism of action is improvement of the epidermal barrier function, presumably by changing the composition of epidermal lipids. Oral fatty acid supplementation has been reported to change cutaneous lipids in Beagle dogs (Campbell and Dorn, 1992).

In contrast to many other symptomatic therapies for CAD, oral supplementation with PUFAs rarely causes adverse effects (Olivry...
et al., 2001; Mueller et al., 2004), although diarrhoea might occur with oral supplementation (Scott et al., 1992). Adverse effects of topically administered PUFAs have not been reported (Tretter and Mueller, 2011). Concurrent treatment with PUFAs might permit reduction of the dosage of other anti-inflammatory medications, such as glucocorticoids, and further improvement in clinical signs (Scott and Miller, 1993; Bond and Lloyd, 1994; Saevik et al., 2004).

Studies on the use of oral fatty acid supplementation have been published (Mueller et al., 2004; Saevik et al., 2004), but reports about the efficacy of topically applied PUFAs or ceramides are rare and describe non-blinded and open trials (Piekutowska et al., 2008; Tretter and Mueller, 2011). The aim of this study was to evaluate the efficacy of a commercial spot-on containing PUFAs and essential oils on the clinical signs of CAD in a prospective, placebo-controlled, randomised trial.

Materials and methods

The study was approved by the Ethics Committee of the Centre for Clinical Veterinary Medicine/Ludwig Maximilian University Munich (Approval number 03-051012). Prior to enrolment, dog owners gave their written consent (Appendix A: Supplementary material).

Study design and study objects

This was a randomized, double-blinded, placebo-controlled multicentre study. Three dermatology referral practices in Germany (Centre for Clinical Veterinary Medicine, Ludwig Maximilian University Munich), the UK (Dermo4Pets Clinic, Buckinghamshire/Berkshire) and the USA (Animal Dermatology Clinic, Tustin, California) participated.

Forty-eight privately owned dogs with atopic dermatitis were included, of different genders, ages and breeds. The treatment group consisted of 23 dogs classified with either moderate to severe CAD (n = 12) or mild CAD (n = 11). There were 25 dogs in the placebo group (16 classified with moderate to severe CAD and nine with mild CAD).

Randomization

The dogs were stratified into two subgroups with mild disease characterized prior to treatment by either low lesion scores i.e. a CAD extent and severity index-03 (CADESI-03 < 60; n = 20), or moderate to severe disease (CADESI-03 > 60; n = 28; Olivery et al., 2008). Separate randomization schedules for both groups and each study centre were created by the study monitor prior to the study according to a computer-generated randomization list.1 Medication and identically packaged placebos were sent to each study centre and each package was specifically marked and dispensed according to the randomization list.

Inclusion criteria

All dogs had been diagnosed with environmentally-induced atopic dermatitis based on history, clinical signs and rule-out differential diagnoses by appropriate means, such as skin cytology, skin scrapings, elimination diets and/or ecotoparase control measures. Dogs with mild disease were treated exclusively with topical therapy, either product or placebo. Antihistamines and other topical therapies were discontinued at least 2 weeks prior to starting the study and glucocorticoids and cyclosporin were discontinued at least 6 weeks prior to enrolment.

In the group with moderate to severe CAD, exclusive treatment with placebo or topical fatty acids/essential oils was considered unethical due to the reported limited improvement seen with oral fatty acid supplementation (Olivery et al., 2001; Mueller et al., 2004). Concurrent low dose glucocorticoids, antihistamines and topical therapy were permitted if they had been administered at an unchanged dose for more than 12 weeks prior to inclusion and during the trial. Diet changes were not permitted within 3 months prior to or during the study. Allergen-specific immunotherapy was permitted in dogs that had been receiving it for at least 12 months prior to inclusion. Dogs with a history or clinical signs of flea bite hypersensitivity received fipronil spot on (Frontline, Merial) or selamectin spot on (Stronghold, Zoetis) once monthly.

Clinical evaluation

A validated lesion score (CADESI-03; Olivery et al., 2007, 2008) was used to determine the severity of skin lesions. If the initial CADESI-03 was >60, dogs were considered to have mild CAD (n = 20). If the CADESI-03 was >60, the disease was categorized as moderate to severe (n = 28), as previously reported (Olivery et al., 2008). Dogs with moderate to severe disease commenced the study after their clinical signs had improved with other therapies (see above) and they were considered stable. Dogs were evaluated at enrolment and after 8 weeks of treatment. The CAD-ESI-03 score was determined by the clinician at each visit. Similarly, owners completed a validated pruritus score at each visit, scoring pruritus from 0 to 10 using a visual analogue scale combined with features of the behaviour and severity-based scales (Hill et al., 2007; Appendix B).

Statistical analyses

Based on data gathered in a recent pilot study (Tretter and Mueller, 2011), it was calculated that with at least 20 dogs in each group (treatment and placebo), a difference of 6 points in CADESI-03 scores and 2 points in pruritus scores could be determined with a power of 90% and a significance level of P < 0.05. To ensure similar groups, initial CADESI-03 scores and pruritus scores were compared using Mann Whitney tests. For the same reason, the age and weight of dogs in both groups were compared with an unpaired t test if data were normally distributed or Mann–Whitney U tests if data were not normally distributed. Gender distribution was analyzed using Fisher’s exact tests. Improvements in pruritus and CADESI-03 scores, respectively, were calculated by subtracting the score at enrolment from the score at the end of the study. This was compared between groups using an unpaired t test with Welch correction (if data were normally distributed) or a Mann–Whitney U test (if data were not normally distributed). The number of dogs improving by at least 50% and the number of dogs deteriorating in the treatment group compared to the placebo group were compared using Fisher’s exact tests. A one-sided P value was chosen, as a previously published pilot study had shown improvement in both pruritus and CADESI-03 scores with this therapy (Tretter and Mueller, 2011) and thus deterioration was not expected in the treatment group compared to placebo. Significance for all tests was set at P < 0.05. The statistical program used was GraphPad Prism 5.0 (GraphPad). Dogs were excluded from the per protocol analysis if they exhibited clinical signs of an adverse reaction to the product, when owner compliance was not satisfactory, or when the clinical signs of atopic dermatitis deteriorated to the point that additional antipruritic therapy was needed. An intention to treat analysis, with the last value carried forward, using all dogs included in the study was performed, as well as a per protocol analysis.

Results

CADESI-03 and pruritus scores

There was no significant difference between treatment and placebo groups with respect to CADESI-03 scores (P = 0.278) or pruritus (P = 0.990) at enrolment. There was also no difference between groups in age (P = 0.735), bodyweight (P = 0.782) or gender distribution (P = 0.785). Because two dogs did not complete the study, per protocol analysis was performed on 46 dogs. As the results of the intention to treat analysis and that of the per protocol analysis were similar, only the results of the intention to treat analysis are reported here.

Study protocol

All dogs were treated with a spot-on preparation once weekly for 8 weeks. The owners applied the product on the dorsal cervical area after being given detailed instructions on how to spread the hair coat and apply the product directly onto the skin. Dogs received either a product containing PUFAs (6 mg/mL of ω-linolenic and 30 mg/mL of linoleic acid), essential oils (neem oil, rosemary extract, lavender oil, clove oil, tea tree oil, oregano extract, peppermint extract and cedar bark extract) and vitamin E (Dermoscent Essential 6 spot-on, LDCA) or a placebo (bio diffusing agents, Dermoscent, LDCA).

Dogs <10 kg received 0.6 mL weekly; dogs weighing 10–20 kg received 1.2 mL weekly, and dogs of 20–40 kg received 2.4 mL weekly. This protocol was according to the manufacturer’s recommendations and the same as the protocol used in a previously published pilot study (Tretter and Mueller, 2011). The commercial product has a distinct odour that was absent from the placebo. However, the owners of placebo treated dogs were not aware of this difference. It was previously established that the odour dissipated within 1 week of application and investigators were unable to detect the odour at the time of scoring, thus keeping the integrity of the blinding intact.

The mean and the confidence intervals of CADESI-03 and pruritus scores pre- and post-therapy are shown in Table 1. Individual improvements in CADESI-03 scores and pruritus scores in each dog were significantly higher in the treatment group than in the placebo group (Mann–Whitney U test, \( P = 0.011 \) and \( P = 0.036 \), respectively). The numbers of dogs improving by at least 50% or 90% are listed in Table 2. More dogs showed an improvement of \( \geq 50\% \) in CADESI-03 and pruritus scores in the treatment group than in the placebo group (Fisher’s exact test, \( P = 0.008 \) and \( P = 0.07 \), respectively). Significantly more dogs deteriorated in the placebo group \((15/25)\) compared to the treatment group \((5/23)\); Fisher exact test, \( P = 0.01 \). Raw data are shown in Appendix B: Supplementary materials.

### Adverse effects and exclusions

All except two dogs completed the study. These had moderate to severe clinical signs and both deteriorated during the first 4 weeks of the study, requiring their concurrent therapy to be changed. One of those dogs was in the treatment group and one was in the placebo group. Adverse effects were not observed in any of the treated dogs.

### Discussion

This study demonstrated that the clinical signs of atopic dermatitis in dogs with stable CAD that met the study entry criteria significantly improved after eight weekly topical treatments of a commercially available compound containing PUFAs and essential oils. The degree of improvement was similar to another randomized, placebo-controlled study where 29 dogs received oral fatty acid supplementation for 10 weeks and showed significant improvement (Mueller et al., 2004). That study used a different scale to measure outcomes, as it preceded the use of the CADESI-03. The improvement in pruritus scores could be perceived as that for glucocorticoids or cyclosporin (Steffan et al., 2006; Olivry et al., 2010, 2011). PUFAs are thought to have lower efficacy than glucocorticoids and cyclosporin (Olivry et al., 2010), but they are a safe alternative to other anti-inflammatory therapies. Adverse effects associated with their use are rare and usually mild (Mueller et al., 2004; Muñoz et al., 2004; Tretter and Mueller, 2011).

The results of the present investigation support the findings of a recent pilot study using the same product (Tretter and Mueller, 2011) and also studies evaluating oral fatty acid supplementation (Olivry et al., 2010). PUFAs are thought to have lower efficacy than glucocorticoids and cyclosporin (Olivry et al., 2010), but they are a safe alternative to other anti-inflammatory therapies. Adverse effects associated with their use are rare and usually mild (Mueller et al., 2004; Muñoz et al., 2004; Tretter and Mueller, 2011), which is particularly important in the long-term treatment of chronic diseases such as CAD. In this work, no adverse effects were noted with short-term use (8 weeks). Widespread use of the product over longer periods of time is needed to make more definitive statements regarding safety.

As this is the first double-blinded, placebo-controlled study evaluating topical therapy with a commercial product containing essential oils and PUFAs, the only comparison possible is with oral PUFA supplementation. There are many studies using oral PUFA supplementation, but only a few that are placebo-controlled and double-blinded. In one such study, there was a significant improvement of clinical signs with commercially available EPA and DHA preparations (Mueller et al., 2004). In another controlled study based on the measurement of serum arachidonic acid before and after the trial, dogs with early CAD showed more improvement than dogs with a longer history of CAD (Abba et al., 2005).

Direct comparison of an oral and topical product is not possible, but our study provides evidence that the efficacy of topical therapy with essential oils and PUFAs appears to be comparable to that reported for studies evaluating oral PUFAs (Mueller et al., 2004; Saevik et al., 2004). With oral fatty acid supplementation, approximately half of the dogs treated with daily fatty acids improved by 50% or more compared to only 10% in the placebo group in an earlier study (Mueller et al., 2004). In the current work, the corresponding results were in the same range for CADESI-03 and pruritus scores, suggesting both types of treatment are suitable for the treatment of CAD, but the success rate for either one is not as high as that for glucocorticoids or cyclosporin (Steffan et al., 2006; Olivry et al., 2010).

It was recommended a decade ago that PUFA supplementation should be administered for at least 12 weeks before assessing the success of treatment (Olivry et al., 2001). This was based on the pharmacokinetics of oral PUFAs (Campbell and Dorn, 1992; Campbell et al., 1995). However, other authors observed effects with daily PUFA supplementation as early as 2 weeks after the initiation of therapy (Scott et al., 1992, 1997; Olivry et al., 2010). In our study, an 8-week supplementation period was chosen because the clinical effects in a pilot study were noted after 8 weeks of administration (Tretter and Mueller, 2011).

In the present study, a validated pruritus score and a CADESI-03 were used. Pruritus and skin lesions are typically considered the most relevant parameters in studies evaluating CAD (Olivry et al., 2010). The scores for lesions and pruritus have been previously validated (Hill et al., 2007; Olivry et al., 2007, 2008). The number of dogs improving by more than 50% and 90%, respectively, was always higher in the treatment group than in the placebo group for both pruritus and CADESI-03 scores (Table 2). However, in the placebo group more dogs improved in pruritus scores than in CADESI-03. The improvement in pruritus scores could be perceived rather than real and might provide evidence for the more subjective nature of the assessment of pruritus.

PUFAs are considered less efficacious than, for example, glucocorticoids (Olivry et al., 2010) or cyclosporin (Steffan et al., 2006) in the treatment of CAD, but have been shown to be successful

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 56</th>
<th>Improvement with treatment</th>
<th>Placebo Day 0</th>
<th>Placebo Day 56</th>
<th>Improvement with placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of dogs</td>
<td>23</td>
<td></td>
<td>25</td>
<td>15 (19–23)</td>
<td>10 (10–20)</td>
<td>25 (1–2)</td>
</tr>
<tr>
<td>Mean CADESI-03 (95% CI)</td>
<td>46 (29–63)</td>
<td>28 (18–39)</td>
<td>18 (4–32)</td>
<td>78 (41–116)</td>
<td>80 (44–115)</td>
<td>80 (44–115)</td>
</tr>
<tr>
<td>Mean pruritus (95% CI)</td>
<td>5.2 (4.2–6.2)</td>
<td>3.9 (2.7–5.2)</td>
<td>1.3 (0.2–2.4)</td>
<td>5.3 (4.3–6.3)</td>
<td>5.0 (4.1–6.0)</td>
<td>5.0 (4.1–6.0)</td>
</tr>
<tr>
<td>Number of dogs with mild AD</td>
<td>9</td>
<td></td>
<td>11</td>
<td>22 (11–32)</td>
<td>34 (21–48)</td>
<td>22 (11–32)</td>
</tr>
<tr>
<td>Mean CADESI-03 (95% CI)</td>
<td>25 (13–36)</td>
<td>15 (9–22)</td>
<td>9 (–1 to 20)</td>
<td>3.8 (2.1–5.5)</td>
<td>4.0 (2.1–5.5)</td>
<td>4.0 (2.1–5.5)</td>
</tr>
<tr>
<td>Number of dogs with moderate–severe AD</td>
<td>12</td>
<td></td>
<td>16</td>
<td>6 (19–30)</td>
<td>6 (19–30)</td>
<td>6 (19–30)</td>
</tr>
<tr>
<td>Mean CADESI-03 (95% CI)</td>
<td>46 (38–94)</td>
<td>40 (22–58)</td>
<td>24 (–1 to 50)</td>
<td>110 (58–163)</td>
<td>105 (53–157)</td>
<td>105 (53–157)</td>
</tr>
<tr>
<td>Mean pruritus (95% CI)</td>
<td>5.6 (4.4–6.8)</td>
<td>4.1 (2.5–5.7)</td>
<td>1.5 (0–3.0)</td>
<td>6.1 (5.1–7.2)</td>
<td>5.6 (4.5–6.8)</td>
<td>5.6 (4.5–6.8)</td>
</tr>
</tbody>
</table>

CADESI-03, canine atopic dermatitis extent and severity index-03; CI, confidence interval.

* Improvement = Score at the beginning of the trial – Score at the end of the trial.
as adjunctive therapy (Saevik et al., 2004). For this reason, additional medications were permitted in dogs with moderate–severe atopic dermatitis, as outlined above. In addition, it was considered unethical to treat dogs with more severe disease exclusively with topical fatty acids/essential oils. Dogs with a CADESI-03 of >60 have been classified as having moderate to severe atopic dermatitis (Olivry et al., 2008). Since the clinical signs in those dogs were unlikely to be controlled by sole therapy with PUFAs/essential oils, the use of concurrent medication was considered ethical and justified. As the dose of concurrent medications was not changed for the 12 weeks preceding the study or during the study, those drugs are unlikely to have influenced the study outcome.

It is not clear how well spot-on preparations containing fatty acids distribute in the epidermis and how long possible changes in epidermal ceramide composition last. One study reported a significant increase in free ceramides at the application site 3 days after the last treatment after twice weekly application of a topical spot-on preparation containing ceramides and free fatty acids for 3 weeks (Popa et al., 2012). The clinical improvement seen in dogs with multifocal to generalized skin disease treated with such products further supports some effect on the epidermis, but more studies are needed to provide details regarding the distribution and mechanism of action of topical essential oils and PUFAs in dogs.

Conclusions

Based on the findings in this study, the application of a spot-on containing PUFAs and essential oils was beneficial in alleviating the clinical signs of CAD. As complete remission was not achieved in the vast majority of dogs, it seems most useful as an adjunctive therapy in this disease.

Conflict of interest statement

The study was financed by Laboratoire de Dermo-Cosmetique France, which had no influence on study design, data evaluation or manuscript preparation. Dr. Blaskovic was financially supported by LCDa France. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgement

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tvjl.2013.10.024.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Treatment improvement</th>
<th>Placebo improvement</th>
<th>Treatment improvement</th>
<th>Placebo improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥ 50%</td>
<td>≥ 50%</td>
<td>≥ 90%</td>
<td>≥ 90%</td>
</tr>
<tr>
<td>CADESI-03 (n)</td>
<td>8/23</td>
<td>1/25</td>
<td>1/23</td>
<td>0/25</td>
</tr>
<tr>
<td>Pruritus score (n)</td>
<td>9/23</td>
<td>4/25</td>
<td>2/23</td>
<td>1/25</td>
</tr>
</tbody>
</table>

CADESI-03, canine atopic dermatitis extent and severity index-03.

References


Evaluating the effect of oral administration of Echinacea hydroethanolic extract on the immune system in dog

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Summary
1. This study was designed to evaluate the effects of oral administration of Echinacea hydroethanolic extract on the dog’s immune system.
2. The study was performed on 14 dogs that were referred to the veterinary clinic. These dogs were randomly allocated to two equal treatment groups. The first group received 1 ml of 5% Echinacea hydroethanolic extract two times a day for 2 months, and the second group received a placebo (water). To do haematology and immunology tests, the dogs were bled on days 0, 30 and 60. Blood tests, including packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), counting neutrophils (Nut), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos), basophils (Baso) and B cell, were performed. Furthermore, safety factor IgM and per cent of phagocytosis and phagocyte were measured from the blood sample.
3. The results showed that in the group which received Echinacea PCV, Hb, RBC count, WBC count, Lym, Nut, the per cent of phagocytosis and IgM significantly increased (P < 0.05). Moreover, positive effects of Echinacea plant on the immune system were observed. There was a significant change in HTC, RBC, Hb over time in the group that received Echinacea and the per cent of phagocytosis and IgM (P < 0.05).
4. The study establishes that these extracts might have appreciable immunostimulatory activity. However, further studies are required to confirm these findings.

Keywords: Echinacea, oral administration, dog, immune system

Introduction
The use of liquid extracts of Echinacea has increased recently and most often used as immunostimulating agents for the treatment (Hermann et al., 2003) and prevention of various infectious disorders in human medicine (Wolfram & Hans-Helge, 1999). This is due to an increase in the clinical importance of herbal drugs in modern medicine with considerable attention being paid to the use of plants as a source of immunomodulators being at the centre stage. Several medicinal herbs have shown to promote immunity in different ways; they have shown to augment specific cellular and humoral immune response (Duke, 1985).

Immunomodulators are agents that can modulate the immune response, and their effect may be stimulatory or suppressive (Ghonime et al., 2011). Echinacea extracts exhibit that potential. Rehman et al. (1999) (Rehman et al., 1999) showed an increase in primary and secondary IgG response in rats treated with Echinacea. A few scientific studies have assessed the efficacy of Echinacea in vivo with varying results (Grimm & Mu¨ller, 1999; Turner et al., 2000). Therefore, there is need to assess the effects of Echinacea hydroethanolic extract on dogs as an animal model in assessing its efficacy as an immunostimulant. This study was designed to evaluate the effects of oral administration of Echinacea hydroethanolic extract on the dog’s immune system.

Materials and methods

Animals
All experiments were carried out under the ethical guidelines of the Islamic Azad University of Shahrekord Branch, for the care and use of animals (Ernest et al., 1993).
The study was performed on 14 male dogs of mixed breeds that were randomly allocated to two equal treatment groups. All dogs were subjected to clinical examination and housed under uniform environment after being treated for internal parasites. The first group received 1 ml of 5% *Echinacea* hydroethanolic extract two times a day for 2 months, and the second group received a placebo (water) instead of *Echinacea* extract.

**Preparation method for *Echinacea* extract**

Fifty grams of powdered plant was added to 700 ml of 50% ethanol (350 ml distilled water and 350 ml ethanol), and Soxhlet apparatus was used to prepare hydroethanolic extract. The solvent was filtered under reduced pressure. The plant ingredient concentration in the final extract was adjusted to the required concentration by adding distilled water to the dried extract. The extract was prepared each week and stored in a refrigerator.

**Sample collection and analyses**

The dogs were bled on days 0, 30 and 60 for haematology and immunology tests, and every blood sample was divided into two equal volumes for further analyses. The blood tests that were carried out include packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), counting neutrophils (Nut), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos) and basophils (Baso).

**Statistical analysis**

Statistical analysis focused on mean analysis for repeated measures that is before intervention, 30 days and 60 days. The following model was used:

\[ Y_{ijkl} = \mu + \tau_i + B_j + T_k + (\tau B)_ijkl + \varepsilon_{ijkl} \]

where \( Y_{ijkl} \) is the response measured on the \( i \)th treatment, \( i = [PCV, \ Hb, \ RBC \ count, \ WBC \ count, \ counting \ Nut, \ Lym, \ Mon, \ Eos, \ Baso, \ B and \ Cell] \), on the \( j \)th dog, at the \( k \)th time, \( \mu \) is the overall mean, \( \tau \) is the mean effect of treatment, \( B \) is the random subject effect, \( T_k \) is the time effect, \( \tau B \) is the interaction effect and \( \varepsilon \) is the error.

**Results**

The total sample size was 14, and each treatment group comprised of seven dogs. Body temperature and respiratory rate of the two groups were significantly different before the intervention Table 1 \((P < 0.05)\). Heart rate was higher in the group that was selected to receive *Echinacea* but not significantly different from the group that received the placebo \((P > 0.05)\). The results showed that in the group which received *Echinacea* PCV, Hb, RBC count, WBC count, Lym, Nut, the per cent of phagocytosis and IgM significantly increased (Table 2 and 3) \((P < 0.05)\). Also, the results indicated effects of *Echinacea* plant on the immune system. There was a significant change over time in the group that received *Echinacea* on HTC, RBC, Hb and the per cent of phagocytosis and IgM \((P < 0.05)\).

**Discussion**

Burger et al. (1997) (Burger et al., 1997) and See et al. (1997) (See et al., 1997) observed that extracts from *Echinacea* have non-specific immunostimulatory properties \(\text{in vitro}\) including increased phagocytosis, cytokine production and natural killer cell activity. The plant and its extracts have been shown to stimulate phagocytosis \(\text{in vitro}\) and \(\text{in vivo}\) in murine (Melchart et al., 1995). Roesler et al. (1991) (Roesler et al., 1991) confirmed activation of human phagocytic function both \(\text{in vitro}\) and \(\text{in vivo}\).

Information generated in past research suggests that the immunostimulatory activity of *Echinacea* depends on the combined action of caffeic acid derivatives and alkylamides (Bauer 1998; Hermann et al., 2003). Moreover, many pharmacological compounds have been isolated from *Echinacea* (San Feliciano et al., 1993), and several constituents are alleged to be immunologically active, including polysaccharides and glycoproteins (Bauer et al., 1988). Sloley et al. (2001) (Sloley et al., 2001) showed that phenylpropanoid glycosides, which are constituents of certain *Echinacea* species, possess antiviral properties and are antioxidants and free radical scavengers and inhibit Fe\(^{2+}\)-induced lipid peroxidation.
Table 2  Comparison of mean ± SD of blood parameters before and after intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Before</th>
<th>1 month</th>
<th>2 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell count (RBC)</td>
<td>Echinacea</td>
<td>43.43 ± 1.11*</td>
<td>47.71 ± 0.606**</td>
<td>48.14 ± 0.837**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>47.39 ± 3.97</td>
<td>46.29 ± 4.539</td>
<td>50.1 ± 3.97</td>
</tr>
<tr>
<td>Haemoglobin (Hb)</td>
<td>Echinacea</td>
<td>7.36± 0.143*</td>
<td>7.86 ± 0.053**</td>
<td>7.94 ± 0.084**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>7.47 ± 0.465</td>
<td>7.50 ± 0.493</td>
<td>7.46 ± 0.178</td>
</tr>
<tr>
<td>Neutrophils (Nut)</td>
<td>Echinacea</td>
<td>14.70 ± 0.235</td>
<td>15.34 ± 0.043</td>
<td>15.10 ± 0.136</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>14.26 ± 0.612</td>
<td>14.36 ± 0.581</td>
<td>14.42 ± 0.274</td>
</tr>
<tr>
<td>White blood cell count (WBC)</td>
<td>Echinacea</td>
<td>12990 ± 445.67*</td>
<td>13290 ± 441.65**</td>
<td>13550 ± 408.54**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>11580 ± 233.467</td>
<td>11890 ± 533.079</td>
<td>11950 ± 328.494</td>
</tr>
<tr>
<td>Basophils (Baso)</td>
<td>Echinacea</td>
<td>0.286 ± 0.747</td>
<td>1.571 ± 0.297</td>
<td>1.571 ± 0.429</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>1.714 ± 0.36</td>
<td>1.714 ± 0.184</td>
<td>1.143 ± 0.508</td>
</tr>
<tr>
<td>Lymphocytes (Lym)</td>
<td>Echinacea</td>
<td>27.143 ± 2.539*</td>
<td>31.714 ± 2.942**</td>
<td>34.286 ± 4.144**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>32.571 ± 2.626*</td>
<td>31.714 ± 2.109*</td>
<td>27.143 ± 3.068**</td>
</tr>
<tr>
<td>Monocytes (Mon)</td>
<td>Echinacea</td>
<td>2.714 ± 0.565</td>
<td>2.857 ± 0.34</td>
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</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3.286 ± 0.522</td>
<td>3.429 ± 0.369</td>
<td>2.857 ± 0.261</td>
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<td>Eosinophils (Eos)</td>
<td>Echinacea</td>
<td>4.143 ± 0.738</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>3.714 ± 1.04</td>
<td>3 ± 0.65</td>
<td>4 ± 0.535</td>
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<tr>
<td>Basophils (Baso)</td>
<td>Placebo</td>
<td>0.313 ± 0.143</td>
<td>0.557 ± 0.143</td>
<td>0.571 ± 0.297</td>
</tr>
</tbody>
</table>

Different superscript letters within the same column indicate significant difference (P < 0.05).

Table 3  Comparison of phagocytes, phagocytosis and IgM before and after intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Before</th>
<th>30 days</th>
<th>60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytes</td>
<td>Echinacea</td>
<td>25.857 ± 2.604*</td>
<td>27.286 ± 2.884**</td>
<td>28.462 ± 2.07**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>34.286 ± 2.427**</td>
<td>32.571 ± 2.776*</td>
<td>27.286 ± 1.886**</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>30.714 ± 4.714</td>
<td>23.143 ± 2.721</td>
<td>26.429 ± 1.478</td>
</tr>
<tr>
<td>IgM</td>
<td>Echinacea</td>
<td>138.714b ± 3.242*</td>
<td>159.857 ± 13.674**</td>
<td>182.857 ± 13.946***</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>150.286a ± 18.774</td>
<td>181.714 ± 7.63</td>
<td>186.429 ± 6.679</td>
</tr>
</tbody>
</table>

Different superscript letters within the same column indicate significant difference (P < 0.05).

The haemoglobin levels were significantly increased in the trial by *Echinacea* extract. This is supported by Anon (1989) (Anon, 1989) who concluded that *Echinacea* extract behave as an agent that improves the quality of blood by increasing haemoglobin levels and the number of erythrocytes therefore, considered to improve parameters of exercise physiology and performance.

Increase in the number of lymphocytes during the treatment phase of the study support the information generated in other investigations (See et al., 1997; Steinmuller et al., 1993) which suggests that *Echinacea* behaves as an immune system stimulant. In addition, previous studies have demonstrated that *Echinacea* has an enhancing effect on lymphocyte function and proliferation (See et al., 1997). Furthermore, *Echinacea* extracts showed protection of immunosuppressed mice against systemic infections with stimulation of macrophage and neutrophil function (Steinmuller et al., 1993).

Increase of neutrophil counts was achieved only after the first month and then decreased after the second month on *Echinacea* treatment group, thereby raising the question as to what other external factors may have contributed to the change. However, Melchart et al. (1995) (Melchart et al., 1994, 1995) and O’Neill et al. (2002) demonstrated the effect of *Echinacea* on the capacity of neutrophils to ingest more foreign particles and stimulatory effect on these cells by improving phagocytic function.

The changes that occurred to the blood parameters measured in the current study are strongly due to the effects of *Echinacea* which acted as an immunomodulator by activating cytotoxic effector cells such as cytotoxic T lymphocytes, natural killer (NK) cells, lymphocytes, macrophages and...
activated neutrophils as observed by Ghonime et al. (2011) (Ghonime et al., 2011).

Administration of *Echinacea* showed increased number of the total WBC count. Similar increase in WBC count was obtained by plant extracts of *Silene nocturna*, *Nigella sativa* and *Matricaria chamomilla* (Ghonime et al., 2011) and *Withania somnifera* (Davis & Kuttan, 2000). This indicates they can stimulate the hemopoietic system. Although significant patterns were observed, nevertheless, one limitation of the study was the insufficient sample to detect small to moderate differences in the parameters measured between the *Echinacea* and the placebo groups. However, the sample size was small and that might have resulted in large discrepancies observed between the two treatments.

**Conclusion**

The study establishes that these extracts have appreciable immunostimulatory activity. However, further studies are required to confirm these conclusions.

**Conflict of interest**

The authors declared no conflict of interests with respect to the research, authorship and/or publication of this article.

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Effect of *Bidens pilosa* on infection and drug resistance of *Eimeria* in chickens

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**A B S T R A C T**

Extensive use of current anti-coccidial drugs together with drug resistance and residue has raised concerns about public health and poultry development. Here, we studied the anti-coccidial properties of *Bidens pilosa*. A phytochemical approach was developed for analysis of *B. pilosa* utilized as a feed additive. The protective effects of *B. pilosa* supplemented chicken diet were evaluated chickens infected with *Eimeria tenella*. *B. pilosa*, at doses of 0.5%, 1% and 5% of the chicken diet, significantly protected against *E. tenella* as measured by reduction in mortality, weight loss, fecal oocyst excretion and gut pathology in chickens. Finally, drug resistance of *E. tenella* to *B. pilosa* was assessed in chickens using the anti-coccidial index. This index showed that *B. pilosa* induced little, if any, drug resistance to *Eimeria* in chickens. Collectively, this work suggests that *B. pilosa* may serve as a novel, natural remedy for coccidiosis with low drug resistance in chickens.

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1. Introduction

Coccidiosis is a disease that has a large economic impact on the poultry industry, causing high mortality, poor growth and high medical costs (Williams, 1998). In chickens, coccidiosis is caused by parasites of the genus *Eimeria* (Coccidia subclass). Currently, the use of anti-coccidial drugs is one common means to prevent and treat coccidiosis. However, massive and long-time use of anti-coccidial drugs has led to the presence of drug-resistant parasites and residual drugs in chicken products, raising concerns about public health and food safety (Chapman, 1997; McDonald and Shirley, 2009; Orengo et al., 2012). In European countries, the use of anti-coccidial and anti-histomonas drugs as feed additives has been strictly limited since 2006 (Regulation 1831/2003 of the European Parliament) and a full ban has been proposed to be effective in 2021 by the Council Directive of 2011/50/EU published in the Official Journal of the European Union, L 104 of 19 April 2011. The utilization of anti-coccidial vaccines is an alternative means to prevent coccidiosis.

Despite the significant progress made over recent years, efficacy, safety and cost effectiveness are still challenges for anti-coccidial vaccines in poultry (Sharman et al., 2010). Given the concern voiced by consumers and poultry farmers about the use of the present anti-coccidial agents, there is an urgent need for novel and alternative approaches to prevent and treat coccidiosis in fowl. Reports have indicated that the use of effective, edible herbs and natural products as coccicidices in poultry production can be easily appreciated and accepted by consumers (Hassan et al., 2008; Orengo et al., 2012). Plants have been an extraordinary source of food and medicines for humans and animals since antiquity. Over the past decade, over 20 herbs have been tested for anti-coccidial activities (Akhtar et al., 2012; Allen, 2003; Allen et al., 1997; del Cacho et al., 2010; Lee et al., 2011; Naidoo et al., 2008; Orengo et al., 2012; Remmal et al., 2011; Youn and Noh, 2001). Although some plants showed high toxicity or little or no anti-coccidial activity (Nwosu et al., 2011), others were found to exert anti-coccidial function via immune action (Akhtar et al., 2012; Allen, 2003; Lee et al., 2011), suppression of oocyst wall formation (del Cacho et al., 2010), oocyst destruction (Remmal et al., 2011), anti-oxidant action (Allen et al., 1997, 1998; Naidoo et al., 2008; Orengo et al., 2012) and other mechanisms (Youn and Noh, 2001). Phytochemicals, saponins and artemisinin have been proposed to be the active compounds against Coccidia (Allen et al., 1997; del Cacho et al., 2010; Mshvidadze et al., 2010).
2000). Despite these initial findings in early studies on anti-coccidial herbs, new anti-coccidial plants are still needed. 

*B. pilosa* (Asteraceae) is an edible plant, commonly utilized as an ingredient in foods and medicines worldwide (Bartolome et al., 2013). The Food and Agriculture Organization of the United Nations advocated the cultivation of *B. pilosa* in Africa because of its high biosafety and easy growth (Young et al., 2010). Around 200 compounds have been identified from this plant including aliphatics, flavonoids, terpenoids, phenylpropanoids, aromatics, porphyrin and many others (Bartolome et al., 2013). The richness and complexity of the phytochemicals in *B. pilosa* may reflect the wide variety of bioactivities that have been reported for this herb, such as antimicrobial, anti-parasoidal, and many other actions (Bartolome et al., 2013). Nevertheless, the anti-coccidial properties of *B. pilosa* have not been evaluated.

In this study, batch consistency and quality control of a preparation of *B. pilosa* were assessed using phytochemical approaches, and the anti-coccidial activities of *B. pilosa* in chickens, as evidenced by survival rate, body weight loss, oocyst shedding and intestine pathology, were examined. Finally, the drug resistance of *B. pilosa* was evaluated.

2. **Materials and methods**

2.1. **Plant preparation and analysis**

The plant processing and analysis were performed similar to a previous publication (Chien et al., 2009). Three batches of the whole plant of *B. pilosa* were collected from Changhua County, Taiwan, and authenticated. After air drying at room temperature, the plant material was ground into a powder and the particles whose size ranges from 0.149 to 0.177 mm were collected for further use. For chemical fingerprint analysis, each batch of the pulverized *B. pilosa* material was extracted in 10-fold volumes of methanol at room temperature for 2 days. The crude extracts were evaporated by a rotary evaporator (Heidolph, Schwabach, Germany). After evaporation, the extracts were dissolved in water and subjected to high pressure liquid chromatography (HPLC) analysis using an RP-18 column (Phenomenex C18), hyphenated with a UV photodiode detector at 254 nm or a mass spectroscope (MS). The solvent gradient for HPLC was 0.1% TFA/acetonitrile (B) in 0.1% TFA/H2O:10–20% of B for 0–10 min, 21–28% of B for 10–15 min, 19–21% of B for 15.35 min, 21–28% of B for 35–47 min, and 28–100% of B for 47–55 min. Commercial standards, chlorogenic acid and isochlorogenic acid C were purchased from Sigma (St. Louis, MO, USA). The pulverized *B. pilosa* material from batch 1 was selected for the chicken diet formulation as described below.

2.2. **Isolation, characterization and sporulation of *E. tenella* oocysts**

Two isolates of *E. tenella* were collected from ceca of infected chickens after sacrifice at local poultry farms. Briefly, to obtain pure lines of *E. tenella*, different individual oocysts were sporulated with potassium dichromate and propagated throughout 2-week-old chicks, one sporulated oocyst per chicken. Two isolates (Et C1, and Et C2) of *E. tenella* with ~20 μm in diameter were obtained and identified by microscopic method (Joyner and Long, 1974) and interspecies molecular characterization (Blake et al., 2008). All sporulated oocysts were maintained in the laboratory of the Department of Veterinary Medicine, National Chung-Hsing University for 3 years without exposure to any anti-coccidial drugs. The survival rate of the Lohmann chickens 7 days after challenge with Et C1 or Et C2 strain (1 × 10^4 sporulated oocysts) was ~60%. The Et C1 strain was used in this study unless indicated otherwise.

2.3. **Animal husbandry, feed formulation and oral infection of *E. tenella***

In Experiment 1, 74 1-day-old disease-free Lohmann chicks from a local hatchery in Taichung, Taiwan, were obtained from a coccidian-free laboratory. To analyze the anti-coccidial action of *B. pilosa*, the chicks were randomly divided into six groups. There were four cages (4, 3, 3 and 3 chicks) in Group 1, four cages (4, 4, 4 and 3 chicks) in Group 2, four cages (4, 4, 4 and 3 chicks) in Group 3, four cages (4, 3, 3 and 3 chicks) in Group 4, three cages (3, 3 and 3 chicks) in Group 5, and three cages (3, 3 and 3 chicks) in Group 6. Chicks in all cages had *ad libitum* access to feeds and water throughout the experiment. Group 1 (UI control) and Group 2 (1 control) had daily access to standard chicken diet (63.5% yellow corn, 16% soybean meal, 10% full fat soybean, 3.5% fish meal, 3% bran, 1.2% soybean oil, 1% calcium carbonate, 1.1% dicalcium phosphate, 0.4% salt, 0.2% lysine, 0.02% vitamin premix, 0.08% mineral premix) from day 1 to day 21. Group 3 (I Mad control) had daily access to the same diet supplemented with maduramicin (6 mg/kg diet). Group 4 (Bp5), Group 5 (Bp1), and Group 6 (Bp0.5) had daily access to the diet supplemented with *B. pilosa* powder at a dose of 5% (50 g/kg diet), 1% (10 g/kg diet) or 0.5% (5 g/kg diet), respectively. Chicks were inoculated on day 14. The chicks in Group 1 (UI control) were administered with 2 ml of phosphate buffered saline (PBS) and those in Groups 2 (1 control), 3 (I Mad control), 4 (Bp5), 5 (Bp1) and 6 (Bp0.5) were infected with *E. tenella* sporulated oocysts (1 × 10^4). All animals were handled according to the guidelines of the National Chung Hsing University Institutional Animal Care and Use Committee (IACUC).

2.4. **Measurement of survival rate, body weight, oocyst numbers, and gross and microscopic lesion scores in animals**

Survival rate and bird appearance were checked daily. All birds in each cage in Experiment 1 were weighed on days 1, 7, 14 and 21 after hatching. Following published protocols in the literature (Conway et al., 1999; Haug et al., 2006), fecal samples were collected daily, from days 3 to 7 post infection, and weighed. Diluted oocyst suspension was prepared by adding water to 1 g of each fecal sample, followed by a serial filtration with W.S. Tyler sieves (1 mm, 250 μm and 45 μm). After centrifugation, the oocysts were suspended in saturated salt solution and mixed thoroughly. The homogenous suspension was transferred into two McMaster chambers for oocyst counts, with three repeats for each sample. Fecal oocyst number was calculated from the average of three counts of each sample. All the chickens in each group were sacrificed on day 21 and their ceca were removed. Gross lesion scores are obtained as described previously (Johnson and Reid, 1970). Briefly, gross lesions in the ceca caused by *E. tenella* were scored based on 5 grades: 0, normal tissue with no gross lesions; 1, very few scattered petechiae on cecal wall with normal cecal contents; 2, more numerous petechiae on thickened cecal wall with normal cecal contents; 3, noticeable cecal cores on greatly thickened cecal wall, large amounts of bloody cecal contents, and 4, greatly distended cecal wall with bloody or large caseous cores or dead birds. Microscopic lesion scores were obtained from the summation of lesion distribution and mucosal severity as published (Goodwin et al., 1998). Briefly, the entire ceca from the birds were fixed with 10% formalin and embedded in paraffin, followed by hematoxylin and eosin staining. The location of cecal lesions and mucosal histology were examined. The distribution of *E. tenella* infection along the observed cecal segment was graded as follows: 0, no *Eimeria* in any microscopic field at 10-fold magnification; 1, *Eimeria* in one field; 2, *Eimeria* in two fields; 3, *Eimeria* in three fields and 4, *Eimeria* in all four fields. The severity score in mucosae was graded as follows: 0, *Eimeria* in 0% of villi; 1, *Eimeria* in < 25% of villi; 2, *Eimeria* in 25 to 50% of villi; 3, *Eimeria* in...
in 51 to 75% of villi; 4, *Eimeria* in > 75% of villi. The microscopic lesion score is the sum of grades (0–4) found in five section slides per cecum.

### 2.5. Development and evaluation of drug resistance in *E. tenella*

In Experiment 2, 169 newly hatched chickens were purchased for drug resistance testing. Drug resistance of *E. tenella* in chickens was induced according to a previously described protocol with slight modification (Bafundo and Jeffers, 1990; Chapman, 1984). Briefly, *E. tenella* was passaged in chickens fed standard diet alone or supplemented with *B. pilosa* (0.5%) from day 0 to day 21 to obtain the first-generation oocysts. Such passage continued until the fifth-generation *Eimeria* oocysts were produced. Similarly, *E. tenella* was passaged in chickens fed a standard diet supplemented with salinomycin (70 ppm) from day 12 to day 21 until the fifth-generation oocysts were obtained. The above three lines were passaged in chickens fed a standard diet alone or supplemented with *B. pilosa* (1%) and salinomycin (140 ppm) for another three rounds, respectively. To assess drug resistance of the lines after eight serial passages, the three *Eimeria* lines were used to infect chickens in Groups *8, 9* and *10* on day 14, Groups *7* (10 chickens, UI control) and *8* (10 chickens, I control) had daily access to standard chicken diet from day 1 to day 21. Groups *9* (15 chickens, Bp1) was fed daily with the diet supplemented with *B. pilosa* at the dose of 1% (10 g/kg diet) from day 1 to day 21. Group *10* (10 chickens, ISalino control) was given the diet supplemented with salinomycin (140 ppm) from day 12 to day 21. Drug resistance of *E. tenella* in the above experiments were assessed by the anti-coccidial index (ACI) based on the following formula (Li et al., 2004; Wang et al., 2006): ACI = \[ \text{relative body weight gain (RBWG, %)} + \text{survival rate (SR, %)} \] – [lesion score index (LSI) + oocyst count index (OI)], where ACI ≥ 160 is defined as sensitive to the anti-coccidial drug, ACI between 120 and 160 is partially resistant to the anti-coccidial drug, and ACI < 120 is resistant to the anti-coccidial drug. RBWG = (100 × BWG per group)/ [BWG of the uninfected unmedicated group (Group 7, UI control)]; SR = (100 × the number of living chickens)/(total number of chickens per group); LSI = 10 × [lesion score per group]; and OI = 100 × 0.4 × [oocyst counts per group]/[oocyst counts for unmedicated-infected group (Group 8, I control)].

### 2.6. Statistical analysis

Data from nine chickens or more in each group of chickens in Experiments 1 and 2 are presented as mean ± standard error (SE). The cage in each group was used as the experimental unit. Pearson's chi-square test was used to determine whether there was a significant difference in the survival rate between treatment groups and control groups. Data on weight gain were subjected to two-way ANOVA with factors group and cage (group) using the GLM procedure of SAS system. The excreted oocyst values were transformed into ln(x + 1) and, in turn, analyzed by ANOVA using the GLM procedure of SAS system under a normal distribution. Leslie scores were analyzed using a chi-square test after multinomial transformation. Actual *P* values are presented in all experiments.

### 3. Results

#### 3.1. Chemical fingerprinting techniques for assessment of batch consistency and quality control of *B. pilosa* added to chicken diets

Batch consistency and quality control of *B. pilosa* in different preparations is important for the success of applications of *B. pilosa* products in chicken diseases. Therefore, we first employed high-performance liquid chromatography (HPLC), ultraviolet (UV) spectroscopy and mass spectroscopy to analyze the chemical fingerprint of three batches of *B. pilosa* preparations. Each preparation was made from a different plant sample. The HPLC profiles of the three batches of *B. pilosa* extracts were highly similar, suggesting good batch consistency among the *B. pilosa* preparations (Fig. 1A).

### 3.2. Effect of *B. pilosa* on survival rate of chickens following *E. tenella* challenge

To examine the anti-coccidial effect of *B. pilosa* as a feed additive on chickens, chickens were given daily access (day 1–21) to standard chicken feed or feed containing maduramicin or *B. pilosa* powder (at doses of 0.5%, 1% and 5% of chick feed) (Fig. 2A). The survival rate of chickens with access to standard feed dropped from 100% (Group 1; UI control) to 60% (Group 2; I control) after *E. tenella* infection (Fig. 2B). As expected, the survival rate of infected chickens with access to feed containing maduramicin was 93% in Group 3 (1 Mad control, Fig. 2B). In contrast, the survival rate was 100% for the infected chickens with access to feed containing 0.5% or more *B. pilosa* (Groups 4 (Bp0.5), 5 (Bp1) and 6 (Bp0.5), Fig. 2B). Furthermore, we examined the anti-coccidial effect of *B. pilosa* on challenge with a mixture of *E. tenella, E. maxima* and *E. acervulina*. We found that *B. pilosa* significantly increased the survival rate of infected chickens (Supplementary Fig. 54).

### 3.3. Effect of *B. pilosa* on reduced weight gain of chickens following *E. tenella* challenge

Next, we monitored body weight of chickens with access to different diets before and after *Eimeria* infection. Instead of using repeated measurement, we used the change in body weight of the chickens as a single measurement variable in the test in which individual chickens with similar initial weights were chosen. This method of measurement avoided time dependent confounding. The body weight gain in chickens of each group from day 21 and day 14 to day 1 is presented in Table 1. Two-way nested ANOVA with factors group and cage (group) was used to compare the body weight gain data. The actual *P* values are indicated in Table 1. There was no significant difference between each cage in each group.

On day 14, there was no significant difference in the body weight gain of the chickens in Group 3 (1 Mad control), Group 4 (Bp5) and Group 5 (Bp1) in comparison with Group 1 (UI control) and Group 2 (I control). In contrast, the body weight gain in chickens of Group 6 (Bp0.5) was significantly different from those of the chickens in Group 1 (UI control) and Group 2 (I control). On day 21, there was a significant difference in the body weight gain of the chickens in Groups 3 (1 Mad control), Group 4 (Bp5), Group 5 (Bp1) and Group 6 (Bp0.5) in comparison with Group 1 (UI control) and Group 2 (I control). Overall, *B. pilosa* significantly ameliorated reduced weight gain caused by *E. tenella* to a greater degree than maduramicin or control feed alone. Part of this amelioration may be attributed to the weight-gaining effect of *B. pilosa*.

### 3.4. Effect of *B. pilosa* on fecal oocyst excretion of chickens following *E. tenella* challenge

To further determine the anti-coccidial effect of *B. pilosa* in chickens, *Eimeria* oocysts in chicken feces, an indicator of *Eimeria* multiplication, was evaluated. No fecal oocysts were detected in the...
uninfected unmedicated controls (Group 1 (UI control), Table 2). Fecal oocyst excretion was first detected on day 4 post-infection in all E. tenella-infected groups and peaked on day 7 post-infection (Group 2 (I control), Table 2). As expected, the infected maduramicin-fed birds in Group 3 (1 Mad control, Table 2) had significantly fewer oocysts per gram of feces than the infected controls in Group 2 (I control, Table 2). Similarly, the infected B. pilosa diet-fed birds in Group 4 (Bp5), Group 5 (Bp1), and Group 6 (Bp0.5) were daily fed with the diet supplemented with B. pilosa powder at the dose of 5% (50 g/kg diet), 1% (10 g/kg diet) or 0.5% (5 g/kg diet), respectively. The number (n) of chickens in each group, cage number in each group and chicken number in each cage are indicated.

Table 1

<table>
<thead>
<tr>
<th>Group* cage no. (chickens)</th>
<th>BWG (g)</th>
<th>P valuea</th>
<th>P valueb</th>
<th>BWG (g)</th>
<th>P valuea</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 14 – 1</td>
<td>Day 14 – 1</td>
<td>Day 14 – 1</td>
<td>Day 21 – 1</td>
<td>Day 21 – 1</td>
<td>Day 21 – 1</td>
</tr>
<tr>
<td>1 (n = 13) 4(4, 3, 3, 3)</td>
<td>75.0 ± 5.1</td>
<td></td>
<td></td>
<td>145.4 ± 5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (n = 15) 4(4, 4, 4, 3)</td>
<td>76.1 ± 3.9</td>
<td>&gt;0.05</td>
<td></td>
<td>111.7 ± 5.5</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>3 (n = 15) 4(4, 4, 4, 3)</td>
<td>77.9 ± 4.0</td>
<td>&gt;0.05</td>
<td></td>
<td>120.1 ± 7.9</td>
<td>&lt;0.0001</td>
<td>0.008</td>
</tr>
<tr>
<td>4 (n = 13) 4(4, 3, 3, 3)</td>
<td>79.0 ± 4.2</td>
<td>&gt;0.05</td>
<td></td>
<td>121.1 ± 2.9</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>5 (n = 9) 3(3, 3, 3)</td>
<td>85.9 ± 12.0</td>
<td>&gt;0.05</td>
<td></td>
<td>121.8 ± 5.7</td>
<td>&lt;0.0001</td>
<td>0.0023</td>
</tr>
<tr>
<td>6 (n = 9) 3(3, 3, 3)</td>
<td>86.1 ± 3.9</td>
<td>0.00095</td>
<td></td>
<td>134.5 ± 5.9</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The differences in body weight gain (g) of the chickens between infected groups (Groups 2–6) and uninfected unmedicated group (Group 1) is analyzed by nested ANOVA and shown by P value.

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Days post-infection</th>
<th>Ln(OPG) + 1</th>
<th>Ln(OPG) + 1</th>
<th>Ln(OPG) + 1</th>
<th>Ln(OPG) + 1</th>
<th>Ln(OPG) + 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 13) 3</td>
<td>0</td>
<td>11.44 ± 0.11</td>
<td>12.18 ± 0.07</td>
<td>13.39 ± 0.11</td>
<td>13.96 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>2 (n = 15) 4</td>
<td>0</td>
<td>11.09 ± 0.05</td>
<td>11.83 ± 0.09</td>
<td>12.37 ± 0.06</td>
<td>13.55 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>3 (n = 15) 5</td>
<td>0</td>
<td>10.53 ± 0.12</td>
<td>11.54 ± 0.16</td>
<td>12.87 ± 0.10</td>
<td>13.21 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>4 (n = 13) 6</td>
<td>0</td>
<td>10.80 ± 0.07</td>
<td>11.72 ± 0.09</td>
<td>13.01 ± 0.03</td>
<td>13.58 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>5 (n = 9) 7</td>
<td>0</td>
<td>9.55 ± 0.40</td>
<td>11.54 ± 0.16</td>
<td>12.87 ± 0.10</td>
<td>13.21 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

The oocysts per gram feces (OPG) of the same chickens from Table 1 in Experiment 1 were counted from day 3 to day 7 post infection. The OPG values (>10³) of the chickens in each group were transformed into Ln(OPG + 1) and analyzed with ANOVA using the GLM procedure of SAS system under a normal distribution. The number (n) of chickens in each group is indicated.

3.5. Effect of B. pilosa on intestinal lesions of chickens following E. tenella challenge

Next, gross examination of the cecum in the animals that had access to different diets was performed 7 days after Eimeria infection. The gross cecal lesions score is shown in Table 3. The uninfected unmedicated control chickens (Group 1 (UI control), Table 3) had no lesions in the ceca (score = 0). In contrast, E. tenella caused more gross cecal lesions in the gut of unmedicated chickens 7 days post-infection, as evidenced by a lesion score close to 4 (Group 1 control, Table 3). Like maduramicin (Group 3 (1 Mad control)), B. pilosa at different doses (0.5%, 1% and 5%) significantly diminished cecal damage in infected chickens (Groups 4 (Bp5), 5 (Bp1) and 6 (Bp0.5), Table 3) as shown by the gross lesion scores of 2–3.

Mucosal damage caused by coccidia was examined by microscope and scored as microscopic cecal lesions based on the distribution and severity of mucosal destruction in chicken cecum (Table 4). No microscopic cecal lesions (score = 0) were observed in the uninfected unmedicated control group (Group 1 (UI control), Table 4), akin to the observation for gross cecal lesions in the same animals (Group 1 (UI control), Table 3). In sharp contrast, the infected unmedicated animals showed serious microscopic lesions (score = 7.8) in ceca 7 days after Eimeria infection (Group 2 (I control), Table 4). Severe ulceration, hemorrhage and decreased villi were also observed in ceca (data not shown). Oocysts, gametocytes and schizonts appeared inside the cecal epithelia (data not shown). The infected maduramicin-fed animals (Group 3 (1 Mad control), Table 4) showed mild improvement in microscopic lesions (score = 7.3) in the cecum from the infected unmedicated animals (Group 2 (I control), Table 4) post infection. However, the infected B. pilosa diet-fed animals (Groups 4 (Bp5), 5 (Bp1) and 6 (Bp0.5), Table 4) showed significantly reduced microscopic lesions (scores of 1.0–1.7) in the cecum. Consistently, B. pilosa decreased ulceration and hemorrhage and preserved more mucosa and villi in chicken ceca than control diets (data not shown). B. pilosa also decreased the number of oocysts, gametocytes and schizonts inside the cecal epithelia to a greater extent than maduramicin and control diets (data not shown). Overall, B. pilosa significantly reduced gut pathology in chickens following E. tenella infection.

Table 4

<table>
<thead>
<tr>
<th>Group</th>
<th>BWG (g)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 13) Day 14 – 1</td>
<td>75.0 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>2 (n = 15) Day 14 – 1</td>
<td>76.1 ± 3.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>3 (n = 15) Day 14 – 1</td>
<td>77.9 ± 4.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>4 (n = 13) Day 14 – 1</td>
<td>79.0 ± 4.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>5 (n = 9) Day 14 – 1</td>
<td>85.9 ± 12.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>6 (n = 9) Day 14 – 1</td>
<td>86.1 ± 3.9</td>
<td>0.00095</td>
</tr>
</tbody>
</table>

The chickens were classified into six groups. Group 1 (UI control) and Group 2 (I control) had daily access to standard chicken diet-fed birds in Group 1 control) as shown in Table 2. Next, gross examination of the cecum in the animals that had access to different diets was performed 7 days after Eimeria infection, as evidenced by a lesion score close to 4 (Group 1 control, Table 2).
In our study, Figs. S1 and S2). We also demonstrated that long-term use of 1% B. pilosa protects chickens against E. tenella infection.

3.6. Drug resistance of E. tenella to B. pilosa in chickens

In parallel, we tested drug resistance of E. tenella to B. pilosa using the ACI, which is a commonly-used index for the assessment of drug resistance (Li et al., 2004; Wang et al., 2006). Weight gain, survival rate, fecal oocyst excretion and lesion scores of the four groups of experimental chickens (Table 5) were used to calculate this index. After eight passages in infected chickens given standard diet for 168 days, the ACI value was 146 and 40, respectively, for chickens given 1% B. pilosa (Group Bp1) and 140 ppm salinomycin (Group Salino control), indicating the high drug resistance to E. tenella induced by salinomycin but not B. pilosa. The overall data demonstrated that long-term use of 1% B. pilosa showed low drug resistance to E. tenella, a superior result to that for salinomycin.

4. Discussion

Avian coccidiosis poses a continuous challenge to the poultry industry. Due to unmet efficacy and side effects of anti-coccidial drugs and vaccines, edible plants are considered possible viable alternatives to replace current anti-coccidial approaches (Orengo et al., 2012; Remmal et al., 2011). Here, we established spectroscopic methods for chemistry, manufacturing and control of B. pilosa (Fig. 1 and Supplementary Figs. S1 and S2). We also demonstrated that B. pilosa protects chickens against Eimeria infection (Fig. 2 and Tables 1–4) and resistance of E. tenella is poorly developed after long-term treatment with B. pilosa (Table 5). This study, for the first time, proved the feasibility of the use of B. pilosa as an anti-coccidial agent in chickens.

Chicken E. tenella infection rate and mortality are 20–100% and 20–60%, respectively. The severity of both indices is dependent on chicken genetics and Eimeria species (Abu-Akkada and Awad, 2012). For example, the mortality of chickens caused by different isolates of E. tenella can reach up to 40% (Dakpogan et al., 2012). In our study, E. tenella isolate, Et C1, was isolated and amplified from a single E. tenella oocyst in chickens. This E. tenella isolate was considered to be a pure strain based on its morphological traits and molecular markers (Supplementary Fig. S3). We found that E. tenella used in this study caused ~40% of chicken death (Fig. 2B). Clearly, this high mortality is attributable to the virulence, but not the impurity, of the E. tenella isolate.

So far, 20 or so plants have been shown to possess anti-coccidial activities. Nevertheless, some of them showed discrepancies in in vitro and in vivo anti-protozoal bioactivities (van der Heijden and Landman, 2008a, 2008b). One explanation could be lack or insufficiency of batch consistency and/or quality control in the preparation of the plant products. In this work, we established a protocol by which to prepare and analyze B. pilosa extracts using phytochemical techniques (Fig. 1 and Supplementary Figs. S1 and S2). These efforts can ensure the quality of B. pilosa as an anti-coccidial formulation.

More importantly, our data showed that B. pilosa is prophylactically effective against E. tenella in young chickens aged 14 days (Fig. 2 and Tables 1–4). Survival rate, body weight, oocyst shedding, and cecal lesions were used as indicators by which to evaluate the anti-coccidial potential and drug resistance to E. tenella of B. pilosa using the same protocols as published elsewhere (Awaits et al., 2011; Table 3 Gross lesion scores in the ceca of chickens fed standard diet alone or supplemented with maduramicin and different doses of B. pilosa 7 days post E. tenella infection.

<table>
<thead>
<tr>
<th>Group b</th>
<th>Gross lesion score a</th>
<th>Average b</th>
<th>P value c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 13)</td>
<td>13 0 0 0 0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>2 (n = 15)</td>
<td>0 0 0 4 11</td>
<td>3.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>3 (n = 15)</td>
<td>0 0 3 6 6</td>
<td>3.2 ± 0.8 0.0876</td>
<td></td>
</tr>
<tr>
<td>4 (n = 13)</td>
<td>0 0 5 5 3</td>
<td>2.8 ± 0.8 0.0003</td>
<td></td>
</tr>
<tr>
<td>5 (n = 9)</td>
<td>0 1 3 4 1</td>
<td>2.6 ± 0.9 0.0091</td>
<td></td>
</tr>
<tr>
<td>6 (n = 9)</td>
<td>0 1 5 3 0</td>
<td>2.2 ± 0.7 0.0008</td>
<td></td>
</tr>
</tbody>
</table>

In Experiment 2, experimental induction and assessment of drug resistance of E. tenella in Groups 7 (uninfected unmedicated chickens, UI control), 8 (infected unmedicated chickens, I control), 9 (infected B. pilosa-fed chickens, Bp1) and 10 (infected salinomycin-fed chickens, I Salino control) are described in the Materials and methods section. The formulae for the RBWG, SR, LSI, OI and ACI values are also indicated in the Materials and methods section. The number (n) of chickens in each group is indicated.

Table 4 Microscopic lesion scores in the ceca of chickens fed standard diet alone or supplemented with maduramicin and different doses of B. pilosa 7 days post E. tenella infection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Microscopic lesion score a</th>
<th>Average b</th>
<th>P value c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 13)</td>
<td>65 0 0 0 0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>2 (n = 15)</td>
<td>0 0 0 0 0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>3 (n = 15)</td>
<td>0 0 0 0 0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>4 (n = 13)</td>
<td>23 0 24 7 11</td>
<td>7.3 ± 1.2 0.0001</td>
<td></td>
</tr>
<tr>
<td>5 (n = 9)</td>
<td>16 0 19 2 8</td>
<td>1.7 ± 1.5 0.0001</td>
<td></td>
</tr>
<tr>
<td>6 (n = 9)</td>
<td>22 0 23 0 0</td>
<td>1.0 ± 1.0 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

In Experiment 2, experimental induction and assessment of drug resistance of E. tenella in Groups 7 (uninfected unmedicated chickens, UI control), 8 (infected unmedicated chickens, I control), 9 (infected B. pilosa-fed chickens, Bp1) and 10 (infected salinomycin-fed chickens, I Salino control) are described in the Materials and methods section. The formulae for the RBWG, SR, LSI, OI and ACI values are also indicated in the Materials and methods section. The number (n) of chickens in each group is indicated.

Table 5 Evaluation of drug resistance of E. tenella after eight serial passages in chickens given salinomycin and B. pilosa.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBWG (%)</th>
<th>SR (%)</th>
<th>LSI</th>
<th>OI</th>
<th>ACI</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (n = 10)</td>
<td>100 100</td>
<td>0 0 200</td>
<td>8 (n = 15)</td>
<td>60.3 60 33.8 40 46.6</td>
<td></td>
</tr>
<tr>
<td>9 (n = 15)</td>
<td>79.5 100 23.3 10 146.2</td>
<td>10 (n = 15)</td>
<td>32.4 80 32.5 40 39.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Hassan et al., 2008). Our study demonstrated that B. pilosa at the dose of 0.5% of chicken feed or more, conferred 100% protection against Eimeria challenge in chickens (Fig. 2B). B. pilosa consistently reduced fecal oocyst excretion (Table 2) and degree of intestine destruction (Table 3 and 4). Accordingly, B. pilosa treatment improved the reduced weight gain in chickens infected with Eimeria (Table 1). This improvement can be attributed to the ability of B. pilosa to control Eimeria infection and, to some degree, to induce weight gain in chickens (Table 1). The modest weight-gaining effect of B. pilosa powder reflects the fact that this plant is used as a food and has nutritional value as described elsewhere (Bartolome et al., 2013). B. pilosa at 0.5% of chicken diet is effective against chicken coccidiosis in our experimental system. However, higher doses of B. pilosa seems bad for coccidiosis control as evidenced by weight gain, gut pathology and oocyst excretion. These detrimental effects may be associated with the higher viscosity of the gut content when more B. pilosa is added to the chicken diet.

Apart from anti-coccidial action, B. pilosa has several other advantages over the anticoccidial drugs, maduramicin and salinomycin. First, B. pilosa is an edible plant and, therefore, there is little concern about biosafety and drug residue in chicken meat. Second, B. pilosa has a novel anti-coccidial action, which is different from that of commercial drugs. Third, long-term use of B. pilosa shows much lower drug resistance than that of salinomycin. This application of B. pilosa may reduce massive use of anti-coccidial drugs, anti-coccidial drug residue in chicken products, generation of drug-resistant mutants and concerns about public health. This work also expands the medicinal utility of B. pilosa in veterinary medicine.

Drug resistance has been reported against almost all anticoccidial drugs and is a major issue for coccidiosis control (Li et al., 2005). The ACI values revealed that drug resistance of E. tenella to salinomycin significantly increased in 168-day induction experiments (Table 5). In sharp contrast, its drug resistance to B. pilosa was poorly developed (Table 5). Of note, the degree of the drug resistance to B. pilosa may be underestimated because the weight-gaining effect of this plant. Nevertheless, the ACI data suggest that B. pilosa induced little, if any, drug resistance. The reason for this may be that this plant possesses multiple bioactive compounds, which may simultaneously inhibit different pathways of E. tenella. It is not hard to image that E. tenella can develop drug resistance to one agent more easily than multiple agents with different chemical structures.

The anti-coccidial mechanism of action of B. pilosa is currently unclear and needs to be ascertained in further studies. Direct chemical destruction and attenuation of invasive sporozoites are the primary reasons for decreases in oocyst excretion, induction of precocious lines and control drug resistance (Li et al., 2004; McDonald and Shirley, 2009). Since B. pilosa significantly reduced the shedding of fecal oocysts (Table 2) and drug resistance (Table 5), it is plausible that the compounds in B. pilosa act to destroy and attenuate Coccidia. Our earlier publications showed that B. pilosa can increase Th2 immunity (Chang et al., 2007a, 2007b). These findings suggest that B. pilosa may promote the clearance of Coccidia via immune regulation. In the future, identification of the active compounds in B. pilosa...
that are active against coccidiosis will be pivotal for unveiling its mode of action.

5. Conclusions

Here, we performed chemical fingerprint analyses to determine batch consistency and quality control of B. pilosa preparations, and demonstrated that B. pilosa, used as a feed additive can protect against E. tenella in chickens by reducing mortality, oocyst excretion and intestinal lesions. In addition, B. pilosa decreased the induction of drug-resistant E. tenella. In summary, this study illustrates the anti-coccidial potential of B. pilosa in chickens.

Acknowledgements

This work was supported by National Chung-Hsing University (100S0901), the National Science Council of Taiwan (NSC97-2320-B-005-001-MY3) and Academia Sinica (101S0010056). The authors thank Dr. Chun-Hou Chen of Academia Sinica for his constructive suggestions.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rvsc.2014.11.002.


The effects of baicalein on canine osteosarcoma cell proliferation and death

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Abstract

Flavonoids are a group of modified triphenolic compounds from plants with medicinal properties. Baicalein, a specific flavone primarily isolated from plant roots (Scutellaria baicalensis), is commonly used in Eastern medicine for its anti-inflammatory and antineoplastic properties. Previous research shows greater efficacy for baicalein than most flavonoids; however, there has been little work examining their effects on sarcoma cells, let alone canine cells. Three canine osteosarcoma cell lines (HMPOS, D17 and OS 2.4) were treated with baicalein to examine cell viability, cell cycle kinetics, anchorage-independent growth and apoptosis. Results showed that osteosarcoma cells were sensitive to baicalein at concentrations from approximately 1 to 25 μM. Modest cell cycle changes were observed in one cell line. Baicalein was effective in inducing apoptosis and did not prevent doxorubicin cell proliferation inhibition in all the cell lines. The mechanism for induction of apoptosis has not been fully elucidated; however, changes in mitochondrial permeability supersede the apoptotic response.

Keywords

apoptosis, baicalein, chemotherapy, doxorubicin, flavones, osteosarcoma

Introduction

Osteosarcoma is the most prevalent canine primary bone neoplasia, accounting for 5% of all canine tumours, with a higher incidence in large breed dogs. It carries a poor long-term prognosis because of metastasis. In the majority of cases at the time of diagnosis, clinically non-detectable micrometastasis is likely, thereby reducing survival times. The median survival times range from 165 to 470 days, depending on treatment modalities utilized. Treatment typically involves surgery (amputation of the affected limb or limb-sparing procedures) and follow-up chemotherapy, and a majority of dogs diagnosed with osteosarcoma do not live more than 2 years past initial diagnosis.

In the past 20 years, there has been increased use of nutraceuticals or herbal remedies as a palliative treatment by owners of dogs with cancer. While many of these nutraceuticals have potential antioxidant properties, data regarding the effects on canine cancer are limited. More importantly, it is unknown whether these alternative treatments enhance or diminish current traditional chemotherapies when used in conjunction. Recent in vitro data show cytotoxic effects when using carotenoids or isoflavones with in vitro canine osteosarcoma and lymphoma cell lines, respectively; yet in vivo data are lacking. Many of the bioactive components in various nutraceuticals and herbal compounds are classified as flavonoids. Flavonoids are polyphenolic compounds found as secondary metabolites of plants, and are ubiquitous in fruits, vegetables and nuts.

Specifically, Scutellaria baicalensis root, also known as Ma-Huang, has been used for centuries in Eastern medicine for multiple ailments. The bioactive flavones found in this root are the phytochemicals wogonin, baicalin and baicalein. The flavone baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) in particular has been reported to have a broad spectrum of physiological effects, which include reducing hypertension, decreasing inflammation and inhibiting neoplastic proliferation. In many instances, baicalein, as a purified compound, has been shown to be
cytostatic in a number of in vitro and xenograft models, and may also induce apoptosis in neoplastic prostate and urinary bladder cell lines.8–11 The mechanism of baicalein’s cytostatic and apoptotic effects appears to be multifaceted. Alterations in mitochondrial-induced apoptosis, p53-induced cell cycle dynamics and MAP kinase signalling cascades are considered targets in baicalein-induced cell senescence and death.12–15 Although considerable work has been performed on human and mouse cancer epithelial cell lines and xenograft models, the lack of empirical data in sarcoma xenografts or cell lines warrants further investigation. Considering the need for advancements in the treatment of canine osteosarcoma and its prevalence in canine oncology, we sought to assess the effect of baicalein on three canine osteosarcoma cell lines. Cell proliferation assays, soft agar growth, cell cycle analysis and time-course Western blotting for proteins associated with cell cycle alterations, mitochondrial-induced apoptosis and MAP kinase signalling were performed. Additionally, cell proliferation assays using baicalein in conjunction with the chemotherapeutic doxorubicin were performed to observe whether dual treatment would enhance or diminish cell survival.

Materials and methods

Cell culture

Three canine osteosarcoma cell lines were obtained from appendicular osteosarcomas; OS 2.4 cells from Dr Katrina Mealy – Washington State University, HMPOS cells from the Cornell University Comparative Oncology Program and D17 cells from the American Type Culture Collection. A-72 fibroblasts derived from a canine thymoma were also obtained from the American Type Culture Collection. Cells were maintained in complete medium that comprised RPMI 1640 (Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Invitrogen) at 37 °C and 5% CO2 for all experiments and for passage of cells, unless indicated otherwise. Baicalein (Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) and stored under nitrogen gas at −80 °C for up to 1 month.

Forty-eight-hour MTT proliferation assay

3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT; Sigma) assays were performed using D17, OS 2.4, HMPOS and A-72 fibroblasts plated at a density of 2500 cells per well in 96-well tissue culture-treated plates (Costar; Fisher Scientific, Waltham, MA, USA) and incubated overnight. The following day cells were treated with vehicle (DMSO) or various concentrations of baicalein ranging from 0.4 to 25 μM in a serial dilution for 48 h. Briefly, MTT assays were performed after 48 h of treatment by adding 20 μL of MTT dye [5 mg mL−1 in phosphate-buffered saline (PBS)] to the incubating cells for an hour at 37 °C in 5% CO2. Media were then decanted, and the cells were washed with sterile PBS solution, immediately solubilized in 200 μL of isopropanol (Sigma) and evaluated by use of a spectrophotometric plate reader (Epoch; Biotek, Winooski, VT, USA) at a wavelength of 540 nm as previously described.16 Each concentration was assayed in triplicate during three separate experiments, and a mean ± standard deviation (SD) was calculated for each time point.

Growth curves

Cells were plated at a density of 1000 cells per well in 96-well tissue culture-treated plates in RPMI with 2% FBS and 1% antibiotic/antimycotic and incubated overnight. Cells were then treated with vehicle control (VC; DMSO) or baicalein at 12.5, 6.25, 3.13, 1.57, 0.79 and 0.40 μM. MTT assays were performed every 2 days for a total of 6 days. MTT assays were performed as described above. Each concentration was run in triplicate during three separate experiments, and a mean ± SD was calculated for each time point.

Soft agar analysis

Soft agar analysis was performed with all three cell lines in accordance with the procedure described previously.17 Briefly, complete medium with 0.3% melted soft agar (Agar VII; Sigma) was plated to create a layer of agar. Once solidified, more soft agar matrix was dissolved in the RPMI medium base, and 5000 cells per well were added with either 1, 5 or...
10 μM of baicalein, VC (DMSO) or medium alone. Each treatment was plated in triplicate. Agar with and without baicalein at appropriate concentrations was refreshed every 3 days for 15 days. On day 15, all colonies were counted using the 40× objective of an inverted microscope for each of the 40-mm wells, and a mean ± SD was calculated for each treatment.

Flow cytometry

All three osteosarcoma cell lines were plated in six-well tissue culture dishes in complete medium. Cells were treated with DMSO or 10 μM baicalein for 24 and 48 h. Cells were then trypsinized, washed twice with PBS and fixed with cold ethanol overnight at 4 °C. Fixed cells were stained with 10 μg mL⁻¹ propidium iodide solution (Sigma), treated with bovine pancreatic RNAse (Sigma) and subjected to flow cytometry as previously described using a FacsCalibur with CellQuestPro Software (BD Biosciences, San Jose, CA, USA). Each treatment was performed in triplicate for each cell line. Cell populations were identified by their distinctive position on forward- and side-scatter plots and gated. For each sample, 10 000 gated events were acquired. Total event counts within the sub-G1, G1, S and G2M phases were used to calculate the percentage of cells within each phase of the cell cycle. Data are presented as the mean ± 1 SD. The exception was sub-G1, which was assessed in comparison with all cells in the cell cycle as a representation of apoptotic debris.

Mitochondrial permeability assays

A MitoPT kit (Immugenetics, St. Paul, MN, USA) was used to evaluate intact non-permeable versus permeable mitochondria. The kit was used in accordance with the manufacturer’s recommendations. The HMPOS and D17 cell lines were chosen because of their potential future use in xenograft models. Briefly, 10 000 cells were plated on chamber slides and allowed to incubate in growth media overnight. The next morning, cells were treated with DMSO or 10 μM baicalein. After incubation for 24, 36 and 48 h, 5 μL of MitoPT solution was added to the cell culture media, and cultures were incubated at 37 °C for 15 min. The chamber slides were then removed from the incubator and washed two times with wash buffer. A cover slip was immediately applied to each chamber slide, and slides were examined via a fluorescent microscope using both 595 and 345 wavelength filters. A total of 400 cells were counted for each chamber; cells were quantified on the basis of staining characteristics (i.e. red or green), and the percentage of green cells was determined at each time point for vehicle-treated control cells and baicalein-treated cells. Each experiment was repeated three times, and a data are represented as mean ± 1 SD.

Cell lysis and Western blot analysis

D17 and HMPOS cells were grown in RPMI with 2% FBS and 1% antibiotic/antimycotic and treated for variable amounts of time with vehicle (DMSO) and 10 μM baicalein. DMSO-treated cells at 24 and 48 h were lysed to serve as control time points. Baicalein-treated cells were lysed at 12, 24, 36 and 48 h of incubation. Cells were lysed in accordance with a protocol described previously. Cell lysates were collected, and protein concentration was determined for each sample by use of the Bradford technique. Lysates were equilibrated to a common volume (μg μL⁻¹ basis) in lysis and loading buffer. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 8, 12 or 15% SDS-PAGE depending on the kilodalton of the protein of interest by loading 30 μg of total protein per well. The gels were then transferred to polyvinylidene fluoride membranes for 1 h at 300 mA and then placed into 5% milk in Tris-buffered saline/0.5% Tween solution (TBST). Membranes were then transferred to primary antibody solutions including extracellular regulated kinase (ERK), p53 (R&D Biosciences, Boston, MA, USA), Bax, Bcl-2, caspase-3, phospho-ERK, protein kinase B (AKT), ser473 phospho-AKT, jun-n-terminal kinase (JNK) and phospho-JNK (Cell Signaling, Danvers, MA, USA) and β-actin (Sigma) overnight on a rocking platform at 4 °C. All primary antibodies were diluted 1:1000 in TBST, except for anti-β-actin (1:10 000) and anti-ERK (1:5000). Membranes were washed twice with TBST and then incubated at 37 °C for 1 h with 1:5000 dilutions of corresponding anti-rabbit.
IgG or anti-mouse IgG horseradish peroxidase-labelled secondary antibodies (Cell Signaling). Blots were again washed three times with TBST and exposed to chemiluminescent reagent (Western lighting reagent; Millipore, Billerica, MA, USA). Digital images were captured using an imaging system (Biospectrum 410; UVP, Upland, CA, USA).

Doxorubicin proliferation assays

Cells were treated with vehicle (DMSO) or various concentrations of baicalein on the basis of previous growth curve evaluations for cell cultures in complete media. All three cell lines were treated with similar concentrations of baicalein as in the aforementioned 48-h MTT cytotoxicity assay. Cells were then incubated with various concentrations of doxorubicin (0.125 μM for OS 2.4, 0.0125 μM for HMPOS and 0.33 μM for D17) to achieve between 40 and 60% proliferation inhibition or DMSO VC for 4 h. Media were then changed to the initial concentrations of baicalein for the remainder of the 48-h incubation. At the end of the 48-h incubation, MTT assays were performed. Optical density for DMSO treatment versus baicalein treatment was compared to doxorubicin with DMSO versus doxorubicin and various concentrations of baicalien to determine if baicalein increased or diminished the proliferative response beyond that of doxorubicin alone. Three replicates of the experiment were repeated in triplicate, and a mean ± SD was calculated.

Statistical analysis

Data analysis for normality was performed utilizing Shapiro–Wilk testing and revealed normality for a majority of the data; therefore, parametric statistics were used. All soft agar results, 48-h proliferation, growth curves and doxorubicin proliferation were evaluated using one-way analysis of variance compared to VC or control cells with Tukey’s post hoc comparisons, with an α set at P ≤ 0.05. All flow cytometry and mitochondrial permeability apoptosis data were compared with VC (DMSO) groups by use of a non-paired Student T-test to determine significant differences at each time point with an α set at P ≤ 0.05. Additionally, probit analysis was performed for each cell line to determine the 50% inhibitory concentration (IC50) for baicalein alone and in conjunction with doxorubicin.

Results

MTT proliferation assay and growth curve

All three canine osteosarcoma cell lines began to show significant decreases in proliferation when treated with between 0.8 and 1.57 μM baicalein when compared with DMSO VC-treated cells in the 48-h proliferation assay. A-72 fibroblast cells showed significantly diminished cell proliferation at 25 μM. All three osteosarcoma cells showed increased sensitivity with IC50s between 3 and 10 μM (Fig. 1). A-72 fibroblasts did not reach an IC50 using this concentration range. The 6-day growth curve data show a similar range of approximately 2–6 μM IC50 for proliferation. The OS 2.4 cell line was most sensitive, with significant decreases in proliferation at 0.8 μM. Higher concentrations were needed to produce a significant decrease in proliferation in HMPOS and D17 cells at approximately 3.13 μM (Fig. 2).

Soft agar analysis

All three cell lines grew successfully in soft agar and had a significant decrease in colony formation when treated with 5 and 10 μM baicalein compared with the VC group. OS 2.4 cells displayed a complete loss of colony formation with 10 μM, indicating that these cells are more sensitive than the other two cell lines (Fig. 3). D17 cells showed no inhibition of colony formation at 1 μM, whereas both OS 2.4 and HMPOS showed significantly diminished colony formation at this concentration (P < 0.05).

Cell cycle analysis

Analysis of all three cell lines revealed no pronounced shifts in cell cycle dynamics during G1/S, S or G2/M attributable to treatment with the vehicle (DMSO) or 10 μM baicalein, except for the OS 2.4 cells, which showed a mild, yet significant increase in G0/G1 and a decrease in G2M (Table 1). All cell lines ranged between 65 and
Baicalein in canine osteosarcoma cells

Figure 1. Canine osteosarcoma cells 48-h proliferation MTT assay: significant antiproliferative activity starts at concentration of 0.8–1.57 μM for all osteosarcoma cell lines, with A-72 fibroblasts requiring a higher concentration (25 μM). Lowest concentrations to show significant \((P \leq 0.05)\) antiproliferative activity are indicated by # for OS 2.4, * for HMPOS, @ for D17 and ^ for A-72.

85% for G1/0, 6 to 20% for S to 9 and 15% for G2M. Treatment of OS 2.4 and HMPOS cells with baicalein induced a significant increase in sub-G1 nuclear debris (apoptosis) compared with VC-treated cells (Table 1).

Western blot analysis

The lack of prominent cell cycle changes and only mild increases in G0/1 in OS 2.4 cells led to immunoblotting for p53 expression and revealed no expression of p53 with or without baicalein treatment in all osteosarcoma cells. The A-72 cells showed a basal expression of p53; however, there was no observed increase in p53 expression after baicalein treatment (Fig. 4). Western blot analysis for markers of apoptosis in HMPOS and D17 cell lines revealed activation of caspase-3 with evidence of the cleaved caspase-3 fragment at 17 kDa by 36–48 h after treatment with baicalein. The increase in caspase-3 was preceded by a decrease in Bcl-2 expression, which inhibits Bax-induced mitochondrial permeability changes. In contrast, the expression of Bax, the major promitochondrial membrane permeability protein, was not significantly altered throughout treatment (Fig. 5). Western blotting for the MAP kinase activation (ERK and JNK) as well as AKT activation showed no appreciable changes because of baicalein treatment, suggesting that baicalein does not positively or negatively affect these cell signalling events (data not shown).

Mitochondrial permeability assays

Mitochondrial permeability was assessed using the MitoPT assay and dual immunofluorescence. Under normal cellular conditions, the electrochemical gradient of intact mitochondria results in aggregation of the dye, which fluoresces red. In contrast, fluorescent dye escapes the depolarized mitochondria of apoptotic cells, and the non-aggregated dye fluoresces green (Fig. 6A). VC-treated cells at 24...
Figure 3. Osteosarcoma soft agar colony formation. Colonies were treated with vehicle control (VC), 1, 5 and 10 μM baicalein. Only HMPOS and OS 2.4 cells showed significant reduction in colony numbers at 1 μM. *indicated \( P < 0.05 \), with all cell lines showing significant colony reduction at 5 and 10 μM.

Table 1. Cell cycle analysis after 24 h of 10 μM baicalein or vehicle control treatment

<table>
<thead>
<tr>
<th>Cell cycle analysis</th>
<th>G1/O (%)</th>
<th>S (%)</th>
<th>G2/M (%)</th>
<th>Sub-G1 (%)</th>
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<tr>
<td>D17 control</td>
<td>65 ± 4</td>
<td>20 ± 4</td>
<td>15 ± 5</td>
<td>18 ± 5</td>
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<tr>
<td>D17 baicalein 24 h</td>
<td>70 ± 2*</td>
<td>18 ± 1</td>
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<td>22 ± 4</td>
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<td>72 ± 1</td>
<td>10 ± 2</td>
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<td>6 ± 2</td>
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<td>HMPOS control</td>
<td>78 ± 3</td>
<td>13 ± 1</td>
<td>9 ± 2</td>
<td>4 ± 1</td>
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<tr>
<td>HMPOS baicalein 24 h</td>
<td>73 ± 1</td>
<td>16 ± 2</td>
<td>11 ± 1</td>
<td>19 ± 1*</td>
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</tbody>
</table>

Only OS 2.4 cells show a modest significant increase in G1/O and a decrease in G2/M. Both OS 2.4 and HMPOS cells had increased sub-G1 populations, which likely reflect apoptotic or necrotic nuclear debris. D17 cells show no alterations in the cell cycle. *P ≤ 0.05 from control cells.

and 36 h showed less than 10% green fluorescing cells when compared with baicalein at 24 or 36 h of treatment which approached 50% by 36 h in both cell lines, which was significantly greater than VC cells at both time points (Fig. 6B).

Figure 4. p53 Immunoblot: A-72 fibroblasts and three canine osteosarcoma cells were treated with vehicle control or 10 μM baicalein for 24 h to examine p53 when compared with control protein. The molecular weight of the native protein is approximately 53 kDa, whereas the control protein with the histidine tag is slightly larger at about 56 kDa. Only the A-72 fibroblasts show appreciable p53 protein expression that does not increase with baicalein treatment.

Figure 5. Time-course immunoblotting for markers of apoptosis and mitochondrial permeability in VC and 10 μM baicalein-treated D17 and HMPOS cells. Notice activation of caspase-3 as indication of end-stage apoptosis, while the antiapoptotic mitochondrial protein Bcl-2 decreases within 24 h, and Bax, the proapoptotic mitochondrial protein, appears unchanged compared to vehicle control-treated cells.

Doxorubicin cell proliferation inhibition

Doxorubicin inhibited cell proliferation in all three cell lines within 48 h after the onset of incubation with selected concentrations of doxorubicin to induce approximately 40–60% inhibition of cell proliferation. Concomitant serial dilutions of baicalein surrounding the IC50 of all three cell lines showed no protective effects of baicalein treatment because there was no increase in proliferation when treated with both doxorubicin and baicalein (Fig. 7A–C). D17 probit analysis for baicalein alone showed an IC50 of approximately 10.0 μM, whereas when treated in conjunction with a sublethal dose of doxorubicin it was 8.9 μM. OS 2.4 cell probit analysis for baicalein alone showed an IC50 of 1.9 μM, whereas in conjunction with doxorubicin it was 5.0 μM. Lastly, HMPOS cells when treated with baicalein alone showed an IC50 of 3.2 μM, whereas when treated in conjunction with a sublethal dose of doxorubicin it was 3.6 μM. Overall, when the concentrations of baicalein reached 3–6 μM in all osteosarcoma cell lines, there was a significant decrease (\( P < 0.05 \)) in cell viability/proliferation regardless of doxorubicin treatment.
Baicalein in canine osteosarcoma cells

Discussion

The use of flavones in vitro has provided ample evidence of antiproliferative effects in many instances; however, the concentrations needed to initiate cytostatic or apoptotic effects tend to be in the mid-to-high micromolar concentrations.\textsuperscript{8–12,20–22} Baicalein as a single flavone has been shown to inhibit cell proliferation and chemically induced carcinogenesis in rodent models.\textsuperscript{8} Additionally, there have been reports of baicalein being effective in slowing tumour or cellular growth at low micromolar concentrations.\textsuperscript{22,23} Our data suggest that canine osteosarcoma cells are sensitive to the cytostatic effects in the 0.8–2 \textmu M range for all three cell lines examined in a 48-h proliferative assay, which is the lowest concentration reported.
Figure 7. Doxorubicin/baicalein proliferation MTT assays. Cell lines (A) OS 2.4, (B) HMPOS and (C) D17 were examined at doses inducing a 30–60% decrease in cell proliferation with doxorubicin for 48 h with and without baicalein or vehicle controls (VC). The results of baicalein (black line) and baicalein with doxorubicin (grey line) remaining at or below dashed line indicate no survival advantage with the addition of baicalein to doxorubicin treatment. *Represents the first concentration, where a significant (\( P < 0.05 \)) decrease in cell proliferation is observed with baicalein treatment. ** Represents a significant (\( P < 0.05 \)) decrease in cell proliferation for the baicalein with doxorubicin treatment line at this concentration and higher.

to date. When performing growth curve assays in lower serum, the antiproliferative activity was slightly similar in the range of 0.8–3 \( \mu \text{M} \). Examination of anchorage-independent growth in soft agar is a potential measure of metastatic capability in vitro. Our results suggest that colony formation was inhibited with approximately 1–5 \( \mu \text{M} \), similar to the growth curve cytostatic effects. A serum concentration of approximately 1 \( \mu \text{M} \) is potentially physiologically achievable based on pharmacokinetics in other monogastric species after oral dosing.\(^{24–26}\) The pharmacokinetics in rats and humans suggest a serum half-life of approximately 2 h, whereas primate studies suggest a longer half-life of 4–6 h after oral dosing of approximately 150–500 \( \text{mg kg}^{-1} \) body weight.\(^{26}\) The serum concentrations in these primate studies peaked at approximately 1–2 \( \mu \text{M} \),\(^{26}\) and although serum concentration may not reflect tissue concentrations, the findings in other monogastrics warrant further oral pharmacokinetic studies in dogs as well as xenografted osteosarcoma bearing nude mouse models to examine baicalein’s potential for efficacy.

Previous studies examining the potential mechanisms by which baicalein exerts its negative effects on cell growth demonstrated alterations in the cell cycle causing stasis in \( \text{G}_{10} \) with relationships to p53 and/or p21 status.\(^{20,21}\) Examination of the cell cycle showed mild induction of apoptosis at 10 \( \mu \text{M} \) within 24 h in the OS 2.4 and HMPOS cell lines with no significant alterations in the cell cycle other than a mild increase in \( \text{G}_{10} \) in the OS 2.4 cells when compared with control cells. Additionally, Western blot analysis of all three cell lines showed no appreciable p53 expression when compared with the A-72 cells, and that baicalein treatment did not alter its expression in all the cell lines examined. These data suggest that p53 upregulation or phosphorylation is not involved in osteosarcoma cell response to baicalein. This lack of p53 is not uncommon in canine osteosarcoma, which points towards other mechanism of apoptosis induction.\(^{27}\)

Apoptosis involves controlled steps that lead to the loss of cell viability. These steps cause changes in the morphology of the dying cell, and include cytoplasmic condensation, DNA fragmentation and subsequent alterations in membrane phospholipids through the intrinsic and/or extrinsic pathways.\(^{28–30}\) The extrinsic pathway is signalled via ligand binding of death receptors such as Fas/CD95 or TNF receptor 1, and the intrinsic pathway is activated by mitochondrial alterations. Both the intrinsic and extrinsic pathways activate aspartate-specific cysteine proteases, known as
caspases, which induce global proteolysis once activated resulting in apoptosis.\(^{30}\) Time-course immunoblotting using the D17 and HMPOS cell lines using caspase 3 showed that 10 μM baicalein induced activation of caspase-3 (17-kDa fragment) within 36–48 h in both HMPOS and D17 cell lines providing evidence that cell death was initiated via apoptosis.

Upstream mechanisms were investigated by examining other known cell signalling pathways previously shown to be affected by baicalein including the phosphorylation status of extracellular regulated ERK, JNK and AKT, all of which are involved in cell proliferation and survival, but showed little alteration. Baicalein-induced alterations in mitochondrial permeability were also assessed through examination of Bcl-2 and Bax whose expression and balance within mitochondria play a role in mitochondrial-induced apoptosis. Bcl-2 and Bax are members of a family of proteins that have conserved BH domains. This family of proteins contains members that are either proapoptotic (Bax, Bak and Bok) or antiapoptotic (Bcl-2 family) and cell survival can depend partly on the activities of these proteins.\(^{31,32}\) Apoptotic members of the Bcl-2 family compete with Bax to prevent oligomerization of the caspase cascade, thereby protecting the cell against programmed cell death.\(^{33}\) Baicalein has been shown to induce the intrinsic pathway of apoptosis through alterations of common mitochondrial proapoptotic and antiapoptotic proteins, primarily through induction of Bax (proapoptotic) and decrease in Bcl-2 (antiapoptotic).\(^{13–15,28,34,35}\) Our findings suggest that canine osteosarcoma cells undergo apoptosis through similar induction by decreasing expression of Bcl-2, whereas Bax expression remains relatively constant or modestly increased and precedes the activation of caspase-3. Additionally, MitoPT staining was performed on D17 and HMPOS cells at 24 and 36 h post-treatment with baicalein-treated cells transitioned from a punctated red mitochondrial staining to a more diffuse green fluorescence, suggesting a similar time frame for mitochondrial permeability and the downregulation of Bcl-2.

Flavones are traditionally thought of as antioxidants and previous studies have shown that baicalein has antiapoptotic effects on myocytes, which is thought to be partially due to baicalein’s antioxidant effects.\(^{36,37}\) Baicalein has also been shown to reduce plasma concentrations of TNF-α, a proinflammatory cytokine that has the potential to trigger apoptosis.\(^{38}\) Therefore, because of these potential cell survival effects it is important to treat cells in combination with a common chemotherapeutic agent known to produce free radical damage. The cardiotoxic side effects associated with doxorubicin are due to free radical damage making it an ideal agent to examine the effects of baicalein during chemotherapeutic treatment of cells.\(^{39}\) Despite the known antiapoptotic effects on myocardial cells and antioxidant effects, baicalein did not hinder doxorubicin’s ability to decrease cell viability in all three osteosarcoma cell lines. Toxicologic probit analysis showed that IC\(_{50}\) of D17 and HMPOS cells with or without doxorubicin remained similar at around 10 and 4 μM, respectively. However, the OS 2.4 cells showed a very mild shift in IC\(_{50}\) from around 1 μM without doxorubicin to 5 μM, suggesting possible similar inhibitory mechanisms for both baicalein and doxorubicin in this cell line. Overall, there were no major shifts in IC\(_{50}\) or enhanced cell survival, suggesting that baicalein does not protect cells from doxorubicin’s inhibitory effects.

In conclusion, the use of baicalein in these \textit{in vitro} systems shows some utility in cytostatic and proapoptotic events. The slowing of growth and apoptosis seems to be initiated by alterations in mitochondrial membrane permeability through decreases in the Bcl-2 family of proteins. Although the concentrations needed ranged between 1 and 10 μM \textit{in vitro}, this may still be within a physiologically achievable range; however, future pharmacokinetic studies are needed to evaluate its therapeutic potential. Oral delivery systems in a xenograft model of D17 and HMPOS cells are warranted to determine whether primary or metastatic growth is diminished with oral baicalein treatment. Baicalein will likely never have primary chemotherapeutic utility to treat canine osteosarcoma. However, baicalein and other flavones may provide some safe and mildly effective treatment options during remission, or for those not electing to undergo traditional treatment. Extensive work is needed to elucidate its potential utility in treating osteosarcoma.
References


apoptosis in human gastric cancer cells. 
Evaluation of antioxidant capacity and inflammatory cytokine gene expression in horses fed silibinin complexed with phospholipid

Eileen S. Hackett, DVM, PhD; Khursheed R. Mama, DVM; David C. Twedt, DVM; Daniel L. Gustafson, PhD

Objective—To evaluate antioxidant capacity and inflammatory cytokine gene expression in horses fed silibinin complexed with phospholipid.

Animals—5 healthy horses.

Procedures—Horses consumed increasing orally administered doses of silibinin phospholipid during 4 nonconsecutive weeks (0 mg/kg, 6.5 mg/kg, 13 mg/kg, and 26 mg/kg of body weight, twice daily for 7 days each week). Dose-related changes in plasma antioxidant capacity, peripheral blood cell glutathione concentration and antioxidant enzyme activities, and blood cytokine gene expression were evaluated.

Results—Plasma antioxidant capacity increased throughout the study period with increasing dose. Red blood cell nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase I activity decreased significantly with increasing doses of silibinin phospholipid. No significant differences were identified in glutathione peroxidase activity, reduced glutathione or oxidized glutathione concentrations, or expression of tumor necrosis factor α, interleukin-1, or interleukin-2.

Conclusions and Clinical Relevance—Minor alterations in antioxidant capacity of healthy horses that consumed silibinin phospholipid occurred and suggest that further study in horses with liver disease is indicated. (Am J Vet Res 2013;74:1333–1339)
beneficial in recognition of foreign antigens and clearing infection. However, prolonged or overexuberant expression of inflammatory cytokines can be detrimental and has been linked to pathological changes associated with disease.12 Hepatocellular injury may be caused by a combination of the primary oxidative effect and the secondary inflammatory response induced by damaged hepatocytes.13 Tumor necrosis factor α is directly toxic to hepatocytes and induces apoptosis.14 Interleukin-1β and IL-6 reduce hepatocyte protein synthesis, carbohydrate metabolism, and cytochrome P450–dependent detoxification.15 Blood chemokine concentration has been associated with severity of hepatic disease in humans.16 Derangement in the balance of pro- and anti-inflammatory serum cytokines is characteristic of alcoholic cirrhosis and is predictive of prognosis and anti-inflammatory serum cytokines is characteristic of alcoholic cirrhosis and is predictive of prognosis and mortality in humans.17 Inhibiting cytokine release and subsequent inflammatory cell recruitment may limit organ damage. Silibinin protects against inflammation by limiting oxidative injury, inhibiting neutrophil migration, and regulating inflammatory mediators in rats.18 Silibinin inhibits expression and synthesis of inflammatory cytokines TNFα, IL-1, and IL-2 in the presence of diseases and inflammatory stimuli in mice.19,10

The purpose of the study reported here was to evaluate antioxidant capacity and inflammatory cytokine gene expression in horses fed silibinin complexed with phospholipid. It was hypothesized that oral silibinin administration would increase antioxidant capacity in the blood of healthy horses, that these effects would be dose dependent, and that oral silibinin phospholipid administration would not alter gene expression of inflammatory cytokines in the blood of healthy horses because of the absence of preexisting disease or inflammation.

Materials and Methods

This study was performed in conjunction with an institutional animal care and use committee–approved phase II pharmacokinetic study.20 Five horses owned by the Colorado State University Veterinary Teaching Hospital and acclimatized to their housing were used with permission, and environmental conditions were not changed. Horses were group housed in a paddock without access to grass. Horses received water ad libitum and Timothy grass hay once daily, providing for consumption of approximately 12 kg of hay/horse per day. All horses were geldings with a mean ± SD age of 13 ± 3 years (median, 14 years [range, 5 to 17 years]) and mean weight of 582 ± SD 63 kg (median, 613 kg [range, 472 to 625 kg]). There were 3 Quarter Horses, 1 Arabian, and 1 Andalusian. Horses were screened prior to inclusion in the study for evidence of gastrointestinal tract or liver dysfunction by use of physical examination and serum biochemical analyses and were selected only if they readily consumed the carrier diet (400 g of pelleted feed, 50 g of wheat bran, and 150 mL of water [per meal]). Body weight was measured with a commercial scale, and signalment was recorded. Horses consumed each dose twice daily each day for 7 days during 4 administration periods, with progressively higher doses of silibinin phospholipid administered during each period and with each period separated by a washout period (minimum of 2 weeks). During week 1, twice daily, horses consumed the carrier diet without silibinin phospholipid. During week 2, horses were fed the diet plus 20 mg of silibinin phospholipid/kg of carrier diet, resulting in a 6.3 mg/kg of body weight dose of silibinin. During week 3, horses were fed the diet plus 40 mg of silibinin phospholipid/kg of diet, resulting in a 13 mg/kg dose of silibinin. During week 4, horses were fed the diet plus 80 mg of silibinin phospholipid/kg of diet, resulting in a 26 mg/kg dose of silibinin. Mean ± SD total dose of silibinin phospholipid administered in feed in week 2 was 11.6 ± 1.3 g (median, 12.3 [range, 9.4 to 12.5 g]), in week 3 was 23.3 ± 2.6 g (median, 24.6 g [range, 18.9 to 25.0 g]), and week 4 was 46.5 ± 5.2 g (median, 49.2 g [range, 37.8 to 50.0 g]). All 5 horses received identical treatments, except on day 7 of week 4 (highest-dose week), when the final meal supplemented with silibinin phospholipid was incompletely consumed (1 horse) or consumed slowly (1 horse).

Sample collection—Blood samples (total volume, 40 mL) were obtained from horses on day 1, prior to administration of unsupplemented diet or carrier diet mixed with silibinin phospholipid, and on day 7, 1 hour following the final meal of the study diet, each week. Blood for antioxidant analysis was collected directly into EDTA tubes. Plasma was immediately separated via centrifugation at 2,500 X g for 10 minutes, transferred to cryovials, submerged in liquid nitrogen until frozen, and stored at –80°C until analysis. Following plasma removal, the buffy coat layer was transferred to a 15-mL tube, and the pelleted RBCs were transferred to a cryovial, submerged in liquid nitrogen until frozen, and stored at –80°C until analysis. Theuffy coat sample was brought to a final 6-mL sample volume with PBS solution, layered onto 4 mL of a mixture of nonionic, synthetic polymer of sucrose6 and sodium diatrizoate,4 and centrifuged at 800 X g for 30 minutes at 20°C. Following centrifugation, the fraction containing PBMCs was collected and washed twice with PBS solution. The PBMCs were counted with a hemacytometer and resuspended in PBS solution prior to freezing at –80°C until analysis. Blood for cytokine gene expression analysis was collected directly into commercially available evacuated tubes containing a proprietary additive to stabilize the in vivo gene transcription profile by reduction of in vitro RNA degradation,7 maintained at 20°C for 60 minutes, and then frozen at –20°C until analysis as per the manufacturer’s instructions.

Protein assay—Protein measurement in plasma, RBC, and PBMC samples was necessary to report protein-corrected values of glutathione and antioxidant enzymes. The RBC and PBMC samples required additional processing prior to analysis and were thawed on ice and diluted in 25mM Tris (pH, 7.4). Samples were then disrupted by sonication8 in three 2-second bursts at 30% power on ice and centrifuged at 15,000 X g; the supernatant was collected for further analysis. Colorimetric measurement of protein was performed by use of bicinchoninic acid and standard curves of bovine serum albumin.8 Samples were added to a 96-well microplate with 200 µL of working reagent containing bicinchoninic acid, and incubated for 30 minutes at 37°C.
Following incubation, absorbance was measured at 562 nm with a microplate reader. Net absorbance was calculated by subtracting values of blank samples from values of bovine albumin standards and test sample replicates. Standard curves were graphed by plotting mean blank-corrected albumin standard values versus concentration and test samples estimated via linear regression. Protein concentration was reported in milligrams per milliliter.

ORAC—The ORAC of plasma was measured with a commercially available assay as described by Ungvari et al. The assay evaluated the ability of plasma samples to delay oxidation of a fluorescent probe by peroxyl radicals relative to known concentrations of a water-soluble vitamin E analog and has been validated in horses and other species. Plasma samples were thawed on ice, vortexed, and diluted 1:100. Fluorescein solution and either plasma or standard curve samples were added to a 96-well microtiter plate and incubated for 30 minutes at 37°C. The plate was read immediately following addition of 2,2′-azobis(2-methylpropionamide) hydrochloride. Fluorescence was recorded every 64 seconds for 1 hour with a fluorescent microplate reader with an excitation wavelength of 485 nm and an emission wavelength of 538 nm. Net AUC for plasma samples was calculated by subtracting blank sample AUC from test sample AUC and then compared with an antioxidant standard curve of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid constructed by graphing the net AUC of vitamin E analog samples of known concentrations. Lower limit of quantitation was 2.5 µM vitamin E analog. Linear regression was used to estimate the vitamin E analog equivalents of plasma samples on the basis of the vitamin E analog standard curve. Plasma ORAC was compared among horses that were administered different doses of silibinin phospholipid.

NQO1 activity—The NQO1 of PBMC and RBC samples was measured by use of the method described by Gustafson et al. The PBMC and RBC lysates were thawed on ice, vortexed, and diluted 1:100. A 25 mM Tris plus 0.7% bovine serum albumin solution was added to a methacrylate cuvette, followed by DCPIP® solution (40 µM final solution) and nicotinamide adenine dinucleotide (200 µM final solution). Samples were evaluated in duplicate and mixed immediately prior to measurement of absorbance at 600 nm for 120 seconds via spectrophotometer, with and without the addition of dicumarol (20 µM final solution). The NQO1 activity was defined as the dicumarol-inhibited decrease in absorbance at 600 nm, or the difference in the change in optical density per minute between dicumarol-negative and dicumarol-positive samples. The NQO1 activity was converted to nmoL of DCPIP reduced/min through calculations that used the extinction coefficient of DCPIP (21 mM−1cm−1). The NQO1 was normalized for protein content and expressed as nmoL of DCPIP/min/mg.

GPOX activity—The GPOX activity of PBMC and RBC samples was measured by use of the method described by Gustafson et al. A GPOX assay has been validated in horses. The reaction mixture consisted of 2.59 mL of 50 mM potassium phosphate buffer (pH 7.0) with 1 mM EDTA, 10 µL of 5 mM sodium azide, 100 µL of 150 mM GSH, 100 µL of 2.2 mM hydrogen peroxide, 5 µL of glutathione reductase, and 100 µL of 8.4 mM NADPH in a methacrylate cuvette. Test samples were added after a linear rate was established at 340 nm absorbance for 240 seconds in a spectrophotometer. The GPOX activity was defined as the rate of NADPH oxidation in the presence of glutathione and glutathione reductase, or the difference in linear rates following sample addition. The GPOX activity was converted to pmol of NADPH reduced per minute through calculations that used the extinction coefficient of NADPH (6.2 mM−1 cm−1). The GPOX was normalized for protein content and expressed as pmol of NADPH/min/mg.

GSH and GSSG concentration—The GSH and GSSG concentration assays used in this study have been validated in horses and were performed according to instructions from a commercially available assay. The PBMC and RBC lysates were thawed on ice and precipitated with 5% sulfosalicylic acid. The PBMC samples were diluted 2-fold or did not require dilution for analysis. The RBC samples were diluted 10- to 100-fold for analysis. Samples were added to a 96-well microtiter plate with a working solution containing 3,5′-dithiobis(2-nitrobenzoic acid) and glutathione reductase. Following 5 minutes of incubation at 20°C, NADPH solution was added and the plate was read immediately. A microplate reader recorded absorption of samples every 60 seconds for 5 minutes at 412 nm. The resulting slope was plotted from the change in absorbance at 412 nm/min. Standard curves of GSH and GSSG were analyzed, and linear regression of the change in absorbance at 412 nm/min was used to estimate the concentration of GSH and GSSG in test samples. Lower limit of quantitation of the assays was 0.5 ng/mL. Estimated concentration of GSH and GSSG was normalized for protein content and expressed in nanomoles per milligram.

RT-PCR assay—An RT-PCR assay was used to quantitate cytokine gene expression in blood samples. Samples were thawed, and total RNA was extracted by use of a commercial kit and manufacturer’s instructions. Samples were converted to cDNA by RT by use of 1.0 µg of RNA sample and RT master mix, with incubation at 42°C for 15 minutes and 95°C for 5 minutes as described. Equine-specific intron-spanning primer and probe sets were used. Cytokines evaluated included TNFα, IL-1, and IL-2. Reaction mixtures composed of 5 µL of cDNA, 6.25 µL of nuclease-free water, 1.25 µL of 20X assay mix for the primer-probe set, and 12.5 µL of master mix were incubated at 95°C for 10 minutes and underwent 40 cycles in a sequence detection system. β-glucuronidase was used as the housekeeping gene. Changes in gene expression (ΔΔCt) were calculated by use of the following formula:

\[
ΔΔC_t = ([\text{Cytokine threshold cycle} – \text{β-GUS threshold cycle}]_{\text{Silibinin dose group}} – [\text{mean cytokine threshold cycle} – \text{mean β-GUS threshold cycle}]_{\text{Day 1 sample}})
\]

Results were reported as relative cytokine gene expression calculated by use of 2−ΔΔCt, calibrated to samples from day 1 prior to administration of the blank diet for each individual gene.
Silibinin—Plasma ORAC was measured for all time points. Plasma ORAC—Plasma ORAC was measured for all time points. Plasma ORAC—Plasma ORAC was measured for all time points.

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<th>GSH (nmol/mg)</th>
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<td>11.1 ± 6.6</td>
<td>2.74 ± 3.63</td>
<td>0.28 ± 0.20</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>0.67 ± 0.57</td>
<td>9.3 ± 2.2</td>
<td>4.48 ± 0.84</td>
<td>0.81 ± 0.44</td>
</tr>
<tr>
<td>6.5</td>
<td>1</td>
<td>27.0 ± 0.7</td>
<td>26.9 ± 24.2</td>
<td>0.32 ± 0.23</td>
<td>6.6 ± 6.1</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>24.8 ± 1.8</td>
<td>25.0 ± 22.2</td>
<td>0.51 ± 0.34</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td>6.5</td>
<td>7</td>
<td>26.4 ± 1.3</td>
<td>26.9 ± 24.2</td>
<td>0.32 ± 0.23</td>
<td>6.6 ± 6.1</td>
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<tr>
<td>13</td>
<td>7</td>
<td>25.8 ± 0.8</td>
<td>25.9 ± 24.8</td>
<td>0.32 ± 0.23</td>
<td>6.6 ± 6.1</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>24.6 ± 1.6</td>
<td>24.1 ± 22.9</td>
<td>0.51 ± 0.34</td>
<td>0.14 ± 0.05</td>
</tr>
</tbody>
</table>

*Results*

All 5 horses completed the study. Each horse was assigned to one of two doses of silibinin phospholipid, 0.05 were considered significant. Differences in ORAC among groups were not detected among groups. Relative quantities of gene expression among groups were not detected among groups. Differences in GSH and GSSG concentrations of PBMC and RBC samples were not detected among groups. Differences in GSH and GSSG concentrations of PBMC and RBC samples were not detected among groups.

Statistical analysis—Changes in plasma anti-

oxidant capacity, glutathione, antioxidant enzymes in RBCs and PBMCs and quantity of cytokine mRNA relative to silibinin phospholipid was well tolerated.

Differences in ORAC among groups were not detected among groups. Relative quantities of gene expression among groups were not detected among groups. Differences in GSH and GSSG concentrations of PBMC and RBC samples were not detected among groups.
and damage secondary to oxidation. Silibinin administered significantly increases RBC GPOX activity in vitro following oxidant-induced stress. In a study of rats that were administered silibinin via the intraperitoneal route, increases in GSH concentration were tissue specific and occurred primarily in the liver and intestines. The authors attributed the tissue-specific effects to the basic pharmacokinetics of silibinin, which undergoes predominantly biliary excretion and is maintained in high local concentrations by enterohepatic circulation. It is presumed that the antioxidant protective effects of silibinin are similarly concentrated in the tissues of the liver and intestines. Therefore, it is expected that the changes evident in the blood of horses consuming silibinin would be greater in the liver, which is the target organ in horses with liver disease. Hepatic antioxidant effects of silibinin are well documented. Less is known regarding the relative antioxidant effect on blood versus hepatic tissues. In experimental carbon tetrachloride-induced hepatitis, hepatic and RBC GSH were both measured and silibinin administration improved GSH concentration in both tissues, but to a greater degree in hepatic tissue.

Much has been learned from in vitro and in vivo experiments with respect to the peripheral anti-inflammatory effects of silibinin administration. Silibinin reverses increases in serum TNFα, IL-1β, and IL-6 expression in rats with sepsis induced by cecal ligation and perforation. A decrease in acute lung and brain injury accompanies this anti-inflammatory effect. In rats with experimental nonalcoholic fatty liver disease, silibinin decreases plasma TNFα expression concurrent with improvements in liver inflammation and fatty infiltration evident via histologic examination. In rats, silibinin significantly reduces serum TNFα and IL-1 expression associated with partial hepatectomy and the resultant inflammatory response. Release of TNFα and cytotoxicity secondary to toxic damage are decreased by silibinin administration in perfused livers and isolated Kupffer cells. In canine hepatocytes, silibinin ameliorates the proinflammatory influence of IL-1β, including production of chemotactic cytokines, and reduces hepatocyte damage. Silibinin also dimin-

Table 3—Blood cytokine gene expression (mean ± SD [median {range}]) of horses orally administered various doses of silibinin phospholipid.

<table>
<thead>
<tr>
<th>Silibinin dose (mg/kg)</th>
<th>Sample day</th>
<th>TNFα</th>
<th>IL-1</th>
<th>IL-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Referent</td>
<td>1.17 (1.15 [0.97–1.84])</td>
<td>1.12 ± 0.23</td>
<td>1.12 ± 0.21</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0.81 ± 0.36 (0.87 [0.33–1.18])</td>
<td>1.06 ± 0.19</td>
<td>1.12 (0.79–1.21)</td>
</tr>
<tr>
<td>6.5</td>
<td>1</td>
<td>0.89 ± 0.56 (0.64 [0.56–1.72])</td>
<td>1.30 ± 0.51</td>
<td>0.68 ± 0.30</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>1.16 ± 0.49 (1.28 [0.50–1.56])</td>
<td>1.49 ± 1.27</td>
<td>1.04 ± 0.28</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>0.85 ± 0.24 (0.98 [0.25–1.18])</td>
<td>1.39 ± 0.20</td>
<td>1.41 ± 0.62</td>
</tr>
<tr>
<td>26</td>
<td>7</td>
<td>1.03 ± 0.64 (1.03 [0.26–1.82])</td>
<td>1.21 ± 0.45</td>
<td>1.31 ± 0.50</td>
</tr>
</tbody>
</table>

Gene expression values indicate relative quantity of gene calibrated to day 1 of the unsupplemented diet (0 mg/kg) week.

Discussion

The primary antioxidant effect identified in the healthy horses fed diets supplemented with silibinin in the present study was alteration in plasma ORAC. Temporal effects and those of the carrier diet may also have been sources of alterations in plasma ORAC in this study. Modest increases in plasma ORAC have also been associated with decreases in plasma lipid hydroperoxides in horses following exercise-induced stress. In a study of rats that were administered silibinin via the intraperitoneal route, increases in GSH concentration were tissue specific and occurred primarily in the liver and intestines. The authors attributed the tissue-specific effects to the basic pharmacokinetics of silibinin, which undergoes predominantly biliary excretion and is maintained in high local concentrations by enterohepatic circulation. It is presumed that the antioxidant protective effects of silibinin are similarly concentrated in the tissues of the liver and intestines. Therefore, it is expected that the changes evident in the blood of horses consuming silibinin would be greater in the liver, which is the target organ in horses with liver disease. Hepatic antioxidant effects of silibinin are well documented. Less is known regarding the relative antioxidant effect on blood versus hepatic tissues. In experimental carbon tetrachloride-induced hepatitis, hepatic and RBC GSH were both measured and silibinin administration improved GSH concentration in both tissues, but to a greater degree in hepatic tissue.

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unsupplemented diet week, were determined (Table 3).

Table 3—Blood cytokine gene expression (mean ± SD [median {range}]) of horses orally administered various doses of silibinin phospholipid.

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<td>1.21 ± 0.45</td>
<td>1.31 ± 0.50</td>
</tr>
</tbody>
</table>

Gene expression values indicate relative quantity of gene calibrated to day 1 of the unsupplemented diet (0 mg/kg) week.
ishes the proinflammatory influence of IL-1 in human hepatic stellate cells.33

Gene expression of the inflammatory cytokines TNFα, IL-1, and IL-2 did not change in the present study of healthy horses fed diets supplemented with silybin phospholipid. In the absence of an active inflammatory stimulus, this was not surprising. However, the effects of silybin on inflammatory cytokines have been detected in the blood of patients with naturally occurring diseases, especially those diseases in which oxidative injury is prominent. In chronic hepatitis C virus infection, silybin administration inhibits TNFα production by PBMCs.34 Production of TNFα by blood lymphocytes in humans with end-stage diabetic nephropathy is significantly reduced following silybin administration.35 Silybin also reduces inflammatory cytokines and disease severity in humans with non alcoholic fatty liver disease.43–47

Results of the present study provide baseline data on the effects of silybin in healthy horses. Minor changes were observed in antioxidant capacity, which was consistent with previous observations in healthy cats.33 Because of the pharmacokinetics of silybin,3 despite low bioavailability, its antioxidant effects will likely be most prominent in the liver and intestinal tract of horses.

References
and activity of glutathione-metabolizing antioxidant enzymes in erythrocytes of young trotters in basic training. 


35. Das SK, Vasudevan DM. Protective effects of silymarin, a milk thistle (Silybum marianum) derivative on ethanol-induced oxidative stress in liver. Indian J Biochem Biophys 2006;43:306–311.


Appendix IX Abstracts of recent journal articles demonstrating breadth current of research

Note abstracts link to pubmed, by clicking on title.
IX-A Veterinary botanical medicine and Aquaculture

Valladão GM, Gallani SU, Ikefuti CV, da Cruz C, Levy-Pereira N, Rodrigues MV, Pilarski F. Essential oils to control ichthyophthiriasis in pacu, Piaractus mesopotamicus (Holmberg): special emphasis on treatment with Melaleuca alternifolia. J Fish Dis. 2016 Jan 18

In vitro effect of the Melaleuca alternifolia, Lavandula angustifolia and Mentha piperita essential oils (EOs) against Ichthyophthirius multifiliis and in vivo effect of M. alternifolia for treating ichthyophthiriasis in one of the most important South American fish, Piaractus mesopotamicus (Holmberg), were evaluated. The in vitro test consisted of three EOs, the results demonstrated that all tested EOs showed a cytotoxic effect against I. multifiliis compared to control groups (P < 0.05). The in vivo treatment for white spot disease was performed in a bath for 2 h day⁻¹ for 5 days using the M. alternifolia EO (50 μL L⁻¹). In this study, 53.33% of the fish severely infected by I. multifiliis survived after the treatment with M. alternifolia (50 μL L⁻¹) and the parasitological analysis has shown an efficacy of nearly 100% in the skin and gills, while all the fish in the control group died. Furthermore, the potential positive effect of M. alternifolia EO against two emergent opportunistic bacteria in South America Edwardsiella tarda and Citrobacter freundii was discussed.


The aim of this study was to evaluate the possible effects of diets supplemented with probiotics and different cinnamon levels (powder and essential oil) on immunological parameters of Nile tilapia after being subjected to acute stress by hypoxia. Three hundred and thirty juvenile male tilapia fish (66.08 ± 2.79 g) were distributed in 30 tanks of 100 L capacity (11/cage) with a water recirculation system. The animals were fed for 71 days with diets containing extruded cinnamon powder at different levels (0.5, 1, 1.5, 2%), cinnamon essential oil (0.05, 0.1, 0.15, 0.2%) and probiotics (0.4%), all in triplicate. At the end of the experiment, the fish (200.36 ± 19.88 g) of the different groups were subjected to stress by hypoxia. Hypoxia was achieved by capturing the animals with a net, keeping them out of the water for three minutes, and then sampling the blood 30 min after the procedure to determine the levels of cortisol, glucose, haematocrit, lysozyme, bactericidal index, total protein, and its fractions. The animals kept blood homeostasis after hypoxic stress. Diet supplementation with 0.5% cinnamon powder improved the fish immune response, since it resulted in an increase of 0.5% in γ-globulin level. Administration of 0.15% cinnamon essential oil resulted in an increase of α1 and α2-globulins, which may be reflected in increased lipid content of the carcass and the hepatosomatic index. More studies are necessary to better understand the effects of these additives for fish immunity.


This study was conducted to evaluate the effects of garlic supplementation on some skin mucous immune parameters, mucus antimicrobial activity and growth performance of the Caspian roach (Rutilus rutilus caspicus) fry. Fish (1 ± 0.07 g) were divided into four groups fed diets containing 0 (control), 5, 10 and 15 g (kg⁻¹) garlic for 8 weeks. The results showed that there was a significant increase in weight gain and specific growth rate in those fish fed garlic diets compared with the control (P < 0.05). Condition factor was not significantly affected by garlic dosage. At the end of trial, the epidermal mucus protein level, alkaline phosphatase and antimicrobial activity against 2 g-negative bacteria (Escherichia coli and Serratia marcescens) and gram-positive bacteria (Streptococcus faecium and Micrococcus luteus) were
measured. Skin mucus alkaline phosphatase, protein levels and antimicrobial activity were increased following garlic administration, and the bacterial growth inhibition zones were significantly elevated in garlic-fed fish \(P < 0.05\). In salinity stress experiment, no differences were observed for survival rate among the experimental diets. No mortality was recorded during the feeding trial. These results indicated that dietary garlic beneficially affects the skin mucus immune parameters and growth performance of the Caspian roach fry.

Kareem ZH, Abdelhadi YM, Christianus A, Karim M, Romano N. Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (Oreochromis niloticus) and their resistance to Streptococcus agalactiae infection. Fish Physiol Biochem. 2016 Apr;42(2):757-69.

A 90-day feeding trial was conducted on the growth performance, feeding efficacy, body indices, various hematological and plasma biochemical parameters, and histopathological examination of the gonads from male and female Nile tilapia fingerlings when fed different crude plant extracts from Cinnamomum camphora, Euphorbia hirta, Azadirachta indica, or Carica papaya at 2 g kg\(^{-1}\) compared to a control diet. This was followed by a 14-day challenge to Streptococcus agalactiae. All treatments were triplicated, and each treatment consisted of 30 fish. Results showed that C. papaya extracts were the most effective at delaying gonadal maturation to both male and female tilapia, as well as significantly increasing \(P < 0.05\) growth performance compared to the control treatment. Similarly, dietary C. camphora and E. hirta extracts also significantly improved growth, while no significant growth effect was detected between the A. indica and control treatments \(P > 0.05\). Further, crude body lipid was lower in the C. camphora, E. hirta and C. papaya treatments, but was only significantly lower for the E. hirta treatment compared to the control. Meanwhile, none of the hematological or biochemical parameters were significantly affected, although plasma ALT was significantly lower for tilapia fed A. indica compared to the control. After the 14-day bacterial challenge, tilapia fed C. camphora supplementation had significantly higher survival, compared to the control, but was not significantly higher than the other supplemented diets. Results indicate that dietary C. papaya extract can significantly promote growth and delay gonadal maturation to both male and female tilapia, while C. camphora was the most effective prophylactic to S. agalactiae and may be a cost-effective and eco-friendly alternative to antibiotics.


Immunostimulation using medicinal plant extracts is a promising approach for prevention and control of diseases with reference to sustainable fish farming. Oreochromis mossambicus, dubbed as aquatic chicken is a cultured fish worldwide and a laboratory model organism. Aeromonas hydrophila is one of the major bacterial pathogens in fish farming that causes huge loss to aquaculture industries. In this study, we investigated the efficacy of methanol extract of Nyctanthes arbor-tristis seeds on disease resistance of O. mossambicus against live virulent A. hydrophila. We also investigated its effect on the non-specific immune parameters such as serum lysozyme, myeloperoxidase, antiprotease and specific immune parameters in terms of specific serum antibody titres assayed by bacterial agglutination test. Our studies indicate that intra-peritoneal administration of 20mg/kg methanol extract increases the Relative Percent Survival (RPS) of O. mossambicus challenged with LD80 of A. hydrophila. Further, both non-specific and specific immune parameters were enhanced by the methanol extract. Further experiments at molecular levels in the laboratory and also efficacy testing at field level are essential before applying this plant product in aquaculture industry.


Monogenean infections of commercially farmed fishes are responsible for significant economic losses. Garlic (Allium sativum) is a well-known spice which also possesses anti-microbial and anti-
parasitical properties. The current work aimed to test the efficacy of garlic-based treatments against infection with monogenean sp. in the guppy (Poecilia reticulata). Clipped sections of tail fins of guppies heavily infected with Gyrodactylus turnbulli were exposed to aqueous garlic extract (7.5 to 30 mL L\(^{-1}\)) and visually observed under a dissecting microscope. Results revealed that exposure to garlic caused detachment of parasite and cessation of movement indicating death. A positive correlation was seen between garlic concentration and time to detachment and death of parasites, which, at the highest concentration of 30 mL L\(^{-1}\), occurred at 4.1 and 8.6 min, respectively. Bathing in aqueous garlic extract (7.5 and 12.5 mL L\(^{-1}\)) was tested in guppies infected with G. turnbulli. Prior acute toxicity tests revealed the maximum tolerance levels of guppies to garlic extract to be 12.5 mL L\(^{-1}\) for 1h. Bathing of infected fish in garlic extract (7.5 and 12.5 mL L\(^{-1}\)) significantly (p<0.05) reduced infection prevalence and intensity as compared to the control. Oral treatments using dry garlic powder-supplemented diet were tested on guppies infected with G. turnbulli and Dactylogyrus sp. Fish were fed with food containing 10% and 20% dry garlic powder for 14 days. Groups fed with garlic supplemented diets showed significantly reduced (p<0.05) mean prevalence and mean intensity of parasites as compared to the control. Dietary application of garlic did not appear to affect palatability. Fresh crushed garlic was added at a level of 1 gL\(^{-1}\) and applied as an indefinite bath for 14 days. This treatment was seen to significantly reduce (p<0.05) parasite prevalence and mean intensity as compared to the control. Histopathology revealed elevated muscular dystrophy in the 20% garlic-fed group, as compared to control. These findings demonstrate the potential of garlic as a natural alternative to currently used chemical treatments for monogenean sp. infection in the guppy.


Traditional compounds used to treat fish diseases in aquaculture and the ornamental fish industry (such as formalin and malachite green) can be more toxic to the hosts than their parasites. With the reviviscence in the use of herbal products, various botanicals have been heralded as cures for particular pathogens, but the efficacy of these compounds for parasitic worms is questionable. Here, we tested a range of garlic (Allium sativum) products against a major aquarium pathogen, Gyrodactylus turnbulli, infecting the guppy (Poecilia reticulata). All garlic products significantly reduced parasite mean survival time in vitro, from 13 h to <1 h. In fully randomised trials, the number of parasites was also significantly reduced on infected fish exposed to garlic from different sources. Two garlic treatments (minced and granule forms) reduced worm burdens by 66% and 75% after three doses, whereas Chinese freeze-dried garlic and allyl disulphide were 95% effective after a single application. In fact, Chinese freeze dried garlic was equally effective as Levamisole, a licensed livestock dewormer that is highly effective against G. turnbulli but not routinely prescribed for use in fish; hence, garlic may be a potential alternative treatment for gyrodactylosis.


Garlic, Allium sativum L., extract administered as a therapeutic bath was shown to have antiparasitic properties towards Neobenedenia sp. (MacCallum) (Platyhelminthes: Monogenea) infecting farmed barramundi, Lates calcarifer (Bloch). The effect of garlic extract (active component allicin) immersion
on Neobenedenia sp. egg development, hatching success, oncomiracidia (larvae) longevity, infection success and juvenile Neobenedenia survival was examined and compared with freshwater and formalin immersion. Garlic extract was found to significantly impede hatching success (5% ± 5%) and oncomiracidia longevity (<2 h) at allicin concentrations of 15.2 μL L(-1), while eggs in the seawater control had >95% hatching success and mean oncomiracidia longevity of 37 ± 3 h. At much lower allicin concentrations (0.76 and 1.52 μL L(-1)), garlic extract also significantly reduced Neobenedenia infection success of L. calcarifer to 25% ± 4% and 11% ± 4%, respectively, compared with 55% ± 7% in the seawater control. Juvenile Neobenedenia attached to host fish proved to be highly resistant to allicin with 96% surviving 1-h immersion in 10 mL L(-1) (15.2 μL L(-1) allicin) of garlic extract. Allicin-containing garlic extracts show potential for development as a therapy to manage monogenean infections in intensive aquaculture with the greatest impact at the egg and larval stages.


The present work was designed to study the prevalence of trichodinosis and gyrodactylosis in Oreochromis niloticus fries, and to test the therapeutic efficacy and preventive efficacy of garlic oil and crushed garlic cloves. Trichodinosis and gyrodactylosis are ectoparasitic diseases that affect most warm freshwater fish, especially fries and fingerlings. In a private O. niloticus fish hatchery, the prevalence of trichodinosis in 5-, 15- and 30-day-old-fries was 37%, 23% and 40.5%, respectively. The highest infection intensity was detected in 30-day-old-fries. The gyrodactylosis was reported only in combination with trichodinosis. In addition, we found that its prevalence in 5-, 15- and 30-day-old-fries was 17%, 19.5% and 29%, respectively. Mortality rate of fry in the first month of life was 53% as a result of injury to these two types of parasites. The garlic oil and crushed garlic cloves were tested in both in vitro and earthen ponds of the hatchery. Using 2-, 2.5- and 3-ppt (parts per thousand) garlic oil for 4h in vitro water bath treatment resulted in 100% recovery, while 1 and 1.5 ppt garlic oil, respectively, needed 24 and 16 h to treat the infected fries. The treatment by 3 ppt garlic oil as a water bath for 1h treated the two diseases in 55% in 7 days from application in the hatchery earthen pond. In the mean time, 300 mg L(-1) crushed garlic cloves as an indefinite bath in the hatchery earthen pond eliminated 68% of the diseases. The same protocol for preventing the two diseases resulted in obtaining 65% and 75% of parasite free fries, for garlic oil and crushed garlic cloves, respectively, compared to 53% of the control fries.


Monogenean infections of commercially farmed fishes are responsible for significant economic losses and existing chemical therapeutants, often stressful to the fish, pose associated risks. As part of a recent trend to move towards the use of alternative, plant-based remedies for commonly occurring aquaculture-related diseases, the efficiency of ginger (Zingiber officinale) was investigated against the monogenean parasite Gyrodactylus turnbulli in the guppy. In vitro trials revealed the clear anti-parasitic effects of ginger. Ethanolic and aqueous extracts, prepared from freeze dried ginger, were tested. An increase in extract concentration was associated with reduced time to parasite immobilisation, with ethanolic extract being more efficient; at 75 and 200ppt aqueous ginger extract parasites died at 65.6±2.8 and 1.8±0.2min, respectively, whereas at 5 and 40ppt ethanolic extract parasites died at 26.1±0.7 and 4.9±0.3min, respectively. Bathing G. turnbulli-infected fish in ethanolic ginger extract (i.e. 5 and 7.5ppt for 90 and 30min, respectively) significantly reduced infection prevalence and intensity when compared to the water and ethanol controls. The higher concentration (i.e. 7.5ppt) proved as equally effective as Praziquantel, the conventionally used chemical treatment for gyrodactylosis, with the fish appearing to be completely cleared of the infection in both cases. Oral treatments of G. turnbulli-infected guppies with diets supplemented with 10 and 20% ginger powder proved to be ineffective in decreasing parasite load. These findings demonstrate that immersion in ginger extract offers an effective, alternative treatment against monogenean infection in fish.
This study investigated effects of dietary Aloe vera on growth performance, some haemato-biochemical parameters and disease resistance against Streptococcus iniae in tilapia (GIFT). Five groups were designed including a basal diet (control) and 100% A. vera powder incorporated in fish feed at 0.5% 1%, 2%, and 4%/kg feed, which were administered for 8 weeks. Fish fed 0.5%, 1%, and 2% A. vera supplemented diet significantly improved (p < 0.05) weight gain, absolute growth rate and specific growth rate. Feed intake significantly increased in fish fed with A. vera diet at 1% and 2%/kg feed. Feed efficiency ratio, feed conversion ratio, and hepatosomatic index were significantly enhanced in 4% A. vera supplemented fish over unsupplemented ones (p < 0.05). Several haemato-biochemical indices were examined before and after fish were challenged with S. iniae pathogen containing 7.7 × 10^6 CFU cells mL(-1). A. vera supplemented fish showed a significant increase (p < 0.05) in red blood cells, hematocrits (Hb), hemoglobin (Hb), white blood cells (WBC), neutrophils, monocytes, eosinophils, serum total protein, glucose and cortisol after challenge when compared to unsupplemented ones. Meanwhile, 4% A. vera supplemented fish showed a decrease (p < 0.05) in RBC, Hb, Ht, WBC, and mean corpuscular hemoglobin (MCH) after challenge compared to unsupplemented ones and other supplemented ones. In addition, lower mean corpuscular volume values (MCV) (p < 0.05) were observed in fish fed with A. vera diet at 2% and 4% A. vera/kg feed than those fed unsupplemented diet. Unchallenged fish fed 0.5%, 1%, and 2% A. vera showed significantly higher values (p < 0.05) of mean corpuscular hemoglobin concentration (MCHC) than those fed unsupplemented diet and 4% A. vera supplemented diet. There was a significant increase (p < 0.05) in the neutrophil to lymphocyte ratio (N/L) within experimental groups after challenge; N/L ratio in A. vera unsupplemented fish and those supplemented with A. vera diet at 1%/kg feed increased significantly (p < 0.05) throughout challenge period; while those fed 4% A. vera supplemented diet maintained higher values at all experimental stages among groups. There was a significant correlation (p < 0.05, r = 0.53) between N/L ratio and glucose concentration, 96 h after challenge. Aloe had no significant effect (p > 0.05) on the survival of the fish when compared to the control; no mortality was recorded in challenge trial. Overall, our results indicated that dietary aloe supplementation could improve growth, feed utilization, and haemato-biochemical parameters of cultured tilapia.
superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA) were measured during test period. After four weeks of feeding, fish were infected with Aeromonas hydrophila and mortalities were recorded. Results of this study showed that feeding Nile tilapia with CHM-supplementation diet stimulated lysozyme activity, SOD activity and POD activity in serum, induced TNF-α and IL-1β mRNA expression in head kidney and spleen, but decreased serum MDA content. All CHM-supplemental groups showed reduced mortalities following A. hydrophila infection compared with the group fed the control diet. These results suggested that this CHM can be applied as a tilapia feed supplement to elevate fish immunity and disease resistance against A. hydrophila.


Ichthyophthirius multifiliis (Ich), an important fish parasite, can cause significant losses in aquaculture. To find efficacious drugs to control Ich, the root bark of white mulberry Morus alba was evaluated for its antiprotozoal activity. Bark was powdered and extracted with 1 of 5 organic solvents: petroleum ether, chloroform, ethyl acetate, acetone, or methanol. The extracts were concentrated, dissolved in 0.1% (v/v) DMSO, and used for anti-Ich trials. Acetone and ethyl acetate extracts significantly reduced the survival of Ich tomonts and theronts. In vitro, acetone extract at 25 mg l-1 killed all non-encysted tomonts, at 50 mg l-1 eradicated all encysted tomonts, and at 8 mg l-1 caused mortality of all theronts. Ethyl acetate extract at 50 mg l-1 eliminated all non-encysted tomonts, at 100 mg l-1 killed all encysted tomonts and terminated tomont reproduction, and at 8 mg l-1 killed all theronts. Low concentrations (2 and 4 mg l-1) of acetone and ethyl acetate extracts could not kill all theronts after 4 h exposure, but a significant decrease in theront infectivity was observed following 30 min of pretreatment with the extracts. The 96 h LC(50) values of acetone and ethyl acetate extracts to grass carp were 79.46 and 361.05 mg l-1, i.e. much higher than effective doses for killing Ich theronts (8 mg l-1 for both extracts) and non-encysted tomonts (12.5 and 25 mg l-1, respectively). Thus M. alba extract may be a potential new, safe, and efficacious drug to control Ich.

Other papers include:


To determine effects of cranberry extract on development of urinary tract infection (UTI) in dogs and on adherence of Escherichia coli to Madin-Darby canine kidney (MDCK) cells. ANIMALS 12 client-owned dogs (in vivo experiment) and 6 client-owned dogs (in vitro experiment). 12 dogs with a history of recurrent UTI received an antimicrobial (n = 6) or cranberry extract (6) orally for 6 months. Dogs were monitored for a UTI. For the in vitro experiment, cranberry extract was orally administered to 6 dogs for 60 days. Voided urine samples were collected from each dog before and 30 and 60 days after onset of extract administration. Urine was evaluated by use of a bacteriostasis assay. An antiadhesion assay and microscopic examination were used to determine inhibition of bacterial adherence to MDCK cells. None of the 12 dogs developed a UTI. The bacteriostasis assay revealed no zone of inhibition for any urine samples. Bacterial adhesion was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results for urine samples obtained before extract administration. Microscopic examination revealed that bacterial adherence to MDCK cells was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results after culture with urine samples obtained before extract administration. Oral administration of cranberry extract prevented development of a UTI and prevented E coli adherence to MDCK cells, which may indicate it has benefit for preventing UTIs in dogs.


The goal of the present study was to compare the antifungal efficacy of an essential oil (EO) shampoo proven to be effective against Microsporum canis with miconazole/chlorhexidine for topical hair coat disinfection in cats treated concurrently with oral itraconazole. Cats received treatment with oral itraconazole (Itrafungol) at a dose of 5 mg/kg/day pulse administration for 1 week, every 2 weeks for at least 6 weeks and were washed twice a week with a neutral shampoo with added EOs of Thymus serpyllum (2%), Origanum vulgare and Rosmarinus officinalis (5% each) for the period of systemic treatment. This protocol was compared with a conventional treatment (oral itraconazole + 2% miconazole/2% chlorhexidine shampoo). The treatment was well tolerated and adverse effects were not recorded. All cats were clinically negative at week 11. With respect to animals with extensive lesions, the speed of resolution was higher in cats with focal lesions. The animals showing diffuse lesions required more than a course of treatment to achieve a mycological cure. There was no significant difference between the number of weeks to obtain mycological cure for cats treated with EOs and animals treated conventionally. The treatment appeared to be effective and well appreciated by the owners. The use of shampoo with the added EOs of T serpyllum, O vulgare and R officinalis would seem an interesting, natural alternative to conventional topical treatment.


Recent studies have shown that immunological aberrations and epidermal barrier defects could be important in the pathogenesis of canine atopic dermatitis (CAD) and that oral polyunsaturated fatty acids (PUFAs) might influence the epidermal barrier. The aim of this study was to evaluate the effects of a spot-on formulation containing PUFAs and essential oils on pruritus and lesions caused by CAD. Forty-eight privately owned dogs of
different breeds, ages and genders diagnosed with atopic dermatitis were included in a randomized, double-blinded, placebo-controlled, multicentre clinical trial. Dogs were treated with a spot-on formulation containing PUFAs and essential oils or placebo on the dorsal neck once weekly for 8 weeks. Before and after the study, CAD extent and severity index-03 (CADESI-03) and pruritus scores were determined by veterinarians and owners, respectively. There was significantly more improvement in CADESI-03 and pruritus scores in the treatment group than in the placebo group (P=0.011 and P=0.036, respectively). Additionally, more dogs improved by at least 50% in CADESI-03 and pruritus scores in the treatment group than in the placebo group (P=0.008 and P=0.070, respectively). No adverse reactions were observed. The topical preparation containing PUFAs and essential oils was a safe treatment and beneficial in ameliorating the clinical signs of CAD.


An oral herb-based natural health product (NHP) was evaluated in the canine natural osteoarthritis model. At baseline, the peak vertical force (PVF, primary endpoint) and case-specific outcome measure of disability (CSOM) were recorded in privately-owned dogs. Dogs (16/group) were randomized to receive NHP formulations or a negative control. The PVF was measured at week (W) 4 and W8. Daily locomotor activity was recorded using accelerometer. The CSOMs were assessed bi-weekly by the owner. The NHP-treated dogs (n = 13) had higher PVF at W4 (p = 0.020) and W8 (p <0.001) when compared to baseline. The changes at W8 were higher than control dogs (n = 14, p <0.027) and consistent with Cohen’s d effect size of 0.7 (95% confidence interval: 0.0-1.5). The NHP-treated dogs had higher locomotor activity at W8 (p = 0.025) when compared to baseline. No significant change was observed for the CSOM. The NHP improved the clinical signs of osteoarthritis in this model.


The hypothesis was that Non-steroidal anti-inflammatory drugs (NSAIDs) may cause gastrointestinal damage in dogs. To determine the extent to which lansoprazole, liquorice extract, and a herbal solution exhibit protective effects on colonic mucosa when administered to dogs concurrently with the NSAIDs carprofen or robenacoxib, thirty-five healthy beagle dogs (15 male and 20 female) aged 13-14 weeks and weighing 4.3-5.5 kg at the beginning of the experiment were included. Endoscopy and biopsy of the caudal gastrointestinal tract were performed pretreatment and on the last day of a 21-day treatment period with (1) oral carprofen; (2) carprofen and the proton-pump inhibitor lansoprazole; (3) carprofen, liquorice extract, and a herbal solution that contained extracts of thyme, icelandic lichen, hyssop, and saponariae root; (4) robenacoxib; (5) robenacoxib and lansoprazole; (6) robenacoxib, liquorice extract, and herbal solution; or (7) an empty gelatin capsule. Statistical analyses were performed with the Kruskal-Wallis, Cochran’s Q, and chi-squared test with p < 0.05 considered significant. Both carprofen and robenacoxib tested damaged the colonic mucosa with most severe microscopic lesions following administration of robenacoxib with lansoprazole. The risk of histopathological lesions in the colon increased most rapidly in robenacoxib with lansoprazole (absolute risk increase -0.85) similar to robenacoxib only (-0.75), whereas the best result was recorded following the plant remedies together with carprofen (-0.15) and the plant remedies together with robenacoxib (-0.2). TConcurrent administration of liquorice extract and an herbal solution with robenacoxib was associated with decreased severity of the NSAID-induced mucosal lesions.


This study was designed to evaluate the effects of oral administration of Echinacea hydroethanolic extract on the dog's immune system. The study was performed on 14 dogs that were referred to the veterinary clinic. These dogs were randomly allocated to two equal treatment groups. The first group received 1 ml of 5% Echinacea hydroethanolic extract two times a day for 2 months, and the second group received a placebo (water). To do haematology and immunology tests, the dogs were bled on days 0, 30 and 60. Blood tests, including packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count
(WBC), counting neutrophils (Nut), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos), basophils (Baso) and B cell, were performed. Furthermore, safety factor IgM and per cent of phagocytosis and phagocyte were measured from the blood sample. The results showed that in the group which received Echinacea PCV, Hb, RBC count, WBC count, Lym, Nut, the per cent of phagocytosis and IgM significantly increased ($P < 0.05$). Moreover, positive effects of Echinacea plant on the immune system were observed. There was a significant change in HTC, RBC, Hb over time in the group that received Echinacea and the per cent of phagocytosis and IgM ($P < 0.05$). The study establishes that these extracts might have appreciable immunostimulatory activity. However, further studies are required to confirm these findings.


Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in animals, especially in dogs, to manage pain due to inflammatory disease. This study investigated whether plant drugs can prevent mucosal injury induced by robenacoxib. We used fifteen healthy beagle dogs (7 male and 8 female) aged 4 months, weighing 4.2-5.1 kg at the beginning of the study. Endoscopy and biopsy of the colon were performed before and on the 21 day treatment with robenacoxib (1), robenacoxib, herbal solution with liquorice extract (2), placebo - an empty capsule (3). There were 5 animals in each group. The greatest microscopic damage in the colon was observed in animals which received robenacoxib. Plant drug administration reduced the severity of lesions in the colon when administered with robenacoxib ($ARI = -0.15$). Conclusion: concurrent administration of liquorice extract and plant solution with robenacoxib was associated with significant decreased severity of the robenacoxib-induced colonic mucosal lesions.


The present study was conducted to investigate the effects of some commonly used herbs namely Nigella sativa, Lepidium sativum and Trigonella foenum-graecum on the pharmacokinetics of sildenafil in beagle dogs. The study design involved four treatments in a non-balanced crossover design. Sildenafil was given one tablet 100 mg orally to each dog and blood samples were obtained. After a suitable washout period, animals were commenced on a specific herb treatment for 1 week. Blood samples were withdrawn at different time intervals and sildenafil was analyzed by HPLC method. Oral administration of Nigella sativa resulted in reduction of $AUC_0-\infty$, $C_{\text{max}}$ and $t_{1/2}$ as compared to the control. Treatment of Lepidium sativum resulted in a significant reduction in the $C_{\text{max}}$ and AUC. There were no significant differences between the rests of the pharmacokinetic parameters relative to those of the control. For Trigonella foenum-graecum, the effects were similar to those obtained in case of Lepidium sativum. It was concluded that concurrent use of investigated herbs alters the pharmacokinetics of sildenafil. Co-administration of investigated herbs should be cautious since their concomitant use might result in decrease in sildenafil bioavailability.


To investigate the efficacy of a standardized infusion of Herniaria hirsuta against cholelithiasis, and evaluation of its genotoxicity. An in vivo experiment to evaluate the cholesterol lowering effect of a infusion of H. hirsuta in the gall bladder of dogs was carried out. Dogs were divided into 3 groups i.e. control dogs (CG), dogs treated with ursodeoxycholic acid (UDCA) ($2\times7.35\text{mg/kg body weight/day}$) and dogs treated with the standardized infusion (HG) ($2\times48.5\text{mg/kg body weight/day}$). Dogs were fed a fatty diet during 120 days after which a diet without additional fat was introduced till day 180. Treatment started 30 days after introduction of the fatty diet and lasted till the end of the experiment. A bile and blood sample of each dog was collected every 30 days, after which the concentration of cholesterol was determined. An Ames test was performed according to the OECD-guidelines. Conclusion: Prolonged use of this standardized H. hirsuta extract resulted in a cholesterol-lowering effect in the dogs. Since this pharmacological effect prevents the formation of gallstones and can contribute to solving existing gallstones, a standardized infusion of H. hirsuta may have a positive effect in the treatment of gallstones in human patients.
Renal fibrosis is common in progressive kidney disease. Transforming growth factors β (TGF-β) are important mediators of all types of fibrosis, including renal fibrosis. Chinese rhubarb has been shown to have antifibrotic properties in part because of inhibition of TGF-β and has slowed the progression of kidney disease in rodent models. The hypothesis is that administration of a Chinese rhubarb supplement will slow the progression of chronic kidney disease (CKD) in cats and the concurrent administration of Chinese rhubarb and benazepril will be more effective than either alone. Cats with naturally Twenty-nine client-owned occurring IRIS Stage 2 or early Stage 3 CKD and without comorbidity such as cancer, urinary tract obstruction, urinary tract infection, poorly controlled hyperthyroidism, or systemic hypertension were enrolled in the study. A randomized, positive-controlled, prospective study was performed. Cats received Chinese rhubarb, benazepril, or both in addition to standard treatment for CKD. Repeated measures ANOVA was used to assess changes in serum creatinine concentration, body weight, hematocrit, urine protein: urine creatinine ratio (UPC), and systemic arterial blood pressure over time between and within treatment groups over an average of 22 months. No significant differences were detected in serum creatinine concentration, body weight, hematocrit, UPC, and systemic arterial pressure over time between or within treatment groups. This study failed to detect a significant difference in the progression of CKD in cats treated with Chinese rhubarb, benazepril, or both. Further study in specific subsets of cats with CKD is warranted.


Yunnan Baiyao is a Chinese herbal medicine that has been utilized for its anti-inflammatory, haemostatic, wound healing and pain relieving properties in people. It has been utilized in the veterinary profession to control bleeding in dogs with hemangiosarcoma (HSA) and has been anecdotally reported to prolong survival times in dogs with this neoplasm. This study evaluated the in vitro activity of Yunnan Baiyao against three canine HSA cell lines after treatment with increasing concentrations of Yunnan Baiyao (50, 100, 200, 400, 600 and 800 µg mL^{-1}) at 24, 48 and 72 h. Mean half maximum inhibitory concentration (IC_{50}) at 72 h for DEN, Fitz, SB was 369.9, 275.9 and 325.3 µg mL^{-1}, respectively. Caspase-3/7 activity increased in correlation with the IC_{50} in each cell line which was confirmed by the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL, APO-BRDU Kit; BD Biosciences, San Jose, CA, USA) assay. VEGF in cell supernatant was also quantified. Overall, the study found that Yunnan Baiyao causes dose and time dependent HSA cell death through initiation of caspase-mediated apoptosis, which supports future studies involving Yunnan Baiyao.


The objective of this study was to evaluate the effectiveness of a topically applied gel containing essential oils (menthol and thymol) and polyphenolic antioxidants (phloretin and ferulic acid) for reducing halitosis in dogs. A blinded crossover clinical trial was conducted. 20 Dogs received a dental cleaning and examination (periodontal examination including periodontal probing and assessments of plaque, calculus, and gingivitis). Owners then applied a gel (active or placebo) to oral soft tissues twice daily for a 4-week period. Teeth of the dogs were cleaned again, and owners applied the other gel for a 4-week period. Clinicians scored halitosis immediately after the initial cleaning and at 4 and 8 weeks, and owners scored halitosis weekly. Halitosis assessment by clinicians revealed that both groups had improvement in halitosis scores. Two dogs were removed because of owner noncompliance. In the active-to-placebo group (n = 9), halitosis was significantly reduced during application of the active gel but increased during application of the placebo. Seven of 9 owners reported increased halitosis when treatment was changed from the active gel to the placebo. In the placebo-to-active group (n = 9), halitosis decreased during application of the placebo and continued to decrease during application of the active gel. Seven of 9 owners reported a decrease in halitosis with the active gel. An oral topically applied gel with essential oils and polyphenolic antioxidants applied daily after an initial professional dental cleaning decreased oral malodor in dogs.
Artemisinin, a constituent of Artemisia annua L., is a well-known antimalarial drug. Artemisinin-type drugs also inhibit cancer growth in vitro and in vivo. Herbal extracts of A. annua inhibit the growth of cancer cell lines. Here, we report on the use of capsules containing powder of Herba Artemisiae annuae to treat pet sarcoma. The surgical tumor removal as standard treatment was supplemented by adjuvant therapy with A. annua. One cat and one dog with fibrosarcoma survived 40 and 37 months, respectively, without tumor relapse. Two other dogs suffering from fibrosarcoma and hemangioendothelial sarcoma also showed complete remission and are still alive after 39 and 26 months, respectively. A. annua was well tolerated without noticeable side effects. These four cases indicate that A. annua may be a promising herbal drug for cancer therapy.

Flavonoids are a group of modified triphenolic compounds from plants with medicinal properties. Baicalein, a specific flavone primarily isolated from plant roots (Scutellaria baicalensis), is commonly used in Eastern medicine for its anti-inflammatory and antineoplastic properties. Previous research shows greater efficacy for baicalein than most flavonoids; however, there has been little work examining their effects on sarcoma cells, let alone canine cells. Three canine osteosarcoma cell lines (HMPOS, D17 and OS 2.4) were treated with baicalein to examine cell viability, cell cycle kinetics, anchorage-independent growth and apoptosis. Results showed that osteosarcoma cells were sensitive to baicalein at concentrations from approximately 1 to 25 μM. Modest cell cycle changes were observed in one cell line. Baicalein was effective in inducing apoptosis and did not prevent doxorubicin cell proliferation inhibition in all the cell lines. The mechanism for induction of apoptosis has not been fully elucidated; however, changes in mitochondrial permeability supersede the apoptotic response.

Abnormal proximal gastric relaxation is one of the causes of functional dyspepsia. The purpose of this study is to use a barostat in conscious dogs to determine the effects of rikkunshito, which is considered to have beneficial effects on functional dyspepsia, on the proximal stomach. Eight beagles were used. A gastrocutaneous fistula and force transducers were surgically implanted in the middle corpus and gastric antrum and duodenum, respectively. After a recovery period, a plastic bag was inserted through the gastrocutaneous fistula and the proximal stomach was distended using a barostat. First, four dogs were used to investigate the pressure-volume relation in the fasted and postprandial phases. Second, the stomachs of four different dogs were continuously distended at minimal distending pressure +2 mmHg, and 5 min later were infused with warmed liquid rikkunshito (2 g/20 mL) or water through the gastrocutaneous fistula. Finally, changes in the proximal gastric volume and gastrointestinal motility were observed. The proximal stomach was significantly more pliable in the postprandial phase than in the fasted phase. The proximal gastric volume increased immediately after liquid infusion under constant pressure in both phases and duodenal motility was accelerated. The effect of rikkunshito was significantly greater and lasted longer than that of water. No significant difference between the effects during the fasted or postprandial phase and no change in the gastric antrum motility were observed when rikkunshito was infused. These results indicate that rikkunshito accelerates duodenal motility and relaxes the proximal stomach.

The aim of this study was to investigate sonographically the effect of Gongronema latifolium (G. latifolium) on gastric emptying of semi-solid meals in healthy dogs. In a randomized, placebo-controlled experiment,
twenty-five clinically healthy dogs were randomly allotted into five groups of five dogs in each group. The placebo group served as the control, and the low, moderate and high dose groups ingested the methanolic leaf extract of G. latifolium in capsules at 100 mg/kg, 250 mg/kg and 500 mg/kg, respectively, while the prokinetic group ingested 0.5 mg/kg capsules of metoclopramide. After a 12-h fast, each group ingested its treatment capsules 30 min before the administration of a test meal. Measurements of gastric emptying and blood glucose levels were obtained 30 min before and immediately after the ingestion of the test meal and thereafter every 15 min for 4 h. This was followed by further measurements every 30 min for another 2 h. The gastric emptying times of the placebo, low dose, moderate dose, high dose and prokinetic dose groups were 127.0 ± 8.2 min, 135.5 ± 3.7 min, 155.5 ± 3.9 min, 198.0 ± 5.3 min and 59.0 ± 2.5 min, respectively. Gastric emptying times of the moderate and high dose groups were significantly slower than in the placebo control group (155.5 ± 3.9 min, 198.0 ± 5.3 min vs 127.0 ± 8.2 min, P = 0.000). No significant difference in gastric emptying between the low dose and placebo control groups was noted (135.5 ± 3.7 min vs 127.0 ± 8.2 min, P = 0.072). Gastric emptying of the prokinetic group was significantly faster than that of the control group (59.0 ± 2.5 min vs 127.0 ± 8.2 min, P = 0.000). The hypoglycaemic effect of G. latifolium and gastric emptying were inversely related (r = -0.95, P = 0.000).


In this retrospective study, the tolerance to subcutaneus mistletoe injections (*Viscum album L*), adverse reactions and possible indications have been evaluated in feline patients of a small animal clinic. Among the 22 cats treated between 2008 and 2013, 4 did not accept the injections done by the owner, 7 showed slight short time adverse reactions, that disappeared spontaneously. No long term (more than 70 days) adverse reaction directly related to the *Viscum album* treatment could be identified. This study shows that Iscador(*) can be injected subcutaneously without a risk of worsening of the clinical signs or exacerbation of tumors. The antitumoral, but also immune-modulating and anti-inflammatory properties offer interesting treatment opportunities for dermatologic, odonto-stomatologic or allergic patients.


Renal fibrosis is common in progressive kidney disease. Transforming growth factors β (TGF-β) are important mediators of all types of fibrosis, including renal fibrosis. Chinese rhubarb has been shown to have antifibrotic properties in part because of inhibition of TGF-β and has slowed the progression of kidney disease in rodent models. The hypothesis was that the administration of a Chinese rhubarb supplement will slow the progression of chronic kidney disease (CKD) in cats and the concurrent administration of Chinese rhubarb and benazepril will be more effective than either alone. Twenty-nine client-owned cats with naturally occurring IRIS Stage 2 or early Stage 3 CKD and without comorbidity such as cancer, urinary tract obstruction, urinary tract infection, poorly controlled hyperthyroidism, or systemic hypertension were enrolled in the study. A randomized, positive-controlled, prospective study was performed. Cats received Chinese rhubarb, benazepril, or both in addition to standard treatment for CKD. Repeated measures ANOVA was used to assess changes in serum creatinine concentration, body weight, hematocrit, urine protein: urine creatinine ratio (UPC), and systemic arterial blood pressure over time between and within treatment groups over an average of 22 months. No significant differences were detected in serum creatinine concentration, body weight, hematocrit, UPC, and systemic arterial pressure over time between or within treatment groups. This study failed to detect a significant difference in the progression of CKD in cats treated with Chinese rhubarb, benazepril, or both. Further study in specific subsets of cats with CKD is warranted.


Traditional Japanese medicine, known as Kampo medicine, consists of mixtures of several medicinal herbs widely used to treat upper gastrointestinal disorders in Japan. Rikkunshito, one of these medicines, has not been evaluated with respect to its influence on gastrointestinal motor activity. We investigated the effect of rikkunshito on upper gastrointestinal motility and plasma ghrelin concentrations in conscious dogs. Contractile
response to intragastric administration of rikkunshito was studied via surgically implanted force transducers. A powdered extract of rikkunshito (1.3, 2.7, and 4.0 g) dissolved in water was administered into the stomachs of normal and vagotomized dogs before feeding and gastric emptying was evaluated. Several inhibitors of gastrointestinal motility (atropine, hexamethonium, and ondansetron) were injected intravenously before intragastric administration of rikkunshito. Plasma acylated ghrelin levels after intragastric administration of rikkunshito were measured. In a fasting state, intragastric administration of rikkunshito induced phasic contractions in the duodenum and jejunum in normal dogs. Rikkunshito-induced contractions were inhibited by atropine, hexamethonium and ondansetron. In vagotomized dogs, rikkunshito induced phasic contractions, similar to normal dogs. Gastric emptying was accelerated by intragastric administration of rikkunshito in a dose-dependent manner. The plasma acylated ghrelin level 150 min after intragastric administration of 4.0 g of rikkunshito was significantly higher than the control value. Intragastric administration of rikkunshito stimulates gastrointestinal contractions in the interdigestive state through cholinergic neurons and 5-HT type 3 receptors. Moreover, rikkunshito increases plasma acylated ghrelin levels. Rikkunshito may alleviate gastrointestinal disorders through its prokinetic effects.


The treatment of dermatophytoses due to Microsporum canis is cumbersome and relapses can occur. Volatile essential oils (EOs) obtained from plants would seem to represent suitable tools to contrast mycoses both in human and animals. The anti-M. canis activity of some EOs chemically characterized was evaluated both in vitro and in vivo. Eleven feline isolates of M. canis were tested by microdilution against EOs extracted from Thymus serpillum, Origanum vulgare, Rosmarinus officinalis, Illicium verum and Citrus limon. A mixture composed by 5% O. vulgare, 5% R. officinalis and 2% T. serpillum, in sweet almond oil was administered to seven infected, symptomatic cats. T. serpillum and O. vulgare showed the lowest MICs, followed by l. verum, R. officinalis and C. limon. The assay performed on mixture showed that antimycotic activity of each component was enhanced. Four out of seven treated cats recovered both clinically and culturally. T. serpillum and O. vulgare EOs showed a strong antifungal activity. Preliminary data suggest a possible application in managing feline microsporiasis. Considering the potential zoonotic impact of this infection, the use of alternative antifungal compounds would be of aid to limit the risk of environmental spreading of arthrospores.


To clarify the interaction between St John’s wort (SJW) and cyclosporine (CsA) in dogs, the pharmacokinetics of CsA before and during the repeated administration of SJW were analyzed. In the SJW group, SJW (300 mg) was given orally to four dogs every 24 h for 14 days. A single dose of CsA (5 mg/kg) was given orally 7 days before and 7 and 14 days after the initiation of the repeated administration of SJW. In the Control group, a single dose of CsA (5 mg/kg) was given orally to four other dogs in accordance with that in the SJW group. Blood samples from both groups were collected, and whole-blood concentrations of CsA were determined using high-performance liquid chromatography with UV detection. The maximum whole-blood concentration and AUC(0-∞) of the SJW group were significantly lower and the CL(tot) /F and V(d) /F were significantly higher than those in the Control group 7 and 14 days after the initiation of repeated SJW. Thus, repeated administrations of SJW affect the pharmacokinetic profiles of CsA in dogs. Further studies are necessary to elucidate the mechanisms of interaction between SJW and CsA in dogs.


Alzheimer’s disease (AD) involves multiple pathological processes in the brain, including increased inflammation and oxidative damage, as well as the accumulation of amyloid-β (Aβ) plaques. We hypothesized that a combinatorial therapeutic approach to target these multiple pathways may provide cognitive and neuropathological benefits for AD patients. To test this hypothesis, we used a canine model of human aging and AD. Aged dogs naturally develop learning and memory impairments, human-type Aβ deposits, and oxidative damage in the brain. Thus, 9 aged beagles (98-115 months) were treated with a medical food cocktail
containing (1) an extract of turmeric containing 95% curcuminoids; (2) an extract of green tea containing 50% epigallocatechingallate; (3) N-acetyl cysteine; (4) R-alpha lipoic acid; and (5) an extract of black pepper containing 95% piperine. Nine similarly aged dogs served as placebo-treated controls. After 3 months of treatment, 13 dogs completed a variable distance landmark task used as a measure of spatial attention. As compared to placebo-treated animals, dogs receiving the medical food cocktail had significantly lower error scores ($t_{11} = 4.3, p = 0.001$) and were more accurate across all distances ($F(1,9) = 20.7, p = 0.001$), suggesting an overall improvement in spatial attention. Measures of visual discrimination learning, executive function and spatial memory, and levels of brain and cerebrospinal fluid $A\beta$ were unaffected by the cocktail. Our results indicate that this medical food cocktail may be beneficial for improving spatial attention and motivation deficits associated with impaired cognition in aging and AD.


Increases in liver enzymes occur in up to 86% of dogs receiving CCNU and can result in treatment delay or early discontinuation of treatment. Denamarin contains S-adenosylmethionine and silybin, both of which have been investigated as treatments for various liver diseases. Dogs on CCNU receiving Denamarin have lower alanine aminotransferase (ALT) activity than dogs not receiving Denamarin. Dogs on Denamarin are less likely to require treatment delay because of hepatopathy and are more likely to complete their prescribed course of CCNU. Dogs with lymphoma, mast cell tumor, or histiocytic sarcoma that were prescribed CCNU with or without corticosteroids and with normal ALT activity were eligible for enrolment. Dogs were prospectively randomized to receive either concurrent Denamarin during CCNU chemotherapy or to receive CCNU alone. Liver-specific laboratory tests were run before each dose of CCNU. Increased liver enzyme activity occurred in 84% of dogs receiving CCNU alone and in 68% of dogs on concurrent Denamarin. Dogs receiving CCNU alone had significantly greater increases in ALT, aspartate aminotransferase, alkaline phosphatase, and bilirubin and a significantly greater decrease in serum cholesterol concentrations than dogs receiving concurrent Denamarin. Dogs receiving CCNU alone were significantly more likely to have treatment delayed or discontinued because of increased ALT activity. Increased liver enzyme activity occurs commonly in dogs receiving CCNU chemotherapy. These results support the use of concurrent Denamarin to minimize increased liver enzyme activity in dogs receiving CCNU chemotherapy. Denamarin treatment also increases the likelihood of dogs completing a prescribed CCNU course.


This double-blind randomized placebo-controlled trial indicates that Phytopica can be an effective glucocorticoid sparing agent in canine atopic dermatitis (AD). Twenty-two dogs with perennal AD [Canine Atopic Dermatitis with Severity Index (CADESI-03) $\geq 60$] were given 200 mg/kg Phytopica or an identical placebo in food once daily for 56 days. All dogs were initially given 0.4 mg/kg methyl-prednisolone once daily, which was then adjusted according to the daily pruritus score (0-100 mm visual analogue scale). The cumulative dose and pruritus score were lower in the Phytopica than the placebo group. There were statistically significant time and treatment effects for the methyl-prednisolone dose and pruritus score, but there were no significant differences between the Phytopica and placebo groups in the proportion of dogs that achieved a $> 50\%$ reduction in dose or pruritus scores at day 56; the mean CADESI-03 scores at days 0, 28 and 56; the numbers achieving $>50\%$ reduction in CADESI-03 at days 28 and 56; or in the owners’ global efficacy score at days 28 and 56. Adverse events included diarrhoea (three Phytopica and one placebo treated dog), polyuria/polydipsia (three dogs in each group), and polyphagia, intermittent anorexia and panting (one dog each in the placebo group). None of these by themselves required withdrawal of treatment.

Canine atopic dermatitis (AD) is common and new therapies are beneficial. This multicentric, randomized, double-blind, placebo-controlled study tested the efficacy of Actinidia arguta (hardy kiwi) (EFF1001) in dogs with mild/moderate AD. The study was divided into two stages. Stage 1 lasted 6 weeks. In the first 2 weeks prednisolone [days 1-3: 0.2 mg/kg twice daily (BID), days 4-14: 0.2 mg/kg every other day (EOD)] was administered. Responsive dogs were placed on prednisolone 0.2 mg/kg EOD + assigned test article [either placebo or EFF1001 (30 mg/kg)] once daily for 4 weeks. Stage 1 responders were advanced to stage 2, which involved 4 weeks of just EFF1001. Clinicians scored lesions using Canine Atopic Dermatitis Extent and Severity Index (CADESI) and owners scored pruritus using a Pruritus Visual Analogue Scale. Seventy-seven dogs were enrolled, 76 were randomized on day 14, and 57 (57/76 = 75%) completed stage 1 (27 in EFF1001 and 30 in placebo). At the end of stage 1, 35 of 57 dogs (35/57 = 61%) responded (18 in EFF1001 and 17 in placebo) and advanced to stage 2. At completion of stage 1, CADESI scores did not significantly differ between groups while pruritus decreased in EFF1001 group and approached significance. At completion of stage 2, 19 dogs (19/35 = 54%) responded (15/19 = 79% had received EFF1001 and 4/19 = 21% placebo in stage 1). After completing stage 2, dogs placed on EFF1001 throughout the study were 3.5 times more likely to either maintain or improve scores than those that started it in stage 2. It is concluded that EFF1001 is beneficial adjunctive therapy after prolonged use.

Other papers include:

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Mitigation of the methane (CH₄) emission from ruminants is needed to decrease the environmental impact of ruminant animal production. Different plant materials and chemicals have been tested, but few are both effective and practical. Medicinal herbs contain biological compounds and antimicrobials that may be effective in lowering the CH₄ production. However, few studies have systematically evaluated medicinal herbs for their effect on CH₄ production or on the rumen microbiota. In this study, extracts from 100 medicinal herbs were assessed for their ability to decrease CH₄ production by rumen microbiota in vitro. The extracts of 12 herbs effectively lowered the CH₄ production, with the extract of Perilla frutescens seeds being the most effective. The major components of P. frutescens seed extract were identified, and the effects of the extract on the fermentation characteristics and populations of rumen methanogens, fungi, protozoa, and select bacteria were also assessed. The decreased CH₄ production induced by the P. frutescens seed extract was accompanied by an increased abundance of Ruminobacter, Selenomonas, Succinivibrion, Shuttleworthia, Pseudobutyrovibrio, Anaerovibrio, and Roseomonas and a decreased abundance of Methanobrevibacter millerae. The abundance of Pedobacter, Anaeroplasma, Paludibacter, Ruminococcus, and unclassified Lachnospiraceae was positively correlated with the CH₄ production, with no effects on volatile fatty acids. This study suggests that medicinal herbs may be used to mitigate the CH₄ emission from ruminants.


Mastitis is considered the most significant and persistent disease in dairy cows, bringing about large economic losses. Subclinical mastitis brings about major cost implications, for it is difficult to detect due to absence of any visible indications and can persist in the mammary tissue throughout lactation. Immunomodulators have been widely used to reduce intramammary infections by modulating bovine mammary gland. Atractylodis macrocephalae Koidz. polysaccharides (RAMP), extracted from herbal medicine, has been used widely especially for its immunomodulatory function for many years. The objective of this study was to estimate an oil emulsified Atractylodis macrocephalae Koidz. polysaccharides (RAMP-O) as a potential therapeutic agent to treat subclinical mastitis by subcutaneous injection of RAMP-O in the area of supramammary lymph node in lactating cows via analysis of SCC, IMIs and injection of RAMP-O in the area of supramammary lymph node significantly reduced milk SCC and NAGase activity compared with control. The quarters with bacterial infection were also progressively reduced in RAMP-O treated cows and only 9 quarters were found to have bacterial infection, while no obvious change was found in the control group. Subcutaneous injection of RAMP-O in the area of supramammary lymph node had therapeutic value in the treatment of bovine subclinical mastitis by reducing SCC, NAGase and IMIs in milk. Considering both the therapeutic effect and the cost of RAMP-O, 32 mg per dose was found most suitable to reduce milk SCC and NAGase. Therefore, RAMP-O deserves further study for its use in treatment of bovine mastitis.


The purposes of this study were to explore TLR2 and TLR4 participation and MyD88 and NF-κB activation in bovine mammary glands (BMG) treated with Panax ginseng (PG) at involution and verify the effect of PG in the cytokine expression. Quarters were infused at the end of lactation with PG solution (3 mg/ml), placebo or kept as uninoculated controls. Cows were slaughtered at 7 d after cessation of milking and mammary tissue
samples were taken. A significant increase of TLR2, TLR4, MyD88, NF-κB, IL-1β, IL-6 and TGF-β1 mRNA expression was observed in PG-treated quarters. Immunostaining of TLR2 and TLR4 was significantly higher in PG mammary tissues. The percentages of immunopositive cells for NF-κB-p65 were significantly higher in PG-treated quarters. The BMG responded to PG extract components possibly by TLR2 and TLR4 signaling pathway. These results provide an insight into potential mechanisms by which PG stimulates innate immunity during BMG involution.


Bovine mastitis is one of the most important infectious diseases in dairy herds, and staphylococci are the most important etiologic agents of this disease. Antibiotics and chemical agents used in livestock for prevention and cure of the disease can accumulate in milk and give rise to food safety concerns. Rhodomyrtus tomentosa leaf extract was studied as an alternative approach to reduce the bacterial infections. The ethanolic extract of this plant demonstrated antibacterial activity with minimum inhibitory concentration (MIC) values as low as 16-64 μg/mL against staphylococcal isolates. In addition, the extract had an effect on the bacterial cell surface properties by increasing its hydrophobicity in a concentration dependent manner. To further extend the antibacterial efficacy, silver nanoparticles synthesized with the extract, a pure rhodomyrton, and liposomal encapsulated rhodomyrton were applied and their inhibitory effects on bacterial adhesion and invasion were determined by ex vivo study in a bovine udder epidermal tissue model. These agents exerted remarkable antibacterial activity against staphylococci and decreased the adhesion of the bacterial cells to the tissues. These results supported that R. tomentosa ethanolic extract could be applied as an alternative agent for bovine udder care in dairy farms.


Methicillin-resistant Staphylococcus aureus (MRSA) is an important nosocomial pathogen that shows resistance to many antibiotics and is usually associated with serious infections. Having the ability for biofilm formation increases resistance to antibiotics. Sanguisorba officinalis L. is a perennial plant that is distributed in the northern districts of China and has been used as a traditional Chinese medicine. In this study, the effect of S. officinalis on MRSA strain SA3 isolated from a dairy cow with mastitis was evaluated by testing the growth and biofilm formation ability of MRSA cultured with or without ethanol extracts of S. officinalis. The results showed that the ethanol extract of S. officinalis strongly inhibited the biofilm formation of MRSA. With a confocal laser scanning microscope system, we observed that the biofilm structure of the test group with the addition of S. officinalis appeared looser and had less biomass compared with the control group without S. officinalis. Furthermore, we found that the transcript levels of the icaADBC operon remarkably decreased upon addition of the ethanol extract of S. officinalis, indicating that S. officinalis inhibits biofilm formation of MRSA in an ica-dependent manner.


Antibiotic residues in dairy products as well as emergence of antimicrobial resistance in foodborne pathogens have been recognized as global public health concerns. The present work was aimed to study a potent antibacterial extract from natural product as an alternative treatment for staphylococcal bovine mastitis. Staphylococcal isolates (n=44) were isolated from milk samples freshly squeezed from individual cows. All staphylococcal isolates were resistant to ampicillin, ciprofloxacin, erythromycin, gentamicin, penicillin, except vancomycin. Rhodomyrtus tomentosa leaf ethanolic extract was accessed for its antibacterial activity and anti-inflammatory potential. The extract exhibited profound antibacterial activity against all of staphylococcal isolates with MIC and MBC values ranged from 16-64 μg/ml and 64->128 μg/ml, respectively. Moreover, the extract also exerted anti-protein denaturation and human red blood cell membrane stabilizing activity. The
results support the use of R. tomentosa extract that could be applied to cure bovine mastitis and to reduce inflammatory injury caused by the bacterial infections.


Berberine is a plant alkaloid with antimicrobial activity against a variety of microorganisms. In this study, the antimicrobial properties of berberine against multi-drug resistant field isolates of Staphylococcus epidermidis were investigated using berberine alone or in combination with a commonly used antibiotics in veterinary clinics, including penicillin, lincomycin, and amoxicillin. The results indicated that the minimum inhibitory concentrations of berberine, penicillin, lincomycin, and amoxicillin against field S. epidermidis isolates were 2-512, 0.8-213, 0.4-1024, and 0.4-256 mg/mL, respectively. Furthermore, the synergistic effects of antimicrobial activity against these multi-drug resistant isolates were observed when the berberine was combined with penicillin, lincomycin, or amoxicillin; no antagonistic effect of the combination was detected in any of the clinical isolates. These observations were further confirmed using a time-killing assay, in which a combination of 2 agents yielded a greater than 2.03-2.44 log10 decrease in colony-forming unit/mL compared with each agent alone. These findings suggest that berberine is a promising compound for preventing and treating multi-drug resistant S. epidermidis infected mastitis in dairy cows either alone or in combination with other commonly used antibiotics, such as penicillin, lincomycin, and amoxicillin.


Bovine mastitis is an inflammation of the mammary glands of cows and causes significant economic losses in dairy cattle. Staphylococcus aureus is one of the microorganisms most commonly isolated. Novel agents are required in agricultural industries to prevent the development of mastitis. The production of biofilm by Staph. aureus facilitates the adhesion of bacteria to solid surfaces and contributes to the transmission and maintenance of these bacteria. The effect of the essential oils of Syzygium aromaticum (clove; EOSA) and Cinnamomum zeylanicum (cinnamon; EOCZ) and their major components, eugenol and cinnamaldehyde, on Staph. aureus biofilm formation on different surfaces was investigated. The results showed a significant inhibition of biofilm production by EOSA on polystyrene and stainless steel surfaces (69.4 and 63.6%, respectively). However, its major component, eugenol, was less effective on polystyrene and stainless steel (52.8 and 19.6%, respectively). Both EOCZ and its major component, cinnamaldehyde, significantly reduced biofilm formation on polystyrene (74.7 and 69.6%, respectively) and on stainless steel surfaces (45.3 and 44.9%, respectively). These findings suggest that EOSA, EOCZ, and cinnamaldehyde may be considered for applications such as sanitization in the food industry.


Puerperal metritis is an important disorder usually within 21 days postpartum in dairy cattle that occurs within 21 days postpartum, and herbal remedies are believed to be beneficial for post partum female livestock. Sheng HuaTang is a prime example of herbal formula used as a therapeutic aid in prevention or control of post partum disease for centuries in China. In the present study, we were to evaluate the efficacy of Sheng HuaTang as a prophylactic strategy for lowering puerperal metritis risks and improving reproductive performance in dairy cows under field conditions. A total of 311 clinically healthy cows were randomly allocated to the intervention group or the control group 2–4 h after delivery. Treated cows (n=158) received Sheng HuaTang with an oral dose of 0.36g crude herb/kg bw once daily for three consecutive days, whereas the controls (n=153) received no treatment. The logistic regression and survival analysis were used to analyse the incidence of puerperal metritis and reproduction parameters of cows between the two groups, respectively. The results showed that there was a significant reduction in the incidence of puerperal metritis (12.1% vs. 33.3%, P=0.01, odd ratio [OR] 2.392) between Sheng Hua Tang group and the control group. The calving-to-first-service
interval (68.97±17.7 days vs. 80.57±26.6 days, \( P < 0.05 \)) and service per conception (1.7 vs. 2.1, \( P < 0.01 \)) were lower in cows in Sheng HuaTang group than the controls. Additionally, Sheng HuaTang treatment effectively elevated the first AI conception proportion (61.1% vs. 51.3%, \( P < 0.05 \)) and proportion of cows that were pregnant at 305 days in milk (89.8% vs. 82.0%, \( P < 0.01 \)) compared with that of controls. The present results would support efforts to the use of Sheng HuaTang immediately after delivery as a prophylactic strategy for lowering puerperal metritis risk and improving the overall reproductive efficiency of dairy herds under these study circumstances. Thus, Sheng HuaTang treatment could represent an effective prophylactic strategy for bovine post partum care.

Jamra N\(^1\), Das G\(^2\), Singh P\(^3\), Haque M\(^1\). Anthelmintic efficacy of crude neem (Azadirachta indica) leaf powder against bovine strongylosis. J Parasit Dis. 2015 Dec;39(4):786-8.

The present study was conducted to evaluate the anthelmintic efficacy of crude neem (Azadirachta indica) leaf powder against strongyle infections in cattle. Based on copro-examination, 30 cattle positive for strongyle infection with at least 250 [eggs per gram (EPG) of faeces] were selected and grouped as A, B and C (10 animals/group). Group A and B were treated respectively with fendendazole and neem leaf powder @ 5 and 500 mg/kg body weight, whereas Group C served as infected untreated control. Faecal sample from each animal of these groups was examined on day 0, 7, 14 and 28 post treatments and EPG was determined. The result showed significant decrease (\( p < 0.05 \)) in EPG in Group A and B after day 7 post treatment but there was no significant variation in terms of EPG in control group. Thus it can be concluded that crude neem leaf powder has anthelmintic property and it can further be studied to isolate the active component to produce herbal anthelmintics.


Escherichia coli O157:H7 (EcO157) shed in cattle manure can survive for extended periods of time and intervention strategies to control this pathogen at the source are critical as produce crops are often grown in proximity to animal raising operations. This study evaluated whether neem (Azadirachta indica), known for its antimicrobial and insecticidal properties, can be used to amend manure to control EcO157. The influence of neem materials (leaf, bark, and oil) on the survival of an apple juice outbreak strain of EcO157 in dairy manure was monitored. Neem leaf and bark supplements eliminated the pathogen in less than 10 d with a D-value (days for 90% elimination) of 1.3 d. In contrast, nearly 4 log CFU EcO157/g remained after 10 d in neem-free manure control. The ethyl acetate extractable fraction of neem leaves was inhibitory to the growth of EcO157 in LB broth. Azadirachtin, a neem product with insect antifeedant properties, failed to inhibit EcO157. Application of inexpensive neem supplements to control pathogens in manure and possibly in produce fields may be an option for controlling the transfer of foodborne pathogens from farm to fork.


Nonantibiotic treatments for mastitis are needed in organic dairy herds. Plant-derived oils may be useful but efficacy and potential mechanisms of action of such oils in mastitis therapy have not been well documented. The objective of the current study was to evaluate the antibacterial activity of the plant-derived oil components of Phyto-Mast (Bovinity Health LLC, Narvon, PA), an herbal intramammary product, against 3 mastitis-causing pathogens: Staphylococcus aureus, Staphylococcus chromogenes, and Streptococcus uberis. Plant-derived oils evaluated were Thymus vulgaris (thyme), Gaultheria procumbens (wintergreen), Glycyrrhiza uralensis (Chinese licorice), Angelica sinensis, and Angelica dahurica. Broth dilution testing according to standard protocol was performed using ultrapasteurized whole milk instead of broth. Controls included milk only (negative control), milk + bacteria (positive control), and milk + bacteria + penicillin-streptomycin (antibiotic control, at 1 and 5% concentrations). Essential oil of thyme was tested by itself and not in combination with other oils because of its known antibacterial activity. The other plant-derived oils were tested alone and in combination for a total of 15 treatments, each replicated 3 times and tested at 0.5, 1, 2, and 4% to simulate concentrations potentially achievable in the milk within the pre-dry-off udder quarter. Thyme oil at concentrations ≥2% completely inhibited bacterial growth in all replications. Other plant-derived
oils tested alone or in various combinations were not consistently antibacterial and did not show typical dose-response effects. Only thyme essential oil had consistent antibacterial activity against the 3 mastitis-causing organisms tested in vitro. Further evaluation of physiological effects of thyme oil in various preparations on mammary tissue is recommended to determine potential suitability for mastitis therapy.


Dry cow therapy, administered at the end of lactation, is aimed at eliminating current and preventing future intramammary (IMM) bacterial infections and typically involves intramammary administration of antibiotics. Certified organic dairies in the United States are restricted from using antibiotics and must consider an alternative therapy or no dry cow therapy. The current study compared 2 herbal products to conventional dry cow therapy and no treatment for a total of 5 treatments over 2 trials. Trial 1 was conducted over 3 yr on 1 research farm and trial 2 included 4 commercial farms plus the research herd over 2 yr. Treatments included (1) a conventional IMM antibiotic and internal teat sealant (penicillin-dihydrostreptomycin and bismuth subnitrate; CON); (2) an herbal IMM product purported to act as a teat sealant (Cinnatube, New AgriTech Enterprises, Locke, NY; CIN); (3) an herbal IMM product (Phyto-Mast, Bovinity Health LLC, Narvon, PA; P-M); (4) Phyto-Mast and Cinnatube (PC); or (5) no dry cow therapy (NT). Each treatment group was balanced by breed, lactation number, due date, herd, and year. However, the CON treatment was used only in the research herd because of the intent to avoid antibiotic usage on the other 4 farms. Comparisons among treatments included the difference between pre- and posttreatment 305-d mature equivalent milk production (trial 1), somatic cell score change from dry-off to freshening at the cow and quarter levels (trials 1 and 2), and milk microbiology change over the dry period (trial 2). We detected no significant differences among treatments for milk yield differences between the lactation following treatment and the lactation preceding treatment.

Changes in somatic cell score from one lactation to the next also did not differ significantly among treatments in either trial. Cure rates were not significantly different among treatments; only 19.6% of all quarters were infected at dry off. The proportion of quarters with new infections at 3 to 5d postcalving did not significantly differ among treatments, except between CIN and NT. Percentages (least squares means ± standard error) of quarters with new infections were 24 ± 21% for CON, 15 ± 7% for CIN, 30 ± 10% for P-M, 32 ± 11% for PC, and 35 ± 11% for NT. The efficacy of the herbal products was similar to that of conventional therapy, and the herbal products had no apparent adverse effects.


Retained placenta remains therapeutic challenge in cattle. Certain traditional medicines are believed to be able to alleviate retained placenta condition and improve overall fertility in cows. The aim of the present study was to evaluate the efficacy of an herbal tincture for treatment of retained placenta. The herbal tincture was extracted from a combination of Herba Leonuri, Angelicae Sinensis Radix, Flos Carthami, Myrrha and Rhizoma Cyperi by percolation with 70% ethanol to a concentration of 0.5g crude herb/ml. Cows diagnosed with retained placenta (n=48) were randomly divided into one of two treatment groups (A and B), with animals in group A (n=26) receiving herbal tincture orally, and cows in group B (n=22) receiving oxytetracycline infusion into the uterus. Eighty six cows with no clinically visible pathological conditions, given birth alone and with no retained placenta diagnosis were included into control group (C). Retained placenta was expelled within 72h following initial treatment in 19 cows in group A, yet no cows in group B were recorded to expel placenta in the same time. The median number of days to first service (70.0 vs. 102.5 days; P<0.05) and median number of days open (76.0 vs. 134.0 days; P<0.01) were lower in group A than in group B. Percentage of cows pregnant within 100 days postpartum was the highest for animals in group A compared to controls (61.5% vs. 39.5%, P<0.05), and for animals in group B (61.5% vs. 22.7%; P<0.01). Herbal tincture used in the present study might facilitate expulsion of retained placenta and improve subsequent fertility, thus could present effective treatment option for retained placenta in cows.

The objective was to evaluate the efficacy of a botanical product (PHYTO-MAST®) for the intra-mammary treatment of clinical mastitis (CM) in dairy cows managed in an organic system. The study involved 194 naturally occurring cases of clinical mastitis. Treatment was applied every 12 hours for 3 days and cows were evaluated for clinical cure starting on day 4. Outcomes of interest consisted of mastitis resolution at day 4, time to resolution, somatic cell score (SCS) after recovery, and bacteriological cure at 14 and 28 d after treatment. There was no significant effect on clinical mastitis resolution at day 4 for treatment compared with the control group. However, there was a faster recovery for the treatment group compared to the control group with median intervals from end of treatment to recovery of 4.6 d and 6.5 d, respectively. There was no effect on the probability of a SCS < 4 (200 000 SC/mL) after treatment. No significant effects were found for treatment on bacteriological cure at days 14 and 28.


The Rhipicephalus microplus tick is globally regarded as the most economically important ectoparasite of livestock, and the evolution of resistance to commercial acaricides among cattle tick populations is of great concern. The essential oil derived from Tagetes minuta may be efficacious against cattle tick infestation, and the results of a cattle pen trial using this essential oil for the control of ticks are reported here. The chemical composition of the essential oil was determined by GC-MS and NMR spectroscopy analyses, which revealed the presence of four major components in the essential oil. These components represent more than 70% of the essential oil: limonene (6.96%), β-ocimene (5.11%), dihydrotagetone (54.10%) and tagetone (6.73%). The results of the cattle pen trial indicated significant differences among the average values of the analyzed biological parameters, including the number of ticks, the average weight of the ticks, the average egg weight per engorged female and larval viability. Treatment with the T. minuta essential oil prepared in this study promoted significant effects on all biological indicators analyzed. Based on the biological indicators, the essential oil showed 99.98% efficacy compared to the control group when used at a 20% concentration. The results obtained in this study suggest that the T. minuta essential oil is a potential R. microplus tick control agent and may be used to mitigate the economic losses caused by tick infestation.


Sheng Hua Tang, a classical herbal formula consisting of Radix Angelicae sinensis, Ligustici rhizoma, Semen persicae, Zingiberis rhizoma, and Radix glycyrrhizae, is known to be beneficial in alleviating postpartum diseases and facilitating a return to normal reproductive function. This study investigated whether the administration of Sheng Hua Tang within 2 to 4 hours after delivery was effective as a preventive treatment for reducing the risk of retained placenta in Holstein dairy cows. A total of 357 cows, each of which had delivered its calf spontaneously, were randomly allocated to one of two groups. In the treatment group, the cows (n = 175) received Sheng Hua Tang with an oral dose of 0.36 g crude herb per kg·body weight once daily for three consecutive days. The controls (n = 182) received no treatment. The placental retention proportion was 4.0% and 17.0% within 12 hours after delivery in the treated and control animals, respectively (P < 0.01). We found decreases in the calving-to-first-service interval (73.2 ± 25.1 vs. 81.9 ± 32.8 days; P < 0.01), calving-to-conception interval (93.4 ± 38.8 vs. 114.6 ± 42.9 days; P < 0.01), and service per conception (1.5 ± 0.8 vs. 1.9 ± 1.0 days; P < 0.01) in the treatment group compared with the control group. The first artificial insemination conception proportion was higher in the treatment group than in the control group (60.4% vs. 41.1%; P = 0.01). Moreover, the between-group difference in the proportion of cows that were pregnant within 180 days postpartum approached statistical significance (88.2% vs. 80.6%; P = 0.07). Sheng Hua Tang showed beneficial effects in reducing the incidence of retained placenta and improving subsequent reproductive performance in cows. This preventive treatment strategy would be effective in improving the management of puerperal health. The potential benefits of Sheng Hua Tang warrant further investigation to determine whether this preventive treatment strategy can be endorsed as a general preventive approach in postpartum cows.
This paper highlights the role of plant volatile organic compounds, found in essential oils, for the treatment of bacteria related inflammation. This report is focused on tea tree oil, particularly its main compound terpinen-4-ol. Analysis of the published literature shows that many essential oils have significant antibacterial, antifungal and anti-inflammatory effects. Some of their major components, such as terpinen-4-ol, act by inhibiting pro-inflammatory cytokine expression while stimulating production of anti-inflammatory cytokines. Such observations may be exploited to encourage biotherapy against mastitis. The use of synthetic antibiotics is being increasingly discouraged because their presence in dairy milk may have potential downstream effects on population health and the agri-food chain. In the context of inflammation and related mammalian responses, understanding the interplay between volatile organic compounds, especially terpinen-4-ol, and cytokines during bacteria related inflammation should clarify their mode of action to control mastitis.

The lack of efficacy of conventional strategies for the maintenance of healthy udders in domestic cattle has prompted studies on the use of immunomodulators or biological response modifiers (BRM) for this purpose. These compounds are agents that modify the host’s response to pathogens leading to beneficial effects on disease outcome. The objective of this study was to evaluate the effects of a single intramammary infusion of Panax ginseng (GS) extract on the amount of pro-inflammatory cytokines and the number of monocytes/macrophages present in bovine mammary tissues at drying off. Eight mammary quarters from six nonpregnant cows in late lactation were infused with 10 mL of GS (3mg/mL), six quarters were treated with 10 mL of placebo (vehicle alone) and six quarters were maintained as uninoculated controls. The analyses of tumor necrosis factor-alpha (TNF-α) by immunohistochemistry revealed that the production of this proinflammatory cytokine significantly increased (P<0.05) in the inoculated mammary glands of cows following BRM inoculation, whereas the interleukin-1 alpha (IL-1α) and IL-6 staining area was not affected by BRM treatment. The number of monocytes/macrophages detected with CD14 antibody was significantly higher (P<0.05) in BRM-treated quarters than in placebo and uninoculated control quarters. These results indicated an immunomodulator potential of the BRM used. The beneficial effect of the extract could be used as alternative therapy in the control of mastitis at drying off, either alone or in conjunction with dry cow antibiotic therapy.

The seeds of *Nigella sativa* Linn. (Ranunculaceae) known as black seed or black cumin, are used in herbal medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma, diarrhea and dyslipidaemia. In this study the effect of intramammary injection of *Nigella Sativa* Extract (NSE) in paraffin on quarter milk, quality and Somatic Cell Count (SCC) and the shedding pattern of *Staphylococcus aureus* were investigated. Thirty Holstein cows, naturally infected with *S. aureus* subclinical mastitis, were subjected to treatment with the NSE at a dose of 10 mL in paraffin (200 mg mL−1) per day for 3 days, or with 10 mL paraffin as control. The injection areas were checked for adverse reactions. The daily milk production was measured before and after treatment. Intramammary injection of NSE caused a remarkable healing. Quarter milk samples were collected for bacteriological analysis and Somatic Cell Counts (SCC). The bacterial count moderately decreased in the treatment group. After the end of the treatment, the numbers of *S. aureus*-infected quarters and milk SCC tended to decrease in the NSE-treated cows. These clarifications were significantly higher one week post-treatment than pretreatment. Similar changes were not observed in the control group.
The results of the present study showed that the NSE has potential as a therapeutic agent for *S. aureus* infection causing subclinical mastitis of dairy cows and may contribute to the cow's recovery from mastitis. In conclusion, the results indicate that *Nigella sativa* might act as an antibacterial *in vivo* in dairy cows.


Despite the recent growth of the organic dairy industry, organic producers and veterinarians have limited information when choosing mastitis treatments for animals in organic dairy production. Organic producers commonly administer homeopathic or other plant-based products without having research evaluating the efficacy of these products and using estimated or no withholding times to treat mastitis and other health problems in their herds. In this pilot study, we attempted to identify several active ingredients of Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), a plant-based mastitis treatment used on organic dairy farms, and to quantify the product residue in milk and plasma after intramammary administration. We developed an assay to quantify thymol (one of the active ingredients in Phyto-Mast) in milk and plasma using gas chromatography and mass spectrometry (GC-MS). Thymol is a volatile aromatic compound with antiinflammatory properties. As a model for dairy cows, 5 healthy, lactating alpine dairy goats were given 5 mL of Phyto-Mast per udder half. For 10 d following treatment, we analyzed blood and milk samples for thymol residues using GC-MS. The GC-MS assay was very sensitive for thymol detection, to a concentration of 0.01 μg/mL in plasma. Using thymol as a marker, Phyto-Mast was detectable and quantifiable in plasma beginning with the 15-min posttreatment sample, but was no longer detectable in the 4-h posttreatment sample. Thymol residues were only detected in the 12-h posttreatment milk sample. An inflammatory response was not evident in the udder following phytoceutical administration. Although this study provides information about the elimination of thymol, the product contains several other active chemicals, which may have different pharmacokinetic behaviors. Further analysis and additional study animals will help to determine a milk withholding time for Phyto-Mast. Given the recent growth of the organic dairy industry, understanding the pharmacokinetics of therapeutics used in organic production and developing accurate withholding recommendations will help to ensure milk safety.

L. Pan et al. Effects of Radix Bupleuri extract supplementation on lactationperformance and rumen fermentation in heat-stressed lactating Holstein cows Animal Feed Science and Technology 187 (2014) 1– 8

Radix Bupleuri extract (RBE) has been shown to mitigate negative effects of high ambient temperature. This experiment was conducted to investigate effects of RBE supplementation on lactation performance and rumen fermentation in Holstein cows under heat stress. Forty Holstein cows (75 ± 15 d in milk, 37.5 ± 1.8 kg of milk/d, and 1.7 ± 0.4 parity) were randomly assigned to one of four groups (n = 10). One of four treatment diets, assigned randomly to one of four groups, consisted of RBE supplementation at 0, 0.25, 0.5 or 1.0 g/kg of the basal diet (concentrate and roughage) based on dry matter (DM). Cows were housed in a tie-stall barn and were individually fed the treatment diets. The experiment lasted for 10 wk in hot summer. During the experiment, average ambient temperatures and temperature-humidity indexes (THI) were respectively 27.5 ± 1.5, 29.8 ± 1.9 and 28.1 ± 1.7 ◦C, and 78.2 ± 2.7, 79.8 ± 3.3 and 78.3 ± 3.4 at 0600, 1400 and 2200 h. Average respiration rates (RR) with RBE at 0.25, 0.50 and 1.0 g/kg were 65.6, 60.3 and 67.4, respectively, vs. 71.4 (breaths/min) for the control (P < 0.01). Average rectal temperatures (RT) were 39.1, 39.0 and 39.1 vs. 39.3 ◦C for the control (P < 0.01). Moreover, cows supplemented with RBE increased dry matter intake (DMI, 22.8, 21.6 and 22.1 vs. 20.9 kg/d)(P < 0.05) and milk production (34.2, 33.4 and 32.4 vs. 31.6 kg/d) (P < 0.01) compared with control. Percentages of milk protein and fat were similar among groups, while milk protein yield increased with increasing level of RBE (0.97, 0.95 and 0.92 vs. 0.89 kg/d for the control)(P < 0.01). Milk fat yield also increased with RBE (1.13, 1.12 and 1.09 vs. 1.02 kg/d for the control) (P < 0.05). There was no treatment effect on diet apparent digestibility or volatile fatty acid (VFA) concentration among groups. Overall, supplemental RBE at 0.25 or 0.5 g/kg could mitigate the negative effects of heat stress on production in lactating Holstein cows.

Two experiments were carried out to investigate the effects of supplemental Chinese herbs, *Fructus Ligustri Lucidi* (FLL), *Radix Astragali* (RA) and *Radix Codonopsis* (RC) on growth performance, blood antioxidant and immune function in Holstein dairy heifers fed high fibre diet. Experiment 1 indicated that the supplementation of the three herbs had no effect on dry matter intake. FLL supplementation increased heifers average daily gain (ADG), final body weight and feed efficiency. Experiment 2 indicated that FLL supplementation improved the blood antioxidant function with higher concentration of superoxide dismutase (SOD) and lower concentration of malondialdehyde (MDA), and improved immune function with lower concentrations of prostaglandin E2 (PGE2) and immunoreactive fibronectin (IFN-γ). Addition of FLL increased apparent digestibility of diet’s dry matter and organic matter than the other groups. It was demonstrated that FLL supplementation improved nutrient digestion, feed efficiency, blood antioxidant function, immune and growth performance for Holstein dairy heifers.


This study evaluated the effects of dietary supplementation of a novel phytobiotics-rich herbal mixture (PRHM) on feed intake, performance, udder health, ruminal fermentation, and plasma metabolites in cows with moderate or high somatic cell counts (SCC) in the milk. Twenty-four Holstein dairy cows (117 ± 26 d in milk and 46.3 ± 4.7 kg of milk/d at the start of the experiment) were blocked by parity and days in milk and split into 2 groups, based on SCC in the milk: 12 cows were with moderate SCC (260,000 < SCC < 500,000 cells/mL), whereas 12 other cows had high levels of SCC (> 500,000 cells/mL) in the milk. Within each SCC group, cows were blocked by milk yield and parity, and were randomly assigned to 2 different feeding regimens. Half of the cows in each SCC group (n = 6) were supplemented with PRHM (185 g/cow per day, providing 12.4 g of phenolic compounds per day), and the other half (n = 6) were not supplemented in their diets. The experiment lasted 36 d, whereby the first 24 d were used for adaptation to the diets and the last 12 d for sampling. Data showed that supplementation of PRHM decreased somatic cell score in the milk, indicating improved udder health of cows with high initial SCC, but not in cows with moderate SCC. Also, cows supplemented with PRHM consumed more feed DM, produced greater amounts of milk, and showed an improvement of feed utilization efficiency. However, these cows also lost more back-fat thickness during the experiment. Supplementation of PRHM increased fat- and energy-corrected milk yields in cows with high initial SCC, but not in cows with moderate SCC. Supplementation of PRHM decreased milk fat content, whereas other milk components were not affected by PRHM feeding. The PRHM supplementation decreased the acetate-to-propionate ratio in the rumen fluid, but increased β-hydroxybutyrate and cholesterol concentration in the plasma, irrespective of the initial SCC level in the milk. Other plasma metabolites and liver enzymes were not affected by PRHM supplementation. Apparent nutrient digestibility did not differ among treatments. Overall, supplementation of PRHM seems to be an effective strategy to enhance performance and lower SCC, particularly in cows having high SCC levels in the milk. Further research is warranted to evaluate long-term effects of PRHM supplementation, especially with regard to metabolic health status and reproduction.


Although adequate colostrum intake and properly used antibiotics can provide much protection for the bovine neonate, increased antibiotic scrutiny and consumer demand for organic products have prompted investigations of natural immunomodulators for enhancing calf health. One plant-based immunomodulator, *Morinda citrifolia* (noni) fruit, is a well-recognized natural product that has a broad range of immunomodulatory effects. The hypothesis was that Neonatal calves fed noni puree would demonstrate whole blood phagocytic capacity in Gram-negative and Gram-positive in vitro assays. Blood samples were taken from 18 neonatal Holstein bull calves. Calves were divided into 2 groups: Group 1 comprised control calves, whereas Group 2 received 30 mL of noni puree twice a day in milk replacer. Day 0 blood samples were obtained between 36 and 48 hours of age before the first feeding of puree. Ethylenediaminetetraacetic acid anticoagulated blood was collected from each calf on days 0, 3, 7, and 14. Bactericidal assays were performed to estimate the percentage killing of *Escherichia coli* and *Staphylococcus epidermidis*. Blood samples from noni puree-fed calves displayed significantly more *E. coli* bacterial killing than did controls on day 14, and although
differences were not significant on days 0, 3, and 7, bacterial killing progressively increased over time. There was no significant difference between the groups for S. epidermidis killing. The immunomodulatory effect of noni puree may prove valuable in the future as production animal antibiotic use becomes more restricted. Additional clinical trials are warranted to investigate the clinical application of noni puree in promoting calf health.


IX-D Veterinary Botanical Medicine and Equids


Chewing lice are widespread and clinically compromising parasites of livestock and equids. Their management is complicated by growing levels of resistance to commonly applied insecticides. Hence, the development of novel approaches to their control is of major clinical interest. The objectives of the study were to assess the effects of incorporating the essential oils of tea tree and lavender into a grooming programme for populations of donkeys with natural infestations of Bovicola ocellatus in the UK and Ireland when louse populations were at their winter seasonal peak. The study design was an in vivo field trial. Suspensions of 5% (v/v) tea tree or lavender oil or an excipient only control were groomed into the coats of winter-housed donkeys (n = 198) on 2 occasions, 2 weeks apart. Louse counts were conducted before each application and 2 weeks later. After 2 applications, the groups groomed with lavender or tea tree oil suspensions had a significant reduction in louse intensity, with a mean decline in louse abundance of 78% (95% confidence interval 76-80%). Louse numbers in the groups groomed with excipient only either did not change or increased significantly. Donkey hair length had no effect on the decline in louse numbers. These results demonstrate that the inclusion of essential oil suspensions during grooming can be used to manage louse populations successfully.


Cyathostomins are the most important gastrointestinal nematode infecting equids. Their effective control is currently under threat due to widespread resistance to the broad spectrum anthelmintics licenced for use in equids. In response to similar resistance issues in other helminths, there has been increasing interest in alternative control strategies, such as bioactive plant compounds derived from traditional ethnoveterinary treatments. This study used an evidence-based approach to evaluate the potential use of plant extracts from the UK and Ethiopia to treat cyathostomins. Plants were shortlisted based on findings from a literature review and additionally, in Ethiopia, the results of a participatory rural appraisal (PRA) in the Oromia region of the country. Systematic selection criteria were applied to both groups to identify five Ethiopian and four UK plants for in vitro screening. These included Acacia nilotica (L.) Delile, Cucumis prophetarum L., Rumex abyssinicus Jacq., Vernonia amygdalina Delile, and Withania somnifera (L.) Dunal from Ethiopia and Allium sativum L. (garlic), Artemisia absinthium L., Chenopodium album L. and Zingiber officinale Roscoe. (ginger) from the UK. Plant material was collected, dried and milled prior to hydro-alcoholic extraction. Crude extracts were dissolved in distilled water (dH2O) and dimethyl sulfoxide (DMSO), serially diluted and screened for anthelmintic activity in the larval migration inhibition test (LMIT) and the egg hatch test (EHT). Repeated measures ANOVA was used to identify extracts that had a significant effect on larval migration and/or egg hatch, compared to non-treated controls. The median effective concentration (EC-50) for each extract was calculated using PROBIT analysis. Of the Ethiopian extracts A. nilotica, R. abyssinicus and C. prophetarum showed significant anthelmintic activity. Their lowest EC-50 values were 0.18 (confidence interval (CI): 0.1-0.3), 1.1 (CI 0.2-2.2) and 1.1 (CI 0.9-1.4) mg/ml, respectively. All four UK extracts, A. sativum, C. album, Z. officinale and A. absinthium, showed significant anthelmintic activity. Their lowest EC-50 values were 1.1 (CI 0.9-1.3), 2.3 (CI 1.9-2.7) and 0.3 (CI 0.2-0.4) mg/ml, respectively. Extract of A. absinthium had a relatively low efficacy and the data did not accurately fit a PROBIT model for the dose response relationship, thus an EC-50 value was not calculated. Differences in efficacy for each extract were noted, dependent on the assay and solvent used, highlighting the need for a systematic approach to the evaluation of bioactive plant compounds. This study has identified bioactive plant extracts from the UK and Ethiopia which have potential as anthelmintic forages or feed supplements in equids.

The pathogenesis of laminitis is not completely identified and the role of endotoxins (lipopolysaccharides, LPS) in this process remains unclear. Phytogenic substances, like milk thistle (MT) and silymarin, are known for their anti-inflammatory and antioxidant properties and might therefore have the potential to counteract endotoxin induced effects on the hoof lamellar tissue. The aim of our study was to investigate the influence of endotoxins on lamellar tissue integrity and to test if MT and silymarin are capable of inhibiting LPS-induced effects in an in vitro/ex vivo model. In preliminary tests, LPS neutralization efficiency of these phytotherapeutics was determined in an in vitro neutralization assay. Furthermore, tissue explants gained from hooves of slaughter horses were tested for lamellar separation after incubation with different concentrations of LPS. By combined incubation of explants with LPS and either Polymyxin B (PMB; positive control), MT or silymarin, the influence of these substances on LPS-induced effects was assessed. In the in vitro neutralization assay, MT and silymarin reduced LPS concentrations by 64% and 75%, respectively, in comparison PMB reduced 98% of the LPS concentration. In hoof explants, LPS led to a concentration dependent separation. Accordantly, separation force was significantly decreased by 10 µg/mL LPS. PMB, MT and silymarin could significantly improve tissue integrity of explants incubated with 10 µg/mL LPS. This study showed that LPS had a negative influence on the structure of hoof explants in vitro. MT and silymarin reduced endotoxin activity and inhibited LPS-induced effects on the lamellar tissue. Hence, MT and silymarin might be used to support the prevention of laminitis and should be further evaluated for this application.


A retrospective questionnaire-based survey was used to determine the perceived efficacy of Newmarket bloodroot ointment in treating equine sarcoids. In 49 horses with 74 sarcoids, 64 sarcoids responded either completely (n = 49) or partially (n = 15) while 10 did not respond or worsened. Sarcoids < 2 cm responded better to treatment (P < 0.001) than did larger sarcoids.


In order to counteract harmful effects of oxidative stress due to pathological conditions or physical exercise, horses are often administered dietary supplements having supposed high antioxidant activities. The aim of the present study was to identify the in vitro antioxidant potential of "ImmuPlus", a polyherbal formulation (Global Herbs LTD, Chichester, West Sussex, Great Britain), containing three medicinal plants (Withania somnifera, Tinospora cordifolia, and Emblica officinalis), known in Ayurveda for their use in human disease treatment. Extracts obtained by different solvents (water, methanol, ethanol, acetone, and hexane) were tested for total antioxidant capacity, total reducing power, scavenging activity against DPPH radical, and total polyphenol and flavonoid contents. Our results showed that, except as regards hexane, all the used solvents are able to extract compounds having high antioxidant activity, even when compared to ascorbic acid. Regression analysis showed significant correlations between antioxidant properties and polyphenol/flavonoid contents, indicating the latter, known for their beneficial effects on health of human and animal beings, as major components responsible for the strong antioxidant capacities. Moreover, obtained results suggest the effective role of the polyherbal mixture as good source of antioxidants in horses.


Infestations by lice can be a significant clinical and welfare issue in the management of large animals. The limited range of commercial pediculicides available and the development of resistance have led to the need to explore alternative louse management approaches. The results of in vitro and in vivo trials undertaken to control populations of the donkey chewing louse, Bovicola ocellatus (Piaget) (Phthiraptera: Trichodectidae)
using the essential oils of tea tree (Melaleuca alternifolia) and lavender (Lavandula angustifolia) are reported here. Results of contact and vapour bioassays showed that 5% (v/v) tea tree and lavender oils resulted in >80% louse mortality after 2 h of exposure. On farms, separate groups of 10 donkeys sprayed with 5% (v/v) tea tree and lavender oil as part of their usual grooming regime showed significant reductions in louse numbers compared with a control group (0.2% polysorbate 80 in water). These findings indicate that tea tree and lavender essential oils can provide clinically useful levels of control of B. ocellatus when used as part of a grooming routine and suggest that with further development could form the basis of an easy to apply and valuable component of a louse management programme for donkeys.

Talbot WA, Pinchbeck GL, Knottenbelt DC, Graham H, McKane SA. A randomised, blinded, crossover study to assess the efficacy of a feed supplement in alleviating the clinical signs of headshaking in 32 horses. Equine Vet J. 2013 May;45(3):293-7

Feed supplements are commonly used by owners to alleviate headshaking; however, randomised, controlled trials are required to assess their efficacy. The object of the study was to determine the efficacy of a feed supplement for alleviation of the clinical signs of headshaking using a randomised, blinded, placebo-controlled trial. Using a crossover design, 44 horses previously diagnosed with chronic idiopathic headshaking received both the supplement and a matching placebo per os for 28 days with a washout period between of 14 days. Video recordings were taken at rest and exercise prior to the study and at the end of both periods of treatment. The degree of headshaking was assessed in a blinded, randomised manner by 2 veterinary surgeons. At the same time points, owners completed a questionnaire to assess the severity of headshaking signs. A Wilcoxon signed rank test was used to compare the scores while on supplement and placebo. Using the video assessments, there was no significant difference between scores while on supplement compared with placebo (P = 0.7). Using the questionnaire responses, there was no significant difference between scores for any activity when the placebo and the supplement were compared with each other. However, owners reported significant improvement during all activities for both placebo and supplement compared with pretreatment scores. The supplement offered no benefit over a placebo in alleviating the clinical signs of headshaking. There appeared to be a significant proxy placebo effect when the outcome was based on subjective owner perception of clinical signs. This study demonstrated no beneficial effect of this supplement on the clinical signs of headshaking. The study did show a significant placebo effect, thereby highlighting the necessity of properly conducted, randomised controlled trials, with blinding, to assess true treatment effects in trials in animals.


To evaluate antioxidant capacity and inflammatory cytokine gene expression in horses fed silibinin complexed with phospholipid. 5 healthy horses were orally administered increasing doses of silibinin phospholipid during 4 nonconsecutive weeks (0 mg/kg, 6.5 mg/kg, 13 mg/kg, and 26 mg/kg of body weight, twice daily for 7 days each week). Dose-related changes in plasma antioxidant capacity, peripheral blood cell glutathione concentration and antioxidant enzyme activities, and blood cytokine gene expression were evaluated. Plasma antioxidant capacity increased throughout the study period with increasing dose. Red blood cell nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase I activity decreased significantly with increasing doses of silibinin phospholipid. No significant differences were identified in glutathione peroxidase activity, reduced glutathione or oxidized glutathione concentrations, or expression of tumor necrosis factor α, interleukin-1, or interleukin-2. Minor alterations in antioxidant capacity of healthy horses that consumed silibinin phospholipid occurred and suggest that further study in horses with liver disease is indicated.


Anthelmintic resistance in gastrointestinal parasites of horses is an increasing problem, particularly in cyathostomins, and there is a need to find alternative means for the control of these parasites. We screened crude extracts from 37 species of Australian native plants for their anthelmintic activity in vitro against cyathostomin larvae (development from egg to third larval stage), with the aim of identifying those species that may be suitable for incorporation into sustainable parasite management programs. Water extracts from
seven species, namely Acacia baileyana, Acacia melanoxylon, Acacia podalyriifolia, Alectryon oleifolius, Duboisia hopwoodii, Eucalyptus gomphocephala and Santalum spicatum completely inhibited larval development (100% inhibition compared to the control), while another 10 species caused 90% inhibition at the initial screening concentration of 1400 μg of extractable solids/mL. The seven most potent extracts produced IC50 values (concentration of extract which resulted in a 50% inhibition of development) in the range 30.9-196 μg/mL. Fourteen extracts were incubated with polyvinylpolypyrrolidone (PVPP) before the assays, which removed the anthelmintic activity from 12 of these extracts, indicating that tannins were likely to be the bioactive compound responsible for the effect, while in two species, i.e. A. melanoxylon and D. hopwoodii, compounds other than tannins were likely to be responsible for their anthelmintic action. Our results suggest that a number of Australian native plants have significant anthelmintic activity against cyathostomin larval development in vitro. There is potential for these plants to be used as part of sustainable parasite control programs in horses, although more research is needed to identify the compounds responsible for the anthelmintic effects and confirm their activity in vivo.


A biological extract of high-rosmarinic acid mint (HRAM) has previously demonstrated inhibitory effects on lipopolysaccharide (LPS)-induced prostaglandin E(2) (PGE(2)), nitric oxide (NO) and glycosaminoglycan (GAG) release in vitro. This study was undertaken to determine whether HRAM added to feed produces similar effects in horses challenged with intra-articular LPS. Eight horses received HRAM (0 or 28.1 ± 1.3 g/day; n = 4 per group) in their feed for 24 days in a blinded manner. On day 21, all horses received an intra-articular injection of LPS (0.3 ng) into their left or right intercarpal joint. Synovial fluid (SF) samples were taken on postinjection day (PID)-21 (i.e. prior to commencement of supplementation), PID0, PID0.25, PID0.5, PID1 and PID3 and analysed for PGE(2), GAG, NO, protein and total nucleated cells counts. Blood biochemistry and haematology screens were conducted at PID-21, PID0, PID1 and PID3. There was a significant reduction in LPS-induced PGE(2) and GAG in SF in horses supplemented with HRAM compared with controls and a tendency to increase complement recognition protein accumulation in synovial fluid of HRAM horses. Plasma from HRAM horses had reduced total white blood cells, segmented neutrophils (compared with baseline concentrations) and lymphocytes (compared with controls), and increased SF nucleated cell count (compared with baseline concentrations and controls). It is concluded that HRAM offered as part of the feed alter biomarkers of inflammation in SF of LPS-challenged horses. Larger studies that seek to clarify effects of HRAM on synovial fluid cell counts and possible role of HRAM-induced interference with complement signalling are warranted.


To determine the oral bioavailability, single and multidose pharmacokinetics, and safety of silibinin, a milk thistle derivative, in healthy horses, 9 healthy horses were initially administered silibinin IV and silibinin phospholipid orally in feed and via nasogastric tube. Five horses then consumed increasing orally administered doses of silibinin phospholipid during 4 nonconsecutive weeks (0 mg/kg, 6.5 mg/kg, 13 mg/kg, and 26 mg/kg of body weight, twice daily for 7 days each week). Bioavailability of orally administered silibinin phospholipid was 0.6% PO in feed and 2.9% via nasogastric tube. During the multidose phase, silibinin had nonlinear pharmacokinetics. Despite this, silibinin did not accumulate when given twice daily for 7 days at the evaluated doses. Dose-limiting toxicosis was not observed. Silibinin phospholipid was safe, although poorly bio-available, in horses. Further study is indicated in horses with hepatic disease.


Acupuncture exerts diffuse analgesic effects through the release of endogenous opioids and other locally and centrally acting mediators. Successful therapeutic interventions for various musculoskeletal conditions in horses are well documented, and acupuncture may significantly enhance performance. The use of acupuncture is specifically supported in treating nonsurgical gastrointestinal disorders, in which specific techniques can alter motility and contribute to visceral analgesia. This article describes the use of acupuncture...
and Chinese herbal medicine for equine reproductive management and for treating respiratory disease. A careful review of available data and ongoing efforts to enhance unbiased research should continue to guide practitioners of evidence-based medicine in refining the most useful applications of acupuncture and Chinese herbal medicine.


Insulin resistance and hyperinsulinaemia increase the risk of laminitis and horse owners and veterinarians should attempt to enhance insulin sensitivity in at-risk groups. In obese animals this may be achieved, in part, by promoting weight loss and increasing exercise, but such intervention may not be appropriate in non-obese insulin-resistant animals, or where exercise is contra-indicated for clinical reasons. An alternative approach to controlling insulin sensitivity in obese and non-obese horses may be the use of certain herbal compounds that have shown promise in humans and laboratory animals, although little is known of the effects of these compounds in horses. The herbs can be grouped according to their primary mechanism of action, including activators of the peroxisome proliferator-activated receptors, anti-obesity compounds, anti-oxidants, compounds that slow carbohydrate absorption, insulin receptor activators and stimulators of glucose uptake, with some herbs active in more than one pathway. Certain herbs have been prioritised for this review according to the quality and quantity of published studies, the reported (or extrapolated) safety profile, as well as potential for efficacy, all of which will hopefully motivate further research in this field.


Various members of the mint family have been used historically in Chinese and Native American medicine. Many of these same family members, including Prunella vulgaris, have been reported to have anti-viral activities. To further characterize the anti-lentiviral activities of P. vulgaris, water and ethanol extractions were tested for their ability to inhibit equine infectious anaemia virus (EIAV) replication. Aqueous extracts contained more anti-viral activity than did ethanol extracts, displaying potent anti-lentiviral activity against virus in cell lines as well as in primary cell cultures with little to no cellular cytotoxicity. Time-of-addition studies demonstrated that the extracts were effective when added during the first four h of the viral life cycle, suggesting that the botanical constituents were targeting the virion itself or early entry events. Further analysis revealed that the extracts did not destroy EIAV virion integrity, but prevented viral particles from binding to the surface of permissive cells. Modest levels of anti-EIAV activity were also detected when the cells were treated with the extracts prior to infection, indicating that anti-EIAV botanical constituents could interact with both viral particles and permissive cells to interfere with infectivity. Size fractionation of the extract demonstrated that eight of the nine fractions generated from aqueous extracts displayed anti-viral activity. Separation of ethanol soluble and insoluble compounds in the eight active fractions revealed that ethanol-soluble constituents were responsible for the anti-viral activity in one fraction whereas ethanol-insoluble constituents were important for the anti-viral activity in two of the other fractions. In three of the five fractions that lost activity upon sub-fractionation, anti-viral activity was restored upon reconstitution of the fractions, indicating that synergistic anti-viral activity is present in several of the fractions. Our findings indicate that multiple Prunella constituents have profound anti-viral activity against EIAV, providing additional evidence of the broad anti-viral abilities of these extracts. The ability of the aqueous extracts to prevent entry of viral particles into permissive cells suggests that these extracts may function as promising microbicides against lentiviruses.


Equine sarcomas (ES) are common, difficult to treat, and have high recurrence rates. Viscum album extracts (VAE) are used in human cancer treatment. The hypothesis is that therapy with VAE (Iscador P) is effective in the treatment of ES. Fifty-three horses (444 ES); 42 were treated with VAE or placebo as monotherapy; 11 were treated with VAE or placebo after selective excision of ES. A prospective, randomised, blinded, clinical trial was carried out. Horses were randomly assigned to treatment (VAE; n=32) or control group (Placebo; n=21). One milliliter of VAE (Iscador P) in increasing concentrations from 0.1 to 20 mg/mL or physiological
NaCl solution was given SC 3 times a week over 105 days. Number, localization, and type of the ES were documented over 12 months. A subset of 163 clinically diagnosed equine sarcoid (CDES) lesions (95 VAE, 68 Placebo) was evaluated in detail, considering clinical findings and tumor volume. No undesired adverse effects were observed except for mild edema at the injection site in 5 of 32 horses (16%). Complete or partial regression was observed in 13 horses of the VAE group (41%) and in 3 of the control horses (14%; P<.05). After VAE treatment, 48 of 95 CDES (67%) showed an improvement compared with 17 of 68 CDES in the control group (40%; P<.01). Twenty-seven CDES had disappeared completely in the VAE group (38%) compared with 9 CDES in the control group (13% NS). VAE (Iscador P) represents a safe and effective treatment for CDES.


Achyrocline satureioides (Asteraceae) is a medicinal plant traditionally used in Argentina for the treatment of intestinal infections and various digestive disorders. Its infusion is widely utilised for respiratory problems and viral infections. The objective of this study was to investigate cytotoxicity, virucidal and antiviral properties of the cold aqueous extract (CAE) and hot aqueous extract (HAE) of this plant against Western equine encephalitis virus (WEEV). Cytotoxicity in Vero cells was evaluated by maximum non-cytotoxic concentration (MNCC), neutral red (NR) uptake and MTT reduction methods. To study the antiviral activity of aqueous extracts, plaque reduction assay was performed after pre-treatment of host cells, adsorption, penetration and post-penetration of the virus. Extracellular virus inactivation was also analysed by the same method. Extracts showed strong inhibitory activity after virus penetration with selective index values of 32 (NR) and 63.3 (MTT) for the CAE, and 16.2 (NR) and 24.3 (MTT) for the HAE. Both extracts exhibited virucidal action with lower efficacy than their antiviral properties. The present results demonstrate that aqueous extracts of A. satureioides are active against WEEV. Further studies are needed in order to identify which compounds could be responsible for this effect, and how they exert antiviral action.


Dermatophytes are a group of keratinophilic and keratinolytic molds, some of which are responsible for ringworm. Among them Trichophyton equinum, which mostly infects equids, can cause extensive outbreaks in stud farms. The conventional treatment of equine trichophytosis is topical application of medicated shampoos to reduce the spread of infection among the animals. Nevertheless the popularity of phytotherapy is at an all-time peak, and the interest for natural alternatives or complements to conventional drug therapy is challenging both in human and veterinary field. Among herbal remedia Tea Tree Oil (TTO) shows a wide range of antimicrobial activities. A randomized open clinical trial was carried out on 60 thoroughbred breeding horses affected by equine ringworm. The animals were randomly divided into 2 groups of 30 subjects. Diagnostic criteria were the presence of clinical signs and positive T. equinum culture. Specificity control using TTO mixture in 5 not dermatophyte affected animals was achieved also. The antymycotic activity against T. equinum of a mixture containing 25% TTO in sweet almond oil, was evaluated in vivo treating 30 subjects, the others were administered eniliconazole 2% solution. The animals of both groups were topically treated twice a day for 15 days with a 25% mixture of TTO diluted in sweet almond oil and every 3 days, four times with eniliconazole rinses, respectively. The clinical and mycological outcome were evaluated at day 30 from the start of the treatments. Data analysis was performed by chi square test. All the treated animals showed complete clinical and aetiological healing. Part of control subjects also, showed an improvement and none of them exacerbate the lesions. This therapeutic protocol appears to be effective and versatile, being applicable immediately after physical examination, prior to have the laboratory response. It could be an alternative for practitioners interested in herbal medicines, contributing to fulfill the gap existing between in vitro and clinical studies.

Standardbred trainers from 1 racetrack and 7 off-track training facilities were surveyed to determine the most common drugs, and prevalence of concurrent herb administration. Furosemide (on-track) and anti-inflammatory drugs (off-track) were the most common drugs administered. Among horses on-track, 9.8% received herbs compared with 13.8% off-track horses; 67% and 58% of these horses, respectively, received concurrent drugs.


Most herbs and functional foods have not been scientifically tested; this is especially true for the horse. This paper reviews some of the literature pertinent to herbal supplementation in horses and other species. Common supplements like Echinacea, garlic, ginseng, and yucca are not regulated, and few studies have investigated safe, efficacious doses. Ginseng has been found to exert an inhibitory effect on pro-inflammatory cytokines and cyclooxygenase-2 expression. Equine studies have tested the anti-inflammatory effects of a single dose of ginger, post-exercise. Echinacea has been reported to have anti-inflammatory and antioxidant properties. Yucca contains steroid-like saponins, which produce anti-inflammatory, antioxidant, and anti-spasmodic effects. However, some herbs have drug-like actions that interact with dietary components and may contain prohibited substances like salicylates, digitalis, heroin, cocaine and marijuana. Horses fed garlic at >0.2g/kg per day developed Heinz body anaemia. Drug-herb interactions are common and caution needs to be taken when implementing ‘natural product’ usage.


LC/ESI-MS n methods have been previously set up to detect the administration of (i) Harpagophytum and (ii) preparations containing a plant capable of anti-stress properties: Eleutherococcus senticosus. Harpagoside has been found to be the main indicator of Harpagophytum administration in the horse. These methods have been applied to a large number of horse urine samples of various origins. Regarding the detection of Harpagophytum administration, harpagoside, harpagide and 8-para-coumaroyl harpagide were detected together in only one sample out of 317. Eleutheroside E was found to be the main indicator of Eleutherococcus senticosus administration. It was detected in post-administration samples collected from two horses having received a feed supplemented containing Eleutherococcus senticosus for several days. Out of the 382 samples tested, eleutheroside E was found in an unexpected large number of urine samples (39%) of various origins and its presence cannot be only due to the sole use of herbal dietary supplements.


Herbs are an increasingly popular treatment option for horses with cartilage inflammation, despite a relative paucity of research demonstrating efficacy. The research objective was to evaluate the differential anti-inflammatory and chondroprotective efficacy of a simulated digest of indomethacin and a commercially available herbal product in a cartilage model of osteoarthritis. Cartilage explant was integrated with simulated digestion of indomethacin and the herbal product in order to account, at least in part, for the actions of major digestive enzymes and pH. The resulting digests were ultrafiltrated (50 kDa), to account for absorption from the GI tract and movement into the cartilage matrix. We hypothesized that (i) a simulated digest of indomethacin would block interleukin 1 beta-(IL-1) dependent formation of prostaglandin E2 (PGE2) and nitric oxide (NO) without protecting cartilage against IL-1-induced glycosaminoglycan (GAG) release, and (ii) the herbal product would reduce PGE2 and NO in IL-1-stimulated explants, and inhibit release of GAG, in IL-1-stimulated explants. Results showed that indomethacin is an effective anti-inflammatory, evidenced by strong...
inhibition of IL-1-induced PGE2 and NO from cartilage explants. However, indomethacin provided no protection against IL-1-induced GAG release. Simulated digest of the herbal extract significantly inhibited IL-1-induced NO production and GAG release, while having a slight increase in PGE2. These data provide evidence for the anti-inflammatory effect of indomethacin on IL-1-stimulated cartilage explants, and the herbal product Mobility may be a useful adjunct in arthritis because of its chondroprotective properties in IL-1-stimulated cartilage.


Recurrent airway obstruction (RAO), known previously as chronic obstructive pulmonary disease (COPD), is a debilitating respiratory condition that significantly contributes to lost training days and illness in racehorses. Herbs are becoming increasingly popular for the prophylaxis or treatment of the clinical signs of RAO despite a paucity of research on efficacy and safety. We evaluated the ability of an herbal composite containing garlic, white horehound, boneset, aniseed, fennel, licorice, thyme, and hyssop to reduce the clinical signs of RAO, hypothesizing that the product would safely reduce signs and would improve the inflammatory cell profile within the lungs. The composite was fed to 6 horses with symptomatic RAO for 21 d in a crossover manner. Ventigraphs were used to record respiratory rate and intrapleural pressure; the proportion of inflammatory cells in fluid aspirated from the trachea was determined. Blood biochemical and hematologic screening was conducted to identify possible adverse effects. Treatment with the composite did not result in statistically significant changes in any of the parameters evaluated. A trend to a decrease in respiratory rate (P = 0.1) and an increase in the proportion of macrophages (P = 0.1) was observed in the horses receiving the herbal composite compared with placebo. These data indicate a potential for the herbal composite to safely reduce the elevated respiratory rate in horses with RAO. Future research with a greater number of horses is warranted to further characterize the effect of this product on horses with RAO.


IX-E Veterinary Botanical Medicine and Sheep and Goats


Rumen microbiome has a great influence on ruminant health and productivity. Different plant extracts have been tested for their ability to modulate the rumen microbiome to improve feed digestion and fermentation. Among the evaluated plant extracts, essential oils, tannins, and saponins appeared to have positive effects on rumen protein metabolism, volatile fatty acids production, and methane and ammonia production. The objective of this study was to evaluate the effect of rosemary (Rosmarinus officinalis L.) leaves and essential oils on rumen microbial populations. Four ruminally cannulated sheep were used in a 4×4 Latin square design fed (21 d/period): 1) a control diet composed of alfalfa hay and concentrate pellet (CTR), 2) CTR supplemented with 7 g/d/sheep of rosemary essential oil adsorbed on an inert support (EO), 3) CTR with 10 g/d/sheep of dried and ground rosemary leaves (RL), and 4) CTR with 10 g/d of dried and ground rosemary leaves pelleted into concentrate (RL pellet). Abundance of total bacteria, archaea, protozoa, and some select bacterial species or groups was quantified using qPCR, while the community of bacteria and archaea was profiled using denaturing gradient gel electrophoresis. No difference in abundance was noted for total bacteria, protozoa, or Ruminococcus flavefaciens between the control and the treatments, but the rosemary leaves, either in loose form or in pellet, decreased the abundance of archaea and the genus Prevotella (P < 0.001). The rosemary leaves in loose form also decreased (P < 0.001) the abundance of Ruminococcus albus and Clostridium aminophilum, while the EO increased (P < 0.001) the abundance of Fibrobacter succinogenes. The community of bacteria and archaea was not affected by any of the supplements. Being able to affect the abundance of several groups of rumen microbes that are known to be involved in degradation of protein and fiber and production of methane and ammonia, rosemary leaves may be used to modulate rumen microbiome and its function.


The aim of this study was to evaluate the effect of rosemary essential oils (REO) and the forage nature on ewes’ performances, immune response and lambs’ growth and mortality. Forty-eight dairy ewes (Sicilo-Sarde) were fed oat-hay or oat-silage supplemented with 400 g of concentrate during pregnancy and 600 g during postpartum. The experimental concentrate contained the same mixture as the control (barley, soybean meal and mineral vitamin supplement) more 0.6 g/kg of REO. Two groups were obtained with each forage (Hay groups: H-C and H-REO; Silage groups: S-C and S-REO). REO increased the dry matter (DM) intake, the nitrogen intake and retention being higher with the silage groups (P < 0.05). REO increased solid non-fat (P = 0.004) and fat contents of colostrum which was higher with hay (P = 0.002). REO decreased lamb mortality (P < 0.05) which averaged 21% for control groups and 6% for H-REO, while no mortality was recorded with S-REO. REO dietary supply improved forage intake and tended to ameliorate colostrum production; it could be a natural additive to improve ewes’ performances.


A number of herbal products with anti-inflammatory, antiseptic and antimycotic properties are available for dermatological usage. The successful treatment of 13 sheep affected by ringworm due to Trichophyton mentagrophytes with a mixture consisting of essential oils (EOs) of Thymus serpillum 2%, Origanum vulgare 5% and Rosmarinus officinalis 5% in sweet almond (Prunus dulcis) oil. The effectiveness of EOs and of the major
components of the mixture (thymol, carvacrol, 1,8 cineole, α-pinene, p-cymene, γ-terpinene) against the fungal clinical isolate was evaluated by a microdilution test. Thirteen animals were topically administered with the mixture twice daily for 15 days. The other sheep were administered with a conventional treatment (seven animals) or left untreated (two animals). Minimum inhibitory concentration (MIC) values were 0.1% for T. serpillum, 0.5% for O. vulgare, 2.5% for I. verum and 5% for both R. officinalis and C. limon. Thymol and carvacrol showed MICs of 0.125% and 0.0625%. A clinical and aetiological cure was obtained at the end of each treatment regimen in only the treated animals. Specific antimycotic drugs licenced for food-producing sheep are not available within the European Community. The mixture tested here appeared to be a versatile tool for limiting fungal growth.


The in vivo pediculicidal effectiveness of 1% and 2% formulations of tea tree (Melaleuca alternifolia) oil (TTO) against sheep chewing lice (Bovicola ovis) was tested in two pen studies. Immersion dipping of sheep shorn two weeks before treatment in both 1% and 2% formulations reduced lice to non detectable levels. No lice were found on any of the treated sheep despite careful inspection of at least 40 fleece partings per animal at 2, 6, 12 and 20 weeks after treatment. In the untreated sheep louse numbers increased from a mean (± SE) of 2.4 (± 0.7) per 10 cm fleece part at 2 weeks to 12.3 (± 4.2) per part at 20 weeks. Treatment of sheep with 6 months wool by jetting (high pressure spraying into the fleece) reduced louse numbers by 94% in comparison to controls at two weeks after treatment with both 1% and 2% TTO formulations. At 6 and 12 weeks after treatment reductions were 94% and 91% respectively with the 1% formulation and 78% and 84% respectively with the 2% formulation. TTO treatment also appeared to reduce wool damage in infested sheep. Laboratory studies indicated that tea tree oil 'stripped' from solution with a progressive reduction in concentration as well as volume as more wool was dipped, indicating that reinforcement of active ingredient would be required to maintain effectiveness when large numbers of sheep are treated. The results of these studies suggest significant potential for the development of ovine lousicides incorporating TTO.

Hawken PA, Fiol C, Blache D. Genetic differences in temperament determine whether lavender oil alleviates or exacerbates anxiety in sheep. Physiol Behav. 2012 Mar 20;105(5):1117-23

Growing concerns about the risk of addiction to benzodiazepines have led to increasing interest in alternative therapies to treat anxiety and depression. Lavender oil (Lavandula augustifolia) is reportedly anxiolytic in a number of species but little is known about how it affects individuals that are more or less anxious when faced with a stressor. In this study, we used changes in locomotor activity and the plasma concentrations of cortisol to test whether lavender oil would reduce behavioral and endocrine correlates of anxiety in calm and nervous sheep exposed to an isolation stressor. During the non-breeding season, 'calm' or 'nervous' female sheep from the UWA temperament flock were exposed to a mask containing either 1 mL of 10% lavender oil (calm: n=8; nervous: n=8) or peanut oil (calm: n=8; nervous: n=8). After 30 min, each sheep was isolated for 5 min and then returned to the group. Blood was sampled prior to the mask, prior to isolation, 1 min and 30 min after isolation to profile changes in the plasma concentrations of cortisol. Agitation score, locomotor activity and vocalizations were recorded as correlates of anxiety associated with the isolation stressor. Irrespective of whether they were exposed to lavender oil, calm sheep had a lower agitation score (P<0.001), crossed the central lines of the isolation box less frequently (P<0.001), expressed fewer vocalizations (P<0.001) and had lower plasma concentrations of cortisol immediately after isolation (P<0.001) than nervous sheep. Exposure of calm sheep to lavender oil decreased the agitation score (P<0.001), frequency of vocalizations (P<0.05), decreased the number of crosses of the central lines of the isolation box (P<0.05), and the plasma concentrations of cortisol prior to isolation (P<0.05) (after mask application) compared to calm control sheep. Exposure of nervous sheep to lavender oil increased the frequency of vocalizations (P<0.05), the number of sheep attempting to escape (P<0.05) and the plasma concentrations of cortisol 30 min after isolation (P<0.05)
compared to nervous control sheep. We conclude that genetic differences in temperament determine whether lavender oil alleviates or exacerbates the behavioral and/or endocrine correlates of anxiety in sheep.


The effect of the addition of an essential oil (EO) preparation (containing a mixture of natural and nature-identical EO) on the performance of dairy ewes of the Chios breed was investigated. Eighty lactating ewes were allocated into 4 equal groups in a randomized block design, each with 4 replicates of 5 ewes housed in the same pen. The 4 groups were fed the same total mixed ration allowance, the roughage being a mixture of corn silage, lucerne hay, and wheat straw, and the concentrate based on cereals and oil cakes. Control ewes were fed their daily allowance of total mixed ration without any EO. The other 3 groups were supplemented with EO at levels of 50, 100, and 150 mg/kg of the concentrated feed, respectively. Individual milk yield was recorded daily and feed refusals were recorded on a pen basis weekly during the first 5 mo of lactation. Milk samples were analyzed for chemical composition, somatic cell count, and urea content. Rumen samples were analyzed for pH, NH(3)-N content, and protozoa, cellulolytic, hyper-ammonia-producing, and total viable bacteria counts. Results showed that inclusion of EO increased milk production per ewe, the effect being dose dependent [1.565, 1.681, 1.876, and 2.119 L/d (standard error of the difference ± 0.176) for the control, 50, 100, and 150 mg of EO/kg of concentrate diets, respectively], and thus improved feed utilization. Although the inclusion of EO did not affect milk composition, it lowered urea concentration and somatic cell count in milk samples at the highest supplementation level compared with the control. Total counts of viable and cellulolytic bacteria and protozoa were not influenced by EO supplementation; however, counts of hyper-ammonia-producing bacteria were decreased at the 2 highest supplementation levels compared with the control group. Rumen pH was not affected by EO supplementation, but rumen NH(3)-N was reduced at the highest EO supplementation level, and acetate rumen concentrations tended to decrease and propionate to increase in a dose-dependent manner. In conclusion, EO supplementation may improve feed utilization and performance of the high-yielding dairy Chios ewes; however, the underlying mechanisms leading to this improvement merit further investigation.

Callander JT, James PJ. Insecticidal and repellent effects of tea tree (Melaleuca alternifolia) oil against Lucilia cuprina. Vet Parasitol. 2012 Mar 23;184(2-4):271-8

Laboratory studies were conducted to assess the effect of tea tree oil (TTO) from Melaleuca alternifolia (terpinen-4-ol chemotype) against different stages of the Australian sheep blowfly Lucilia cuprina. When applied to wool, 3% TTO formulation repelled gravid female L. cuprina and prevented oviposition for six weeks. Formulations containing 1% TTO caused 100% mortality of L. cuprina eggs and 1st instar larvae and 2.5% TTO caused mortality of most second and third instar larvae in agar feeding assays. In experiments where third instar larvae were dipped in TTO formulations for 60s, concentrations of up to 50% TTO gave less than 50% kill. TTO at concentrations of 0.5%, 2% and 5% was strongly repellent to third instar larvae and caused them to evacuate treated areas. Inclusion of TTO in formulations with diazinon, ivermectin and boric acid reduced mortality in comparison with the larvicides used alone, at least partially because of avoidance behaviour stimulated by the TTO. Addition of TTO to wound treatments may aid in wound protection and myiasis resolution by preventing oviposition by L. cuprina adults, insecticidal action against L. cuprina eggs and larvae, stimulating larvae to leave the wound and through antimicrobial and anti-inflammatory properties that aid in wound healing.

This study was aimed to evaluate the efficacy of crude aqueous-methanol and aqueous extracts of neem (Azadirachta indica) seed kernel against sarcoptic mange of sheep. Crude aqueous-methanol (AME) and aqueous extracts (AE) of neem seed kernel (NSK) were prepared and formulated as 10% and 20% ointments (w/w), using Vaseline as vehicle. Forty-two lambs of Pak Karakul breed, having natural infection of sarcoptic mange were divided into seven experimental groups. Skin scrapings and clinical examination were carried out at scheduled intervals after treatment. Ivermectin (positive control) completely cleared infesting mites from animals after 10 days and 20% AME after 16 days. While, clinical mange was completely cured after 16 and 20 days with ivermectin and 20% AME, respectively, under field conditions. Only the higher concentration (20% AME) of NSK extracts completely cured the clinical mange, suggesting a dose-dependent response. Our results consolidate the belief that use of folk remedies can provide an effective and economic way of combating sarcoptic mange in sheep.


IX-E Veterinary Botanical Medicine and Poultry


The traditional Chinese medicinal plant Brucea javanica has received much attention for its significant antiprotozoal effects in recent years; however, little is known about its potential anticoccidial functions. In the present study, a series of experiments was conducted to investigate the prophylactic and therapeutic effects of ethanol extract from B. javanica on coccidiosis induced by Eimeria tenella in broiler chickens. Chickens infected with E. tenella were treated with B. javanica extract and compared either with broilers treated with the anticoccidial halofuginone hydrobromide (Stenorol) or with control groups that consisted of infected-unmedicated and uninfected-unmedicated broilers. The experiments revealed that the B. javanica extract could significantly (P<0.05) reduce bloody diarrhea and lesion scores. Additional, OPG output in these plant extract treated groups was reduced in comparison with non-treated groups (P<0.05). However, there was no evidence to show that the extract could promote BWG. Histological data showed that the number of second-generation schizonts in the medicated groups was substantially less than that in the infected-unmedicated control. In summary, our work showed that B. javanica extract exerted considerable anticoccidial effects, supporting its use as a promising therapeutic in controlling avian coccidiosis.


The development of antibiotic resistant pathogens has resulted from the use of sub-therapeutic concentrations of antibiotics delivered in poultry feed. Furthermore, there are a number of consumer concerns regarding the use of antibiotics in food animals including residue contamination of poultry products and antibiotic resistant bacterial pathogens. These issues have resulted in recommendations to reduce the use of antibiotics as growth promoters in livestock in the United States. Unlike conventional production, organic systems are not permitted to use antibiotics. Thus, both conventional and organic poultry production need alternative methods to improve growth and performance of poultry. Herbs, spices, and various other plant extracts are being evaluated as alternatives to antibiotics and some do have growth promoting effects, antimicrobial properties, and other health-related benefits. This review aims to provide an overview of herbs, spices, and plant extracts, currently defined as phytobiotics as potential feed additives.


Comfrey (Symphytum officinale), a commonly used herb, contains dehydropyrrolizidine alkaloids that, as a group of bioactive metabolites, are potentially hepatotoxic, pneumotoxic, genotoxic and carcinogenic. Consequently, regulatory agencies and international health organizations have recommended comfrey be used for external use only. However, in many locations comfrey continues to be ingested as a tisane or as a leafy vegetable. The objective of this work was to compare the toxicity of a crude, reduced comfrey alkaloid extract to purified lycopsamine and intermedine that are major constituents of S. officinale. Male, California White chicks were orally exposed to daily doses of 0.04, 0.13, 0.26, 0.52 and 1.04 mmol lycopsamine, intermedine or reduced comfrey extract per kg bodyweight (BW) for 10 days. After another 7 days chicks were euthanized. Based on clinical signs of poisoning, serum biochemistry, and histopathological analysis the reduced comfrey extract was more toxic than lycopsamine and intermedine. This work suggests a greater than additive effect of the individual alkaloids and/or a more potent toxicity of the acetylated derivatives in the reduced comfrey extract. It also suggests that safety recommendations based on purified compounds may underestimate the potential toxicity of comfrey.
Aromatic herbs as feed additives in animal production are encountering growing interest, but data on the fate of the aromatic compounds from the plant in the animal body are very scarce. In the present study, thyme (Thymus vulgaris) herb consisting of leaves and flowers without stems was used as an ingredient in the diet for broilers. The herb was fed for 35 days to five groups of broilers (0, 0.1, 0.2, 0.3, and 1% w/w in the diet). Animal performance and the concentrations of the main essential oil component from thyme, thymol, were measured in gut contents, plasma and liver and muscle tissues using solid phase microextraction and gas chromatography/mass spectrometry. There were no differences between the groups in feed intake, daily weight gain, feed conversion and slaughter weight. Thymol was detected in gut contents, plasma and liver and muscle tissues. Increased intestinal thymol concentrations were found in the group with 1% thyme compared with the other groups (P < 0.05). In liver and muscle tissues the thymol levels were close to the limit of quantification. The data do not indicate a positive effect of thyme on animal performance. With high dietary levels of thyme herb, thymol concentrations increased in gut contents and plasma but were very low in edible tissues such as liver and flesh. © 2014 Society of Chemical Industry.

Eimeriosis, a widespread infectious disease of livestock, is caused by coccidian protozoans of the genus Eimeria. These obligate intracellular parasites strike the digestive tract of their hosts and give rise to enormous economic losses, particularly in poultry, ruminants including cattle, and rabbit farming. Vaccination, though a rational prophylactic measure, has not yet been as successful as initially thought. Numerous broad-spectrum anti-coccidial drugs are currently in use for treatment and prophylactic control of eimeriosis. However, increasing concerns about parasite resistance, consumer health, and environmental safety of the commercial drugs warrant efforts to search for novel agents with anti-Eimeria activity. This review summarizes current approaches to prevent and treat eimeriosis such as vaccination and commercial drugs, as well as recent attempts to use dietary antioxidants as novel anti-Eimeria agents. In particular, the trace elements selenium and zinc, the vitamins A and E, and natural products extracted from garlic, barberry, pomegranate, sweet wormwood, and other plants are discussed. Several of these novel anti-Eimeria agents exhibit a protective role against oxidative stress that occurs not only in the intestine of Eimeria-infected animals, but also in their non-parasitized tissues, in particular, in the first-pass organ liver. Currently, it appears to be promising to identify safe combinations of low-cost natural products with high anti-Eimeria efficacy for a potential use as feed supplementation in animal farming.

Intensive poultry production systems depend on chemoprophylaxis with anticoccidial drugs to combat infection. A floor-pen study was conducted to evaluate the anticoccidial effect of Artemisia annua and Foeniculum vulgare on Eimeria tenella infection. Five experimental groups were established: negative control (untreated, unchallenged); positive control (untreated, challenged); a group medicated with 125 ppm lasalocid and challenged; a group medicated with A. annua leaf powder at 1.5% in feed and challenged; and a group treated with the mixed oils of A. annua and Foeniculum vulgare in equal parts, 7.5% in water and challenged. The effects of A. annua and oil extract of A. annua + F. vulgare on E. tenella infection were assessed by clinical signs, mortality, fecal oocyst output, faeces, lesion score, weight gain, and feed conversion. Clinical signs were noticed only in three chickens from the lasalocid group, six from the A. annua group, and nine from the A. annua + F. vulgare group, but were present in 19 infected chickens from the positive control group. Bloody diarrhea was registered in only two chickens from A. annua group, but in 17 chickens from the positive control group. Mortality also occurred in the positive control group (7/20). Chickens treated with A. annua had a significant reduction in faecal oocysts (95.6%; P = 0.027) and in lesion score (56.3%; P = 0.005) when compared to the positive control. At the end of experiment, chickens treated with A. annua leaf powder had the highest...
body weight gain (68.2 g/day), after the negative control group, and the best feed conversion (1.85) among all experimental groups. Our results suggest that A. annua leaf powder (Aa-p), at 1.5% of the daily diet post-infection, can be a valuable alternative for synthetic coccidiostats, such as lasalocid.


Forsythia suspensa extract (FSE) has been demonstrated to attenuate physiological stress induced by high temperature or high stocking density. This experiment was conducted with 144 male Arbor Acre broilers (1-d-old, weighing 42.7 ± 1.7 g) to determine the effects of FSE on performance, nutrient digestibility, antioxidant activities, serum metabolites, and immune parameters for birds treated with corticosterone (CS). The birds were randomly allotted to 1 of 4 treatments in a 2 × 2 factorial arrangement that included FSE supplementation (0 or 100 mg/kg) and CS administration (0 or 20 mg/kg of diet for 7 consecutive days starting on d 14). The feeding program consisted of a starter diet from d 1 to 21 and a finisher diet from d 22 to 42. Corticosterone administration decreased (P < 0.01) ADG and impaired (P < 0.01) feed conversion ratio in both phases and overall, which were alleviated (P < 0.01) by dietary FSE supplementation in the finisher phase and overall. At d 21, CS administration caused decreases (P < 0.05) in the apparent digestibility of energy, relative weight of bursa and thymus, total antioxidant capacity, superoxide dismutase (SOD) activity, and antibody titers to Newcastle disease virus (NDV); however, serum malondialdehyde and uric acid were increased. All of these changes were attenuated (P < 0.05) by dietary FSE supplementation. At d 42, FSE supplementation improved (P < 0.05) the apparent digestibility of DM and CP, relative weights of bursa, SOD activity, and antibody titers to NDV, which were impaired by CS administration. Interactions (P < 0.05) were noted between CS and FSE for ADG and feed conversion ratio in the finisher phase and overall, as well as total antioxidant capacity, SOD activity, uric acid, and antibody titers to NDV at d 21, as well as relative weights of thymus at d 42. In conclusion, dietary FSE supplementation enhanced nutrient digestibility and performance of broiler possibly by reducing oxidative stress and immune depression challenged by CS.


Marek's disease (MD) seriously threatens the world poultry industry and has resulted in great economic losses. Chinese medicinal herbs are a rich source for lead compounds and drug candidates for antiviral treatments. The object of the study was to investigate the anti-MDV activity and mechanism of 20 compounds extracted from Chinese medicinal herbs. Antiviral assay, time of addition experiments, and virucidal assay were performed on chicken embryo fibroblast cells. The 50% cytotoxic concentration and 50% effective concentration were determined and, accordingly, selectivity index and inhibition ratio were calculated. Antiviral assay showed dipotassium glycyrrhizinate (DG) and sodium tanshinone IIA sulfonate (STS) exhibited significantly inhibitory activity against MDV in a dose-dependent manner. EC50 of DG and STS were 893.5 ± 36.99 µg/mL and 54.82 ± 2.99 µg/mL, and selective index (SI) were >3.36 and >9.12, respectively. Time of addition experiment and virucidal assay demonstrated DG inhibited viral replication in the full replication cycle and inactivated MDV particles in non-time-dependent manner, but STS interfered with the early stage of MDV replication and inactivated MDV particles in a time-dependent manner. Moreover, both DG and STS promoted apoptosis of cells infected by MDV. DG and STS have great potential for developing new anti-MDV drugs for clinic application.


After a ban on the use of antibiotics as growth promoters in farm animals in the European Union in 2006, an interest in alternative products with antibacterial or anti-inflammatory properties has increased. In this study, we therefore tested the effects of extracts from Curcuma longa and Scutellaria baicalensis used as feed additives against cecal inflammation induced by heat stress or Salmonella Enteritidis (S. Enteritidis) infection in
chickens. Curcuma extract alone was not enough to decrease gut inflammation induced by heat stress. However, a mixture of Curcuma and Scutellaria extracts used as feed additives decreased gut inflammation induced by heat or S. Enteritidis, decreased S. Enteritidis counts in the cecum but was of no negative effect on BW or humoral immune response. Using next-generation sequencing of 16S rRNA we found out that supplementation of feed with the 2 plant extracts had no effect on microbiota diversity. However, if the plant extract supplementation was provided to the chickens infected with S. Enteritidis, Faecalibacterium, and Lactobacillus, both bacterial genera with known positive effects on gut health were positively selected. The supplementation of chicken food with extracts from Curcuma and Scutellaria thus may be used in poultry production to effectively decrease gut inflammation and increase chicken performance.


The traditional Chinese medicinal plant Brucea javanica has received much attention for its significant antiprotozoal effects in recent years; however, little is known about its potential anticoccidial functions. In the present study, a series of experiments was conducted to investigate the prophylactic and therapeutic effects of ethanol extract from B. javanica on coccidiosis induced by Eimeria tenella in broiler chickens. Chickens infected with E. tenella were treated with B. javanica extract and compared either with broilers treated with the anticoccidial halofuginone hydrobromide (Stenorol) or with control groups that consisted of infected-unmedicated and uninfected-unmedicated broilers. The experiments revealed that the B. javanica extract could significantly (P<0.05) reduce bloody diarrhea and lesion scores. Additional, OPG output in these plant extract treated groups was reduced in comparison with non-treated groups (P<0.05). However, there was no evidence to show that the extract could promote BWG. Histological data showed that the number of second-generation schizonts in the medicated groups was substantially less than that in the infected-unmedicated control. In summary, our work showed that B. javanica extract exerted considerable anticoccidial effects, supporting its use as a promising therapeutic in controlling avian coccidiosis.


Aflatoxins as potent mycotoxins can influence vital parameters in chickens. Turmeric was used in decreasing toxic effect of mycotoxins on vital organs, traditionally. The study compared the protective effect of turmeric and Mycoad(TR) in broilers exposed to aflatoxin. Chickens (270) were divided into six groups. The chickens were fed a basal diet, turmeric extract (5 mg/kg diet), Mycoad(TR) (25 mg/kg diet), productive aflatoxin (3 mg/kg diet), aflatoxin plus turmeric extract (3 versus 5 mg/kg diet), and aflatoxin plus Mycoad(TR) (3 versus 25 mg/kg diet) in basal diet. At 28 d old, we determined plasma concentration of total protein, albumin, triglyceride, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium, potassium, phosphorous, uric acid, aspartate transferase (AST), and alanine aminotransferase (ALT). Furthermore, liver and kidney were sampled for pathological examination. Chickens fed turmeric with aflatoxin had significant lower ALT, AST, and uric acid than chickens fed aflatoxin (11.4 ± 0.79, 228 ± 9, and 6 ± 0.4 versus 17.2 ± 1.7, 283 ± 5, and 7.7 ± 0.1) whereas, total protein, calcium, and HDL values in chickens fed aflatoxin plus turmeric increased significantly (2.66 ± 0.16, 8.4 ± 0.2, and 920 ± 4.1 versus 1.7 ± 0.17, 7 ± 0.2, and 690 ± 4.8). Pathological examination revealed severe congestion, degeneration, and necrosis in liver and kidney in chickens that received aflatoxin. The study showed that turmeric may provide protection against the toxic effects of aflatoxin on liver and kidney.


The economic impact of the poultry red mite, Dermanyssus gallinae, the lack of new acaricides, the occurrence of resistance and tighter legislation have all led to the need to find new ways to control this pest. One promising alternative method of control focuses on employing repellent and/or toxic effects of selected plant essential oils against D. gallinae. Ten essential oils (basil, thyme, coriander, eucalyptus, lavender, lemon, fir tree, oregano, mint, and juniper) were tested for the persistence of toxic and repellent effects. In filter-paper toxicity bioassays against D. gallinae, the best results were observed for lavender (more than 97% mortality...
after 48 and 72 h) and thyme (84% at 72 h) at a dose of 0.12 mg/cm(2). In addition, two oils showed significant persistent toxic effects 15 and 30 days post application to filter papers. Thyme was the most effective (100% mortality at 72 h), followed by lavender (nearly 80% mortality after 72 h). Out of the ten oils tested for their repellent effect, thyme was the strongest, with nearly 80% of the tested area avoided by mites; oregano caused a 60% avoidance and lavender exhibited an effect close to 40%. All other oils exhibited a repellent effect of less than 30%. None of the experiments showed a repellent effect for HM (commercial alimentary oil) or negative controls. We found that the thyme and lavender essential oils exhibited promising results when tested in vitro for toxic and repellent effects against D. gallinae; thus, we suggest that future experiments focus on in vivo tests using these oils in farm units.


Three commercial broiler breeds were fed from hatch with a diet supplemented with Capsicum and Curcuma longa oleoresins, and co-infected with Eimeria maxima and Clostridium perfringens to induce necrotic enteritis (NE). Pyrotag deep sequencing of bacterial 16S rRNA showed that gut microbiota compositions were quite distinct depending on the broiler breed type. In the absence of oleoresin diet, the number of operational taxonomic units (OTUs), was decreased in infected Cobb, and increased in Ross and Hubbard, compared with the uninfected. In the absence of oleoresin diet, all chicken breeds had a decreased Candidatus Arthromitus, while the proportion of Lactobacillus was increased in Cobb, but decreased in Hubbard and Ross. Oleoresin supplementation of infected chickens increased OTUs in Cobb and Ross, but decreased OTUs in Hubbard, compared with unsupplemented/infected controls. Oleoresin supplementation of infected Cobb and Hubbard was associated with an increased percentage of gut Lactobacillus and decreased Selenihalanaerobacter, while Ross had a decreased fraction of Lactobacillus and increased Selenihalanaerobacter, Clostridium, Calothrix, and Geitlerinama. These results suggest that dietary Capsicum/Curcuma oleoresins reduced the negative consequences of NE on body weight and intestinal lesion, in part, through alteration of the gut microbiome in 3 commercial broiler breeds.


The effect of dietary thyme-oil extract (TOE) supplementation on immune functions of broilers were assessed by feeding graded levels (50, 100, 200, or 400 ppm) of TOE to male broiler chicks during a 42-d feeding trial compared with negative- or positive-control diets. Dietary control treatments included a negative-control diet with no feed-additive supplementation and 2 positive-control groups supplemented with either virginiamycin or zinc bacitracin. In total, 300 1-day-old Ross × Ross male broilers were randomly assigned to 6 dietary treatments that consisted of 5 replicates of 10 birds each. On d 21 and 42, 2 birds from each replicate were killed by cervical cutting to measure the relative weights of spleen and bursa of Fabricius. At 25 d of age, chicks were injected with 0.5 mL of 10% SRBC suspension. Broilers fed with 200 ppm of TOE had heavier weights of bursa of Fabricius than those fed other dietary treatments at d 42 of age. Furthermore, dietary inclusion of 100 ppm of TOE resulted in higher (P < 0.05) total immunoglobulin response in primary antibody titer against sheep erythrocytes compared with other dietary treatments. On the other hand, diet modifications had no significant effect on blood leukocyte subpopulations and heterophil-to-lymphocyte ratio. These results suggest that dietary supplementation with TOE, especially at the level of 100 ppm, can improve immunological responses of broiler chicks.


After a ban on the use of antibiotics as growth promoters in farm animals in the European Union in 2006, an interest in alternative products with antibacterial or anti-inflammatory properties has increased. In this study,
we therefore tested the effects of extracts from Curcuma longa and Scutellaria baicalensis used as feed additives against cecal inflammation induced by heat stress or Salmonella Enteritidis (S. Enteritidis) infection in chickens. Curcuma extract alone was not enough to decrease gut inflammation induced by heat stress. However, a mixture of Curcuma and Scutellaria extracts used as feed additives decreased gut inflammation induced by heat or S. Enteritidis, decreased S. Enteritidis counts in the cecum but was of no negative effect on BW or humoral immune response. Using next-generation sequencing of 16S rRNA we found out that supplementation of feed with the 2 plant extracts had no effect on microbiota diversity. However, if the plant extract supplementation was provided to the chickens infected with S. Enteritidis, Faecalibacterium, and Lactobacillus, both bacterial genera with known positive effects on gut health were positively selected. The supplementation of chicken feed with extracts from Curcuma and Scutelleria thus may be used in poultry production to effectively decrease gut inflammation and increase chicken performance.


The effects of dietary garlic bulb were studied separately on hematological parameters, ascites incidence, and growth performance of an ascites susceptible broiler hybrid under both standard temperature conditions (STC:) and cold temperature conditions (CTC:). A total of 336 one-day-old male broiler chickens were allocated to 4 experimental groups with 4 replicates of 21 birds each under STC. In addition, the same grouping with another 336 birds was used for CTC. Under CTC, the birds were exposed to cold temperatures for induction of ascites. Experimental groups were defined by the inclusion of 0 (control), 5, 10 or 15 g/kg garlic bulbs in the diets under both STC and CTC. Growth performance, systolic blood pressure (as a measure of systemic arterial blood pressure), physiological and biochemical parameters, as well as ascites indices (right ventricle [RV:], total ventricle [TV:] weights, and RV/TV:) were evaluated. Systolic blood pressure was determined using an indirect method with a sphygmomanometer, a pediatric cuff, and a Doppler device. The final body weight decreased quadratically (P = 0.003), with increasing garlic bulb levels in the diets under STC. The feed conversion ratio showed no significant differences among all groups under both STC and CTC. No significant differences were observed in total mortality and ascites-related mortality in all groups under STC, although total mortality (L: P = 0.01; Q: P = 0.001) and ascites-related mortality (L: P = 0.007; Q: P = 0.001) were significantly different among the diets under CTC. Under STC, the systolic blood pressure, packed cell volume, hemoglobin, RV, TV, and RV/TV did not vary significantly among the diets. However, red blood cell count and erythrocyte osmotic fragility decreased linearly (P < 0.005) with increasing garlic bulb levels in the diets under STC. Under CTC, the systolic blood pressure, packed cell volume, red blood cell count, and erythrocyte osmotic fragility decreased (P < 0.05) with increasing garlic levels. It is concluded that the inclusion of 5 g/kg garlic bulb in susceptible broiler chicken diets has a systemic anti-hypertensive effect and could decrease ascites incidence without impairing broiler chicken performance.


The development of antibiotic resistant pathogens has resulted from the use of sub-therapeutic concentrations of antibiotics delivered in poultry feed. Furthermore, there are a number of consumer concerns regarding the use of antibiotics in food animals including residue contamination of poultry products and antibiotic resistant bacterial pathogens. These issues have resulted in recommendations to reduce the use of antibiotics as growth promoters in livestock in the United States. Unlike conventional production, organic systems are not permitted to use antibiotics. Thus, both conventional and organic poultry production need alternative methods to improve growth and performance of poultry. Herbs, spices, and various other plant extracts are being evaluated as alternatives to antibiotics and some do have growth promoting effects, antimicrobial properties, and other health-related benefits. This review aims to provide an overview of herbs, spices, and plant extracts, currently defined as phytobiotics as potential feed additives.

In this study, the effect of chlorogenic acid extract from Lonicera japonica Thunb. on Mycoplasma gallisepticum infections and the performance of broiler flocks was investigated. A total of 360 Ross-308 broiler chicks taken from M. gallisepticum seropositive flocks were divided equally into three groups designated as control (nothing administered), antibiotic (Tylosin tartrate given for the first 3 d and d 20-22) and test group (chlorogenic acid extract given twice a day on d 16 and 22). Broiler performance analysis, serological tests (slide agglutination), molecular identification (polymerase chain reaction) and histopathological examination were performed to detect M. gallisepticum. The results show that chlorogenic acid not only increases live body weight but is also an alternative treatment option in M. gallisepticum-infected broiler flocks.


Newcastle disease virus (NDV) belonging to the Paramyxovirinae subfamily is one of the most devastating pathogens in poultry. Although vaccines are widely applied to control the infection, outbreaks of Newcastle disease (ND) repeatedly happen. Currently, there are no alternative control measures available for ND. In the present study, we found that sulfated Chuanmingshen violaceum polysaccharide (sCVPS) were potent inhibitors of NDV in specific pathogen free chickens infected with a virulent strain. With sCVPS treatment, the survival rate increased by almost 20% and virus titers in test organs, including brain, lung, spleen and thymus, were significantly decreased. The sCVPS also exhibited the ability to prevent viral transmission by reducing the amount of virus shed in saliva and feces. Higher concentrations of interferon α and γ in serum were detected in chickens treated with sCVPS, indicating that one of the antiviral mechanisms may be attributed to the property of immunoenhancement. Histopathological examination showed that sCVPS could alleviate the tissue lesions caused by NDV infection. These results suggest that sCVPS are expected to be a new alternative control measure for NDV infection and further studies could be carried out to evaluate the antiviral activity of sCVPS against other paramyxoviruses.


Extensive use of current anti-coccidial drugs together with drug resistance and residue has raised concerns about public health and poultry development. Here, we studied the anti-coccidial properties of Bidens pilosa. A phytochemical approach was developed for analysis of B. pilosa utilized as a feed additive. The protective effects of B. pilosa supplemented chicken diet were evaluated chickens infected with Eimeria tenella. B. pilosa, at doses of 0.5%, 1% and 5% of the chicken diet, significantly protected against E. tenella as measured by reduction in mortality, weight loss, fecal oocyst excretion and gut pathology in chickens. Finally, drug resistance of E. tenella to B. pilosa was assessed in chickens using the anti-coccidial index. This index showed that B. pilosa induced little, if any, drug resistance to Eimeria in chickens. Collectively, this work suggests that B. pilosa may serve as a novel, natural remedy for coccidiosis with low drug resistance in chickens.


The objective of this study was to determine the effect of per os administration of 3 various dosages of a Citrosept preparation (a grapefruit extract) to growing turkey hens on changes in their selected haematological and immunological blood indices. An attempt was also undertaken to select the most efficient dose of the preparation with respect to the mentioned indices in turkey hens. The experiment was conducted on 180 turkey hens allocated at random to 4 groups, 45 birds in each group. Samples of their full blood were analyzed for haematological indices, such as red blood cell count (RBS), haemoglobin content (Hb), haematocrit value (Ht), and white blood cell count (WBC). Samples of blood plasma were assayed to determine the activity of lysozyme (chamber-diffusive method) and heterophilis capability to reduce nitro blue tetrazolium (stimulated and spontaneous NBT test). Phagocytic activity of leucocytes against Staphylococcus aureus 209P strain was assessed and expressed as the percentage of phagocytic cells (% PC) and phagocytic index (PI). The administration of the grapefruit extract to turkey hens with drinking water caused a significant increase in
haemoglobin content in blood, as well as an increase in non-specific humoral immunity marker (activity of lysozyme) and non-specific cellular immunity marker (percentage of phagocytic cells; \( P \leq 0.05 \)). The results obtained enabled the positive evaluation of the advisability of applying the Citrosept preparation in the feeding of turkey hens at the age of 6-9 weeks. Among the doses examined, the most efficient with respect to the stimulation of the non-specific humoral and cellular immunity was the dose of 0.021 ml/kg of body weight.


Avian pathogenic Escherichia coli (APEC) causes inflammation in multiple organs of chickens called avian colibacillosis, and results in serious economic loss to the chicken industry. Polyphenolic compounds possess a wide range of physiological activities that may contribute to their beneficial effects against inflammation-related diseases. In this study, the curative effect and mechanism of action of the polyphenolic extracts from Punica granatum L. and Terminalia chebula Retz. in chickens challenged with APEC were studied. Specific-pathogen-free white Leghorn chickens (males, 21-d old) were challenged with APEC and then given oral administration of extracts of P. granatum and T. chebula. The extracts decreased the morbidity and inflammation induced by APEC. Data from quantitative real-time polymerase chain reaction and enzyme-linked immunosorbent assay showed that the extracts of P. granatum and T. chebula polyphenols (GCP) reversed the over-expression genes of the Toll-like receptor (TLR) 2, 4, and 5, down-regulated the activation of nuclear factor-kappa B signal transduction pathways, and inhibited the production of pro-inflammatory cytokines. Naturally occurring GCP may be a potential alternative medicine for the prevention or treatment of avian colibacillosis.


Heat shock proteins (HSPs) are highly conserved proteins, shown to protect organisms against physical and physiological stress. TEX-OE(®) is a patented total extract of the fruit of Opuntia ficus indica, which has been demonstrated to accelerate the development of HSPs in several animal species. One-day-old commercial broiler chicks were treated with TEX-OE(®); HSP was measured by enzyme-linked immunosorbent assay (ELISA), and a large commercial field trial investigated key performance indicators (KPIs) in treated versus untreated controls chicks. TEX-OE(®) significantly increased HSP concentrations in treated chicks versus controls. Final cumulative mortality, liveweight and percentage factory-rejects were better than in controls. The accelerated HSP response may enable chicks to cope with early stressors, which is reflected in improved KPIs.


Infectious bursal disease (IBD), caused by infectious bursal disease virus (IBDV), is an immunosuppressive infectious disease of global economic importance in poultry. This study was designed to evaluate the effect of oral administration of ginseng stem-leaf saponins (GSLS) on humoral and gut mucosal immunity in chickens vaccinated with live IBDV vaccine, and furthermore, to test its protective efficacy against virulent IBDV challenge following vaccination. In experiment 1, chickens were orally administered with GSLS at 5 mg/kg of BW for 7 d, and then immunized with live IBDV vaccine via the oral route. Serum was sampled on 0, 1, 2, 3, 4, and 5 wk postvaccination for detecting antibody titers by ELISA, and intestinal tissues were collected on 0, 1, 3, and 5 wk postvaccination for measurement of IgA-positive cells and intestinal intraepithelial lymphocytes by immunohistochemical and hematoxylin-eosin staining, respectively. Result showed that antibody titers, IgA-positive cells and intestinal intraepithelial lymphocytes were significantly higher in chickens drinking GSLS than the control, suggesting an enhanced effect of GSLS on humoral and gut mucosal immune responses. In experiment 2, chickens were delivered with GSLS and then vaccinated in the same way as in experiment 1. The
Birds were challenged with virulent IBDV at wk 3 postvaccination. Then the birds were weighed, bled, and necropsied at d 3 postchallenge and the bursae were sampled for gross and histopathological examination. Results demonstrated that GSLS provided a better protection against virulent IBDV challenge following vaccination than the control. In conclusion, oral administration of GSLS enhances both humoral and gut mucosal immune responses to IBDV and offers a better protection against virulent IBDV challenge. Considering its immunomodulatory properties to IBDV vaccine, GSLS might be a promising oral adjuvant for vaccination against infectious diseases in poultry.


This experiment was conducted to evaluate the effects of different levels of sweet orange (Citrus sinensis) peel extract (SOPE) on humoral immune system responses in broiler chickens. Three hundred 1-day broilers (Ross-308) were randomly allocated to treatments varying in supplemental SOPE added in the drinking water. The experimental groups consisted of three treatments fed for 42 days as follows: a control treatment without feed extract, a treatment containing 1000 ppm of SOPE and a treatment containing 1250 ppm of SOPE. All treatments were isocaloric and isonitrogenous. Broilers were vaccinated with Newcastle disease virus (NDV), avian influenza (AI), infectious bursal disease (IBD) and infectious bronchitis virus (IBV) vaccines. Antibody titer response to sheep red blood cells (SRBC) was higher in the group fed 1250 ppm of SOPE (P < 0.05) as well as for immunoglobulin G (IgG) and IgM. Similarly, antibody titer responses to all vaccines were constantly elevated (P < 0.05) by SOPE enrichment in a dose-dependent manner. Relative weights of spleen and bursa of Fabricius were unaffected by treatments. Dietary SOPE supplementation may improve the immune response and diseases resistance, indicating that it can constitute a useful additive in broiler feeding. Thus, supplying SOPE in rations may help to improve relative immune response in broiler chickens.


Marek’s disease (MD) seriously threatens the world poultry industry and has resulted in great economic losses. Chinese medicinal herbs are a rich source for lead compounds and drug candidates for antiviral treatments. This study was to investigate the anti-MDV activity and mechanism of 20 compounds extracted from Chinese medicinal herbs. Antiviral assay, time of addition experiments, and virucidal assay were performed on chicken embryo fibroblast cells. The 50% cytotoxic concentration and 50% effective concentration were determined and, accordingly, selectivity index and inhibition ratio were calculated. Antiviral assay showed dipotassium glycyrrhizinate (DG) and sodium tanshinone IIA sulfonate (STS) exhibited significantly inhibitory activity against MDV in a dose-dependent manner. EC50 of DG and STS were 893.5 ± 36.99 µg/mL and 54.82 ± 2.99 µg/mL, and selective index (SI) were >3.36 and >9.12, respectively. Time of addition experiment and virucidal assay demonstrated DG inhibited viral replication in the full replication cycle and inactivated MDV particles in non-time-dependent manner, but STS interfered with the early stage of MDV replication and inactivated MDV particles in a time-dependent manner. Moreover, both DG and STS promoted apoptosis of cells infected by MDV. DG and STS have great potential for developing new anti-MDV drugs for clinic application.


Organic farming of poultry has increased in recent years as the prophylactic use of antibiotics has come into disfavor. This study was conducted to explore the antiparasitic effect of a methanolic extract of Peganum harmala in broilers challenged with coccidiosis. For this purpose, 200 1-week-old broiler chicks were divided into five treatments: negative control (basal diet, Ph-0/NC), positive control (basal diet with coccidiosis challenge, Ph-0/C), and three groups challenged with coccidiosis and supplemented with P. harmala at the rate of 200 mg L(-1) (Ph-200), 250 mg L(-1) (Ph-250), and 300 mg L(-1) (Ph-300) drinking water. Each group had three replicates of ten chicks each. Challenge with standard dose of the larvae of coccidiosis and supplementation of P. harmala were initiated on day 14 until 35 days of age. As expected, the results revealed...
that weight gain, feed intake, and feed conversion ratio (FCR) were depressed significantly in Ph-0 group with significant mortality percentage. Weight gain, total body weight, and FCR increased linearly with increasing dose of P. harmala with the exception of feed intake. The growth and feed efficiency of Ph-0/NC was better in Ph-0/NC compared to that in Ph-0/C and comparable to that in P. harmala-treated birds. Similarly, mean oocysts per gram (OPG) decreased linearly ($P < 0.05$) in supplemented groups compared to that in Ph-0/C. Histological evidences showed that cecal lesion and leucocyte infiltration decreased markedly in supplemented groups of P. harmala specifically the Ph-300 group compared to those in Ph-0/C. From the present experiment, we concluded the anticoccidial effect of P. harmala in broiler chicks.


*Forsythia suspensa* extract (FSE) has been demonstrated to attenuate physiological stress induced by high temperature or high stocking density. This experiment was conducted with 144 male Arbor Acre broilers (1-d-old, weighing 42.7 ± 1.7 g) to determine the effects of FSE on performance, nutrient digestibility, antioxidant activities, serum metabolites, and immune parameters for birds treated with corticosterone (CS). The birds were randomly allotted to 1 of 4 treatments in a 2 × 2 factorial arrangement that included FSE supplementation (0 or 100 mg/kg) and CS administration (0 or 20 mg/kg of diet for 7 consecutive days starting on d 14). The feeding program consisted of a starter diet from d 1 to 21 and a finisher diet from d 22 to 42. Corticosterone administration decreased ($P < 0.01$) ADG and impaired ($P < 0.01$) feed conversion ratio in both phases and overall, which were alleviated ($P < 0.01$) by dietary FSE supplementation in the finisher phase and overall. At d 21, CS administration caused decreases ($P < 0.05$) in the apparent digestibility of energy, relative weight of bursa and thymus, total antioxidant capacity, superoxide dismutase (SOD) activity, and antibody titers to Newcastle disease virus (NDV); however, serum malondialdehyde and uric acid were increased. All of these changes were attenuated ($P < 0.05$) by dietary FSE supplementation. At d 42, FSE supplementation improved ($P < 0.05$) the apparent digestibility of DM and CP, relative weights of bursa, SOD activity, and antibody titers to NDV, which were impaired by CS administration. Interactions ($P < 0.05$) were noted between CS and FSE for ADG and feed conversion ratio in the finisher phase and overall, as well as total antioxidant capacity, SOD activity, uric acid, and antibody titers to NDV at d 21, as well as relative weights of thymus at d 42. In conclusion, dietary FSE supplementation enhanced nutrient digestibility and performance of broiler possibly by reducing oxidative stress and immune depression challenged by CS.


Intensive poultry production systems depend on chemoprophylaxis with anticoccidial drugs to combat infection. A floor-pen study was conducted to evaluate the anticoccidial effect of *Artemisia annua* and *Foeniculum vulgare* on *Eimeria tenella* infection. Five experimental groups were established: negative control (untreated, unchallenged); positive control (untreated, challenged); a group medicated with 125 ppm lasalocid and challenged; a group medicated with *A. annua* leaf powder at 1.5% in feed and challenged; and a group treated with the mixed oils of *A. annua* and *Foeniculum vulgare* in equal parts, 7.5% in water and challenged. The effects of *A. annua* oil extract of *A. annua + F. vulgare* on *E. tenella* infection were assessed by clinical signs, mortality, fecal oocyst output, faeces, lesion score, weight gain, and feed conversion. Clinical signs were noticed only in three chickens from the lasalocid group, six from the *A. annua* group, and nine from the *A. annua + F. vulgare* group, but were present in 19 infected chickens from the positive control group. Bloody diarrhea was registered in only two chickens from the *A. annua* group, but in 17 chickens from the positive control group. Mortality also occurred in the positive control group (7/20). Chickens treated with *A. annua* had a significant reduction in faecal oocysts (95.6%; $P = 0.027$) and in lesions score (56.3%; $P = 0.005$) when compared to the positive control. At the end of experiment, chickens treated with *A. annua* leaf powder had the highest body weight gain (68.2 g/day), after the negative control group, and the best feed conversion (1.85) among all experimental groups. Our results suggest that *A. annua* leaf powder (Aa-p), at 1.5% of the daily diet post-infection, can be a valuable alternative for synthetic coccidiostats, such as lasalocid.

This study was carried out to evaluate the impact of ginger (Zingiber officinale) feed supplementation on growth performance, antioxidant status, carcass characteristics and blood parameters in broiler chicks under conditions of heat stress (32 ± 2ºC for 8 h per d). 2. A total of 336 d-old male broiler chicks (Cobb-500) were randomly assigned to one of 6 dietary groups representing: basal diet with no supplement as control, basal diet containing 100 mg/kg vitamin E as positive control, basal diets containing either 7.5 or 15 g/kg of ginger root powder, and diets containing 75 or 150 mg/kg of ginger essential oil. 3. The results indicated that at 22 d of age, the group receiving 7.5 g/kg of ginger root powder experienced significantly increased body weight (BW) and body weight gain (BWG) compared to the control group. There were no significant difference among the diet groups regarding BW, BWG, feed intake (FI) or feed conversion ratio (FCR) at 42 and 49 d of age. 4. The inclusion of powder and essential oil of ginger in broiler diets did not affect carcass characteristics and blood parameters of the chickens. However, in the group receiving 150 mg/kg ginger essential oil, the total superoxide dismutase (TSOD) activity in liver increased compared to the control group. Malondialdehyde (MDA) concentrations in liver also decreased in the groups receiving ginger powder and essential oil compared to that in the control group. There were no significant difference between experimental groups regarding glutathione peroxidise (Gpx), TSOD and catalase (CAT) enzymes in red blood cells. All dietary groups increased total antioxidant capacity (TAC) and decreased MDA concentration in serum compared to the control group. 5. The results of this study suggest that ginger powder and essential oils may be a suitable replacement for synthetic antioxidants in broiler diets. Results also suggest that ginger powder might be better than extracted essential oil for improving antioxidant status in broilers.


This paper reviews the use of botanical extracts in the control of coccidial infection in poultry. 2. Some plants and their respective volatile oils and extracts have the potential to alleviate coccidiosis and reduce its severity. 3. Most plant bioactives improve some, but not all, aspects of coccidiosis with variable effectiveness against different species of Eimeria. 4. Difficulties in comparing research findings have arisen from the use of different experimental models, different active components and infectious dose of Eimeria. 5. Current knowledge of their potential anti-coccidial effects may provide guidance for the use of botanical extracts in the control of the coccidiosis.


The present study was designed to study the protective effect of sea buckthorn (SBT) against renal damage induced by ochratoxin A (OTA) in Japanese quail. Day-old quail chicks were divided into six groups and fed a basal quail chick mash containing 2% SBT leaf powder (group SX), OTA at a dietary level of 3 ppm (group OX), 25 ppm L-beta-phenylalanine (Phe) plus 3 ppm OTA (group OP), 2% dietary level of SBT leaf powder plus 3 ppm OTA (group OS), SBT leaf extract at a level of 10%/L of drinking water plus 3 ppm OTA (group OSS), and a standard toxin-free feed (group CX, control) for 21 days. OTA at 3 ppm level in diet grossly revealed mild to moderate renal swelling in OX birds, and the severity was less in the case of OS, OSS, and OP birds. Microscopically, degenerative, necrotic, and inflammatory changes were observed in OX birds, but the changes were less severe in OS, OSS, and OP birds. Ultrastructural studies revealed remarkable and consistent changes in the proximal convoluted tubules (PCTs), with severe damage of mitochondria and endoplasmic reticulum in OX birds, whereas SBT-treated birds (groups OS, OSS) had mild changes in mitochondria. A moderate to marked increase in number of peroxisomes in the cytoplasm of PCTs was a consistent finding in the Phe- and SBT-treated groups kept on OTA in comparison to the group fed OTA alone. In conclusion, the inclusion of 2% SBT leaf powder in feed and SBT leaf extract in water provided partial protection against OTA-induced nephropathy in Japanese quail.
Due to an increasing demand for natural products to control coccidiosis in broilers, we investigated the effects of supplementing a combination of ethanolic extracts of Artemisia annua and Curcuma longa in drinking water. Three different dosages of this herbal mixture were compared with a negative control (uninfected), a positive control (infected and untreated), chemical coccidiostats (nicarbazin+narazin and, later, salinomycin), vaccination, and a product based on oregano. Differences in performance (weight gain, feed intake, and feed conversion rate), mortality, gross intestinal lesions and oocyst excretion were investigated. Broilers given chemical coccidiostats performed better than all other groups. Broilers given the two highest dosages of the herbal mixture had intermediate lesion scores caused by Eimeria acervulina, which was higher than in broilers given coccidiostats, but less than in broilers given vaccination, oregano and in negative controls. There was a trend for lower mortality (P = 0·08) in the later stage of the growing period (23-43 days) in broilers given the highest dosage of herbal mixture compared with broilers given chemical coccidiostats. In conclusion, the delivery strategy of the herbal extracts is easy to implement at farm level, but further studies on dose levels and modes of action are needed.


The effects of dietary supplementation with an organic extract of Curcuma longa on systemic and local immune responses to experimental Eimeria maxima and Eimeria tenella infections were evaluated in commercial broiler chickens. Dietary supplementation with C. longa enhanced coccidiosis resistance as demonstrated by increased BW gains, reduced fecal oocyst shedding, and decreased gut lesions compared with infected birds fed a nonsupplemented control diet. The chickens fed C. longa-supplemented diet showed enhanced systemic humoral immunity, as assessed by greater levels of serum antibodies to an Eimeria microneme protein, MIC2, and enhanced cellular immunity, as measured by concanavalin A-induced spleen cell proliferation, compared with controls. At the intestinal level, genome-wide gene expression profiling by microarray hybridization identified 601 differentially expressed transcripts (287 upregulated, 314 downregulated) in gut lymphocytes of C. longa-fed chickens compared with nonsupplemented controls. Based on the known functions of the corresponding mammalian genes, the C. longa-induced intestinal transcriptome was mostly associated with genes mediating anti-inflammatory effects. Taken together, these results suggest that dietary C. longa could be used to attenuate Eimeria-induced, inflammation-mediated gut damage in commercial poultry production.


Intestinal helmintic infection, continue to be a cause of major concern in several parts of the world, particularly in the developing nations. The use of plant extracts to control poultry helminths is increasing in different rearing systems. The anthelmintic activity of ginger and curcumin was studied on the nematode Ascaridia galli. In vitro and in vivo studies were allocated. Live parasites for in vitro studies were collected from the intestine of naturally infected chickens. Some living worms were incubated at 37 °C in media containing ginger at three concentration levels (25, 50, and 100 mg/ml), and others were incubated in media containing curcumin at the same concentration levels. Another living worm group was incubated in media containing albendazole at a dose of 7.5 mg/ml. The extracts' efficacy was exhibited in a concentration-time-dependent manner mainly at 100 mg/ml and after 48 h. The in vivo study takes place on experimentally infected chickens. Group of infected chickens was treated with ginger extract at dose of 100 mg, another group was treated with curcumin extract at dose of 100 mg, and a third group was treated with albendazole at dose of 7.5 mg. In vivo study of ginger and curcumin recorded lower mortality rates than the in vitro study. It is concluded that ginger and curcumin extracts have potential anthelmintic properties against A. galli. Ginger in all concentrations used exhibited a higher death rate observed than curcumin. Their wormicidal effect is concentration-time dependent.

The effects of Dangguibuxue Tang (DBT) on growth performance and immunity response in immunosuppressed broiler chicks were investigated in this study. 240 one-d-old broiler chicks (DaHeng S01) were randomly divided into 4 groups, 2.0% DBT-treatment (A), 0.5% DBT-treatment (B), cyclophosphamide-control (C), and control group (D). From 4 d to 7 d of age, chicks in group A, B and C were given cyclophosphamide (CY) at a dosage of 100mg/kg body weight (BW) daily by intraperitoneal injection to induce immunosuppression. Chicks in group D were given an equal volume of physiological saline daily by intraperitoneal injection and considered normal chicks. Groups A and B were supplemented with 2.0% or 0.5% of DBT in the drinking water from 8 d to 42 d of age. Groups C and D did not receive any additional medication. The results revealed that chicks from group B had lower feed:gain rate (FGR), lower total mortality, higher immunity organ indexes, higher levels of Newcastle disease (ND) antibody and infectious bursal disease (IBD) antibody, higher interleukin-2 and interleukin-6 levels, and greater lymphocyte proliferative responses to concanavalin A (ConA) during the experiment than those from group C. However, no significant difference in the immunity status in the two levels of DBT-treatment was observed. These results indicate that supplementation of 0.5% of DBT can improve both cellular immunity and humoral immunity in immunosuppressed broiler chicks.


The leaves and berries of sea buckthorn (SB; Hippophae rhamnoides; family Elaeagnaceae) are medically claimed as having phytoantioxidant, anti-inflammatory, and anticancerous properties in humans. This study evaluated the hepatoprotective activity of oil from SB berries against toxicity induced by aflatoxin B1 (AFB1) in broiler chickens. The toxicity of AFB1 led to lower total serum proteins and specifically reduced albumin (P < 0.001). Serum aspartate aminotransferase increased from 191.14 ± 11.56 to 218.80 ± 13.68 (P < 0.001). When chickens were simultaneously dosed with AFB1 and an extract of SB berries, subsequent histology of the liver showed a significant reduction of necrosis and fatty formation compared with chickens treated with AFB1 alone. Immunohistochemical results indicated that COX2, Bcl-2, and p53 were highly expressed in the liver of AFB1-treated chickens and their expression was significantly reduced by SB oil supplementation. The levels of AFB1 residues in chickens livers were significantly reduced by SB oil from 460.92 ± 6.2 ng/mL in the AFB1 group to 15.59 ± 6.1 ng/mL in the AFB1 and SB oil group. These findings suggest that SB oil has a potent hepatoprotective activity, reducing the concentration of aflatoxins in liver and diminishing their adverse effects.


Hypericum perforatum extract (HPE) has been proved a drug effective to many viral diseases. The purpose of this paper was to investigate the therapeutic efficacy and immuno-enhancement of HPE for chickens which were already challenged with infectious bursal disease virus (IBDV BC-6/85). Chickens infected with IBDV were treated with HPE for 5 consecutive days, the observation of immune organ indexes and pathological changes index, determination of IFN-α and detection of IBDV with RT-PCR were employed to assess in vivo whether or not HPE had the certain therapeutic efficacy on infectious bursal disease (IBD), and if HPE was able to improve the immunologic function. The results showed that 1330 and 667.9 mg/kg body weight (BW) per day of HPE had significant therapeutic efficacy and improvement immunologic functions for chickens infected experimentally with IBDV.

The study was conducted on broiler birds to evaluate the anticoccidial efficacy of an extract of Chinese traditional herb Dichroa febrifuga Lour. One hundred broiler birds were assigned to five equal groups. All birds in groups 1-4 were orally infected with $1.5 \times 10^4$ Eimeira tenella sporulated oocysts and birds in groups 1, 2 and 3 were medicated with 20, 40 mg extract/kg feed and 2 mg diclazuril/kg feed, respectively. The bloody diarrhea, oocyst counts, intestinal lesion scores, and the body weight were recorded to evaluate the anticoccidial efficacy. The results showed that D. febrifuga extract was effective against Eimeria infection; especially 20 mg D. febrifuga extract/kg feed can significantly increase body weight gains and reduce bloody diarrhea, lesion score, and oocyst excretion in comparison to infected-unmedicated control group.


This paper reports the immunostimulatory and protective effects of Aloe vera extracts (aqueous and ethanolic) against coccidiosis in industrial broiler chickens. The study was divided into two experiments. Experiment-I was conducted for the evaluation of immunostimulatory activity of A. vera and experiment-II demonstrated the protective efficacy of A. vera extracts against coccidiosis in chickens. Results of the experiment-I revealed significantly higher ($p<0.05$) lymphoproliferative responses in chickens administered with ethanolic extract of A. vera as compared to those administered with aqueous extract and control group. Microplate haemagglutination assay for humoral response on day 7th and 14th post primary and secondary injections of sheep red blood cells (SRBCs) revealed significantly higher ($p<0.05$) anti SRBC antibody (total Igs, IgG and IgM) titers in chickens of experimental groups as compared to the control group. None of the extracts, however, demonstrated significant effects on the development of lymphoid organs. Results of experiment-II revealed maximum protection (60%) in chickens administered with aqueous Aloe extract as compared to the ethanolic extract administered chickens (45%). Mean oocysts per gram of droppings in the control group was significantly higher ($p<0.05$) as compared to the chickens in both the experimental groups. Chickens administered with aqueous Aloe extract showed a minimal mean lesion score (2.3) followed by those administered with ethanolic Aloe extract (2.6) and control chickens (3.05) for caeca, and a similar pattern was observed for intestinal lesion scoring. Further, significantly higher weight gains and antibody titers ($p<0.05$) were observed in chickens administered with A. vera extracts as compared to those in the control group. It was concluded that A. vera may be a potential and valuable candidate to stimulate the immune responses and can be used successfully as an immunotherapeutic agent against coccidiosis in industrial broiler chickens.
IX-G Veterinary Botanical Medicine and Swine


Chinese patent medicines play an important role in veterinary clinical use. The aim of this study is to research the anti-infection effect of Chinese patent medicine "Wuhuanghu" for the treatment of porcine infectious pleuropneumonia and to evaluate the safety of "Wuhuanghu" in order to provide a comprehensive understanding of its toxicity. The anti-infection results showed that the treatment with "Wuhuanghu" could significantly inhibit pneumonia and decrement of the pneumonia in high, medium and low doses of "Wuhuanghu" groups were 70.97%, 61.29% and 58.06% respectively. The acute toxicity test showed that rats in the highest group (5000mg/kg) had no death and no abnormal response, suggesting the LD50 of "Wuhuanghu" was more than 5000mg/kg. The subchronic toxicity study showed that hematology indexes in all groups had no obvious differences; blood biochemical index, only albumin and total cholesterol in middle and low doses of "Wuhuanghu" groups were significantly decreased when compared with control group. The clinical pathology showed that the target organ of "Wuhuanghu" was liver. The safety pharmacology study indicated that "Wuhuanghu" had no side effects on rats. In conclusion, "Wuhuanghu" has therapeutic and protective effects to porcine infectious pleuropneumonia in a dose-dependent manner and "Wuhuanghu" is a safe veterinary medicine.


To investigate the effects of Centella asiatica (L.) on growth performance, nutrient digestibility and blood composition in piglets, 32 nursery pigs were fed 0.0, 0.5, 1.0 and 2.0% dietary C. asiatica (L.) from 15 to 90 kg BW. At 30 kg BW, nutrient digestibility was measured and at 35 kg BW piglets were vaccinated with Mycoplasma hyopneumoniae. Hematological parameters were checked at 40 and 80 kg BW. Compared with the control, growth performance was not affected. The ether extract, ash and calcium digestibility were lower at 0.5%, and dry matter, crude protein, crude fat, phosphorus and energy digestibility were lower at 1.0% (P<0.05). On hematological values, at 40 kg hematocrit, total white blood cells, neutrophils, eosinophils, basophils, monocytes and lymphocytes were higher at the 2.0% level (P<0.05). Most of these values except basophils and monocytes continued until at 80 kg, at which total white blood cells, neutrophils, eosinophils and lymphocytes were higher even at 1.0% (P<0.05); neutrophil-to-lymphocyte ratio tended to be higher at 2.0% (P<0.03). Cholesterol, triglycerides and antibody levels against M. hyopneumoniae did not differ except that at 40 kg the cholesterol of 0.5% was lower (P<0.05) and M. hyopneumoniae-specific antibodies tended to be higher with increasing levels of C. asiatica (L.) (P<0.07). The result that C. asiatica (L.) could not improve growth performance but increased values of serum hematocrit and white blood cells, and mycoplasma immunity to M. hyopneumoniae might suggest that C. asiatica (L.) has no function to elevate body weight but has the potential to enhance innate immunity.


The objective of the study was to evaluate the protective effect of Calendula officinalis propylene glycol extracts against oxidative DNA damage and lipid peroxidation induced by high polyunsaturated fatty acid (PUFA) intake in young growing pigs. Forty young growing pigs were assigned to five treatment groups:
control; oil (linseed oil supplementation); C. officinalis 1 and 2 groups (linseed oil plus 3 ml/day of C. officinalis propylene glycol extracts); and vitamin E group (linseed oil plus 100 mg/kg of vitamin E). Lymphocyte DNA fragmentation and 24-h urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) excretion were measured to determine DNA damage. Lipid peroxidation was studied by analysing plasma and urine malondialdehyde (MDA), and urine isoprostane concentrations (iPF2α-VI), total antioxidant status of plasma and glutathione peroxidase (GPx) assays. C. officinalis 1 (extract from petals) effectively protected DNA from oxidative damage. It indicated a numerical trend towards the reduction of plasma MDA and urinary iPF2α-VI excretion. Its effect was comparable with that of vitamin E. C. officinalis 2 (extract from flower tops) showed less antioxidant potential than the extract from petals. We can conclude that the amount of C. officinalis extracts proposed for internal use by traditional medicine protects the organism against DNA damage induced by high PUFA intake.


Two experiments were performed to determine the anthelmintic effect of some phytogenic feed additives on a mild infection of Ascaris suum in growing and finishing pigs. Usually, an infection of A. suum is controlled by using conventional synthetic drugs. Organic farmers, however, prefer a non-pharmaceutical approach to worm control. Therefore, phytotherapy could be an appropriate alternative. In the first experiment, a commercial available organic starter diet was supplemented with 3% of a herb mixture, adding 1% Thymus vulgaris, 1% Melissa officinalis and 1% Echinacea purpurea to the diet, or with 4% of a herb mixture, thereby adding the mentioned herbs plus 1% Camellia sinensis (black tea). A negative control group (no treatment) and a positive control group (treatment with conventional synthetic drug flubendazole) were included. In the second experiment, the anthelmintic properties against A. suum of three individual herbs, Carica papaya, Peumus boldus and Artemisia vulgaris, each in a dose of 1%, were tested. Pigs were infected with 1000 infective worm eggs each. Each experiment was performed with 32 individually housed growing pigs (8 replicates/treatment), which were monitored for 67 days. It was hypothesized that the herbs would block the cycles of the larvae, thereby preventing the development of adult worms. Therefore, phytogenic feed additives were not supplied during the whole experimental period, but only from the start until D39. Pigs were inoculated with infective worm eggs during five consecutive days (D17-D21). At D67 all pigs were dissected, whereafter livers were checked for the presence of white spots. Also numbers of worms in the small intestine were counted. In experiment 1, the numbers of worm-infected pigs were similar for both the herb supplemented (groups 3 and 4) and the unsupplemented (group 1) treatments (5-6 pigs of 8), while the treatment with flubendazole (group 2) resulted in 0 infected pigs. In experiment 2, herb addition (groups 2-4) did not significantly reduce the number of worm-infected pigs compared to the negative control (group 1). It can be concluded that the tested herb mixtures and individual herbs in the diets of growing and finishing pigs did not decrease the number of pigs which were infected with A. suum, although the herb mixture without black tea and also boldo leaf slightly (P<0.10) reduced the number of worms in the intestinal tract. The tested herb mixtures and individual herbs did not affect the performance of the pigs.


The objective of this study was to evaluate effects of a combined use of extracts of medicinal herbs Taraxaumi mongolicum, Viola yedoensis Makino, Rhizoma coptidis, and Radix isatidis (MYCI) on porcine epidemic diarrhea (PED). Twenty-two 3-day-old piglets received an oral challenge with 3 x 10(3.5) TCID50 of the virulent PED virus (PEDV) in PBS or PBS only and daily oral administration of 60 mg of the MYCI mixture suspended in milk replacer or the vehicle for 7 days in a 2 x 2 factorial arrangement of treatments. Average daily gain (ADG) increased (p < 0.05) in response to the MYCI treatment in the PEDV-challenged piglets (-18 vs. 7 g for the vehicle- vs. MYCI-administered group), but not in unchallenged animals (27 vs. 28 g). Diarrhea score and fecal PEDV shedding, however, were not influenced by the MYCI treatment. The PEDV challenge caused severe intestinal villus atrophy and crypt hyperplasia, both of which were alleviated by administration of the MYCI mixture as indicated by an increase in the villus height and a decrease in the crypt depth due to the treatment. Overall, medicinal herb extracts used in this study ameliorated impaired growth performance and intestinal
lesion of newborn piglets challenged with the virulent PEDV. Therefore, our results suggest that the MYCI mixture could be used as a prophylactic or therapeutic agent against PED.


Many health effects can be attributed to the Mediterranean herb oregano (Origanum vulgare L.) and several studies demonstrated the improving effect on performance, changes in blood count, antibacterial, antifungal and immunomodulating abilities. The majority of these investigations were carried out with processed essential oil, while whole plant material was only used in a few studies. Thus, the aim of the present experiment was to test the effect of increasing proportions of dried oregano in piglet feed on health and performance, with a special focus on immune modulation. A total of 80 male castrated weaned piglets (body weight [BW] 7.9 kg ± 1.0 kg) were used in a feeding experiment lasting 5 weeks. They were assigned to 4 experimental groups: a control diet, and three diets with an oregano supplementation at 2 g, 4 g and 8 g per kg feed, respectively, corresponding to 23.5 mg, 46.9 mg and 93.9 mg carvacrol/kg DM. After 3 weeks, half of each group was challenged with 5 µg lipopolysaccharides (LPS) per kg BW. Blood samples were collected 2 h after LPS stimulation and analysed for T-cell phenotypes, granulocyte activity, clinical-chemistry as well as white and red blood count. The results indicate no effects of oregano on performance. In contrast, oregano altered the lymphocyte proportion and the ratio of CD4(+) and CD8(+) T-cells as well as the triglyceride concentration in the serum of non-stimulated and in LPS-stimulated piglets. In conclusion, whole plant supplementation of oregano to piglet feed altered immune-related parameters, but did not modulate the acute inflammatory response induced by LPS stimulation.

Malo C1, Gil L, Cano R, Martínez F, Galé I. Antioxidant effect of rosemary (Rosmarinus officinalis) on boar epididymal spermatozoa during cryopreservation. Theriogenology. 2011 Jun;75(9):1735-41

The objective of the present study was to evaluate the ability of rosemary to protect epididymal boar spermatozoa from freeze-thaw damage. Testis from eight boars were collected at the slaughterhouse in two trials. In the laboratory, sperm from epididymis were recovered by flushing and cryopreserved in lactose-egg yolk solution supplemented with various concentrations (low; medium; high) of rosemary. After thawing, total motility, viability, acrosome integrity, response to hypoosmotic swelling test (HOST) and malonaldehyde (MDA) concentration were assessed. The results showed that there was an increase in motility at 1, 2 and 3 h in the presence of rosemary. The addition of this herb provided a significant beneficial effect on viability at 2 h of incubation, compared to the control group. Conversely, acrosome status was not affected by any extender. Higher concentration of rosemary produced significant improvement in percentages of positive HOST at 0 and 1 h, whereas no impact was observed at the end of incubation. Considering membrane lipid peroxidation, a greater decrease in MDA production was observed when rosemary content was raised. Rosemary-enriched freezing extender improved the post-thaw epididymis boar spermatozoa quality, showing a significant correlation between rosemary concentration and concentration of MDA. Further studies are needed to define the active component in rosemary that prevents peroxidation.


Salmonella enterica serovar Choleraesuis, a host-adapted pathogen of swine, usually causes septicemia. Lactic acid bacteria (LAB) strains have been widely studied in recent years for their probiotic properties. In this study, a mouse infection model first screened for potential agents against infection, then a pig infection model evaluated effects of LAB strains and herbal plants against infection. Scutellariae radix (SR) and Gardeniae fructus (GF) showed abilities to reduce bacteria shedding and suppressing serum level of TNF-α induced by infection in swine. Bioactivities of SR and GF were enhanced by combining with LAB strains, which alone could speed up the bacteria elimination time in feces and boost immunity of infected pigs. Baicalein and genipin exhibited stronger cytotoxicity than baikalin and geniposide did, as well as prevent Salmonella from invading macrophages. Our study suggests LAB strains as exhibiting multiple functions: preventing infection, enhancing immunity to prepare host defenses against further infection, and adjusting intestinal microbes' enzymatic
activity in order to convert herbal compounds to active compounds. The SR/GF-LAB strain mixture holds potential infection-prevention agents supplied as feed additives.


Since rotavirus is one of the leading pathogens that cause severe gastroenteritis and represents a serious threat to human and animal health, researchers have been searching for cheap, safe, and effective anti-rotaviral drugs. There is a widespread of interest in using natural products as antiviral agents, and among them, licorice derived from Glycyrrhiza spp. has exerted antiviral properties against several viruses. In this study, anti-rotaviral efficacy of Glycyrrhiza uralensis extract (GUE) as an effective and cheaper remedy without side-effects was evaluated in colostrums-deprived piglets after induction of rotavirus diarrhea. Colostrums-deprived piglets were inoculated with porcine rotavirus K85 [GSP(7)] strain. On the onset of diarrhea, piglets were treated with different concentration of GUE. To evaluate the antiviral efficacy of GUE, fecal consistency score, fecal virus shedding and histological changes of the small intestine, mRNA expression levels of inflammation-related cytokines (IL8, IL10, IFN-β, IFN-γ and TNF-α), and transcription factor (NFκB) in the small intestine and spleen were determined. Among the dosages (100-400 mg/ml) administrated to animals, 400 mg/ml of GUE cured diarrhea, and markedly improved small intestinal lesion score and fecal virus shedding. mRNA expression levels of inflammation-related cytokines (IL8, IL10, IFN-β, IFN-γ and TNF-α), signaling molecules (p38 and JNK), and transcription factor (NFκB) in the small intestine and spleen were markedly increased in animals with RVA-induced diarrhea, but dose-dependently decreased in GUE treated animals after RVA-induced diarrhea. GUE cures rotaviral enteritis by coordinating antiviral and anti-inflammatory effects. Therapy of this herbal medicine can be a viable medication for curing rotaviral enteritis in animals and humans.


The objective of this study was to determine the effects of feeding Chinese herbal ultra-fine (CHU) powder to sows during the last week of gestation and during the lactation period on immunological performance of the offspring. In this experiment, 15 pregnant sows (mean BW = 235.6 ± 3.7 kg) were randomly assigned to 1 of 3 treatments including no additive (Control), 0.75% CHU powder (Group A), or 1.5% CHU powder (Group B) added to a maize- and soybean meal-based diet. Blood from 10 piglets per group was collected at d 7, d 14, or d 21 of age to measure serum metabolites, lymphocyte proliferating activity, and serum antibody and cytokine concentrations. Dietary supplementation of sows with CHU powder increased (P < 0.05) serum concentrations of total protein, albumin, and triglycerides of offspring, whereas the concentration of glucose was reduced (P < 0.05) compared with Controls. The CHU powder enhanced (P < 0.05) serum concentrations of IgG in Group B offspring on d 7 and IgM in Group A offspring on d 7 and d 14, increased IL-10 in Group A offspring on d 7, as well as IL-2 in offspring from Groups A and B on all days of determination. The CHU powder increased interferon gamma in Group A offspring on d 14 and in Group B offspring on d 14 and d 21, and increased tumor necrosis factor alpha in offspring of Group A on d 14 and in Group B on all days surveyed. Compared with Controls, a greater number (P < 0.05) of T lymphocyte subpopulations were detected in Group A and B offspring including CD4+ cells in Group A on d 7 and d 21, CD4+ cells in Group B on d 14 and d 21, and CD8+ cells in Group A on d 7 and d 14. Collectively, these findings indicate a beneficial effect of CHU powder treatment of sows in later gestation and during lactation on serum metabolism and cellular and humoral immune responses of their offspring.


The effect of by-products of oriental medicinal plants (OMP; T1) containing 0.03% herb extracts (T2) or 0.1% aminolevulinic acid (T3) on the production performance of swine during the finishing period and on its meat quality were investigated. No significant differences were found in the weight gain, feed intake and feed conversion rate among the tested groups (P > 0.05). But the treated group showed higher (P < 0.05) moisture and ash and lower protein than the control group. The T3 group showed a lower meat cholesterol
content (38.42 mg/100 g) compared to the other groups (P < 0.05). The vitamin E content of the muscle in the treated groups was higher compared to the control group. No antibiotic content was detected in all treated and control samples. The values of the volatile basic nitrogen (VBN) and thiobarbituric acid reactive substance (TBARS) of the treated groups were significantly lower (P < 0.01) than the control group. The treated groups had significantly better (P < 0.05) sensory-test scores for color, flavor, off-flavor and total acceptability compared to the control group.


Many traditional Chinese medicine (TCM) decoctions are proven to have multiple functions in animal production. These decoctions are seldom recognized by the international scientific community because the mechanisms of action are not clearly elucidated. According to TCM theory, Cortex Phellodendri (COP), Rhizoma Atractylodes (RA), Agastache Rugosa (AR), and Gypsum Fibrosum (GF) can be used to formulate a medicinal compound that prevents or cures animal disease caused by heat stress. The aim of this research was to study the regulatory functions of the active components of TCM and to elucidate the effects of different TCM decoctions on antioxidant activity and lipid peroxide content, using in vitro and in vivo models of heat stress. For in vitro experiments, intestinal crypt-like epithelial cell line-6 (IEC-6) cells were employed to evaluate the effects of the active components of COP, RA, AR, and GF. For in vivo experiments, forty-eight 2-mo-old Chinese experimental mini-pigs (7.20 ± 0.02 kg) were randomly assigned to 4 groups: a normal-temperature group (NTG); a high-temperature group (HTG); HTG treated with COP, RA, AR, and GF (1:1:1:1, TCM1); and HTG treated with COP, RA, AR, and GF (1:1:1:0.5, TCM2). Results showed that the active components of the COP, RA, AR, and GF increased (P < 0.05) the proliferation and viability of heat-stressed IEC-6 cells and that the most effective treatment doses of COP alkaloid, RA Aetherolea, Herba Agastachis Aetherolea, and GF water extract were 200, 100, 100, and 200 µg/mL, respectively. All 4 active components increased (P < 0.05) superoxide dismutase, glutathione peroxidase activities, and glutathione content, and decreased (P < 0.05) malondialdehyde content with respect to the heat-stressed group to concentrations similar to those seen in NTG. In vivo experiments demonstrated that TCM1 and TCM2 improved (P < 0.05) the poor growth performance seen in HTG pigs. The superoxide dismutase, glutathione peroxidase activities, and malondialdehyde content in porcine jejunum treated with TCM1 and TCM2 were not different (P > 0.05) from those seen in the NTG and were better (P < 0.05) than results seen in the HTG. Overall, it appeared that TCM2 was more effective than TCM1 in ameliorating the effects of heat stress in pigs. In conclusion, this study revealed that the active components of common TCM decoctions have antioxidant functions.


The purpose of this study was to investigate the effects of a traditional Chinese herbal medicine complex supplementation on the growth performance, immunity and serological traits of pigs, and the feasibility of its use as a substitute for antibiotics. Thirty-six weaned pigs LYD with average initial body weight of 10 ± 0.55 kg were randomly divided into three treatments with three replicates. These constituted the control, the antibiotics group (chlortetracycline 100 µg/kg, oxytetracycline 100 µg/kg), and 0.3% Chinese herbal medicine complex group (CHM). Experiment results indicated that the CHM group exhibited significantly increased average feed intake and peripheral blood CD3(+)CD8(+) T cell percentage as compared with those of the antibiotics group (P < 0.05). High-density lipoprotein (HDL) level was greater while low-density lipoprotein + very low-density lipoprotein (LDL + VLDL) level was lower in the CHM group than the control group (P < 0.05). The in vitro results indicated that peripheral blood mononuclear cells (PBMC) stimulated by Con-A produced a greater interleukin (IL)-6 level in the CHM group and IL-6 level stimulated by lipopolysaccharide was greater than the antibiotics groups (P < 0.05). Above all, this study has indicated that the addition of Chinese traditional herbal complex to pigs’ diets has beneficial results.

The purpose of this study was to evaluate the effects of dietary Chinese medicinal herbs (CMH) supplementation composed of Panax ginseng, Dioscoreaceae opposite, Atractylodes macrocephala, Glycyrrhiza uralensis, Ziziphus jujube and Platycodon grandiflorum, on the performance, intestinal tract morphology and immune activity in weanling pigs. Two hundred and forty weaned pigs were assigned randomly to four dietary groups including the negative control (basal diet), 0.1% CMH, 0.3% CMH and 0.114% antibiotic (Chlortetracycline calcium Complex, Sulfathiazole and Procaine Penicillin G) supplementation groups for a 28-day feeding trial. Results indicated that both CMH supplementation groups had a better gain and feed/gain than control group (CT) during the first 2 weeks of the experimental period. The 0.3% CMH had a significant decrease in the diarrhoea score in first 10 days of experimental period when compared with other groups. The CMH supplementation groups had a higher villous height, increased lactobacilli counts in digesta of ileum and decreased coliform counts in colon compared with CT. The immune activities of polymorphonuclear leucocytes (PMNs), including the respiratory burst and Salmonella-killing ability, were significantly enhanced in CMH supplementation groups at day 7 of experiment period. The CMH and antibiotic supplementations increased the nutrient digestibility such as dietary dry matter, crude protein and gross energy in weanling pigs. In conclusion, the dietary CMH supplementation improved intestinal morphology and immune activities of PMNs, thus giving rise to nutrient digestibility and reduce diarrhoea frequency in weanling pigs.


Tanshinone IIA (STS), an active ingredient of the Chinese herb Danshen (Salvia miltiorrhiza) for angina and stroke in adults, has been reported to inhibit platelet function. However, its effect on platelet and underlying mechanism remain largely unknown, particularly in neonates. To investigate the effect of STS on the platelet aggregation and its interaction with various platelet activation pathways, platelet aggregatory function was studied in whole blood stimulated by collagen (2-10 μg/ml) ex vivo in newborn piglets receiving intravenous STS (0.1-10mg/kg, n=8) and in vitro in whole blood from newborn piglets (n=6) incubated with STS (0.1-100 μg/ml). The respective morphological changes of platelets were also examined by scanning electron microscopy. Plasma levels of nitrite/nitrate (NOx) and thromboxane B(2) (TxB(2)), matrix metalloproteinase (MMP)-2 and -9 activities were also examined. To further delineate the mechanistic pathway, the effect of STS on endothelial microparticles release from cultured human umbilical vein endothelial cells (HUVECs) was quantified by flow cytometry. STS impaired the ex vivo, but not in vitro, collagen-stimulated platelet aggregation. Infusion of STS elevated the plasma level of TxB(2) at 10mg/kg. However, STS had no effect on NOx level. Incubating cultured HUVECs with STS (1 and 10 μg/ml) caused a significant release of endothelial microparticles. Morphologically, STS elicited platelet activation in vivo, but not in vitro. STS impairs the ex vivo whole blood platelet aggregatory function by activating platelet in vivo in healthy newborn piglets. It implies that STS may elicit its effects by stimulating endothelial microparticles production and eicosanoid metabolism pathway.


Chinese herbal medicine (CHM) is often used as dietary supplements to maintain good health in animals and humans. Here, we review the current knowledge about effects of CHM (including ultra-fine Chinese herbal powder, Acanthopanax senticosus extracts, Astragalus polysaccharide, and glycyrrhetinic acid) as dietary additives on physiological and biochemical parameters in pigs, chickens and rodents. Additionally, we propose possible mechanisms for the beneficial effects of CHM on the animals. These mechanisms include (a) increased digestion and absorption of dietary amino acids; (b) altered catabolism of amino acids in the small intestine and other tissues; (c) enhanced synthesis of functional amino acids (e.g., arginine, glutamine and proline) and polyamines; and (d) improved metabolic control of nutrient utilization through cell signaling. Notably, some phytochemicals and glucocorticoids share similarities in structure and physiological actions. New research findings provide a scientific and clinical basis for the use of CHM to improve well-being in livestock species and poultry, while enhancing the efficiency of protein accretion. Results obtained from animal studies also have important implications for human nutrition and health.
**Effects of herbal preparation on libido and semen quality in boars.**


The objective of this study was to investigate the effects of a preparation from herbal extracts (PHE) on libido and semen quality in breeding artificial insemination boars. Ten fertile boars were divided into control and experimental groups according to significant difference of libido. There were no differences in semen quality between groups. Animals were fed a commercial feeding mixture for boars. The feeding mixture for the experimental group was enriched with PHE, which was prepared from Eurycoma longifolia, Tribulus terrestris and Leuzea carthamoides. Duration of the experiment was 10 weeks. Samples of ejaculate were collected weekly. Libido was evaluated according to a scale of 0-5 points. Semen volume, sperm motility, percentage of viable spermatzoa, sperm concentration, morphologically abnormal spermatzoa, daily sperm production and sperm survival were assessed. Amounts of mineral components and free amino acids were analysed in seminal plasma. Significant differences were found in these parameters: libido (4.05 ± 0.22 vs 3.48 ± 0.78; p < 0.001), semen volume (331.75 ± 61.91 vs 263.13 ± 87.17 g; p < 0.001), sperm concentration (386.25 ± 107.95 vs 487.25 ± 165.50 × 10^3 /mm(3); p < 0.01), morphologically abnormal spermatzoa (15.94 ± 11.08 vs 20.88 ± 9.19%; p < 0.001) and Mg concentration (28.36 ± 11.59 vs 20.27 ± 13.93 mm; p < 0.05). The experimental group's libido was increased by 20% in comparison with the beginning of the experiment. Results of this study showed positive effect of PHE on libido and some parameters of boar semen quality.

**Achyranthes bidentata polysaccharide enhances immune response in weaned piglets.**

Immunopharmacol Immunotoxicol. 2009 Jun;31(2):253-60.

The acquired immunity is underdeveloped at 3-4 weeks of age when piglets are usually weaned on commercial farms, and weaning is associated with compromised immunity. Dietary supplementation with immunomodulatory phytochemicals may enhance immune responses in the weaned piglets. This study is conducted to investigate the effects of dietary supplemental achyranthes bidentata polysaccharide (ABP) on proliferation activity of lymphocytes, and production of antibodies, complements and cytokines in weaned piglets. Results showed that lymphocyte proliferation activity in piglets fed diets supplementing with 1000 and 1500 mg/kg ABP increased (P < 0.05) on days 14 and 28 compared with the non-additive piglets, as well as serum contents of IgG, IgA, IgM, C(3), C(4), IL (interleukin)-2 and IFN (interferon)-gamma. The ABP had dose-dependent immunomodulatory activity and the dose of 1500 mg/kg presented the strongest stimulating activity in vivo. In addition, the ABP increased (P < 0.05) the proliferation activity and production of IL-2 and IFN-gamma of cultured lymphocytes in dose- or time-dependent manner. The proliferation activity of peripheral T cells and splenic lymphocytes in 400 microg/ml of ABP group arrived at their peak values, as well as the production of IL-2 and IFN-gamma at 72 and 12 h after the treatment, respectively. Collectively, these findings suggested that dietary supplementation with ABP to weaned piglets enhances cellular and humoral immune responses, and ABP addition to culture medium also increases the proliferation activity and cytokine production of lymphocytes cultured in vitro, which indicate that dietary supplementation with the herbal polysaccharide may offer an effective alternative to antibiotics for weaned piglets.

**Dietary supplementation with Chinese herbal powder enhances ileal digestibilities and serum concentrations of amino acids in young pigs.**


This study was designed to determine the effect of ultra-fine Chinese herbal powder as a dietary additive on serum concentrations and apparent ileal digestibilities (AID) of amino acids (AA) in young pigs. In Experiment 1, 60 Duroc x Landrace x Yorkshire piglets weaned at 21 days of age were randomly assigned to one of three treatments, representing supplementation with 0 or 2 g/kg of the powder, or 0.2 g/kg of colistin (an antibiotic) to corn- and soybean meal-based diets (n = 20 per group). Blood samples from five piglets per group were collected on days 7, 14, and 28 to determine serum AA concentrations. In Experiment 2, 12 barrows with an average initial body weight of 7.64 kg were randomly assigned to one of the three dietary treatments, followed by surgical placement of a simple T-cannula at the terminal ileum. All of the diets contained 0.1% titanium oxide as a digestibility marker. The samples of terminal ileal digesta were collected on day 7 for determining AID of AA. Results show that dietary supplementation with the herbal powder increased (P < 0.05) serum concentrations and AID of most AA by 10-50% and 10-16%, respectively. As an indicator of improved intestinal function, AID values of calcium were also enhanced in piglets supplemented with the herbal powder. Dietary supplementation of colistin increased serum concentrations and AID values of some AA by 8-44% and
10-15%, respectively, in comparison with the non-supplemented group. These novel findings demonstrate that
the herbal powder can enhance the digestibility of dietary protein and the intestinal absorption of AA into the
systemic circulation in post-weaning pigs, therefore providing a new mechanism for its growth- and immunity-
promoting efficacy.

Xiao C\(^1\), Rajput ZI, Liu D, Hu S. Have Enhancement of serological immune responses to foot-and-mouth disease

Foot-and-mouth disease (FMD) is a highly contagious disease affecting cloven-hoofed animals. Vaccination
against FMD is a routine practice in many countries where the disease is endemic. This study was designed first
to investigate the extract of the seeds of Momordica cochinchinensis (Lour.) Spreng. (ECMS) for its adjuvant
effect on vaccination of inactivated FMDV antigens in a guinea pig model and then to evaluate the supplement
of ECMS in oil-emulsified FMD vaccines for its immunopotentiation in pigs. The results indicated that ECMS
and oil emulsion act synergistically as adjuvants to promote the production of FMDV- and VP1-specific
immunoglobulin G (IgG) and subclasses in guinea pigs. A supplement of ECMS in a commercial FMD vaccine
significantly enhanced FMDV-specific indirect hemagglutination assay titers as well as VP1-specific IgG and
subclasses in pigs. Therefore, ECMS could be an alternative approach to improving swine FMD vaccination
when the vaccine is poor to induce an effective immune response.

Other papers

E. Peeters, B. Driessen and R. Geers Influence of supplemental magnesium, tryptophan, vitamin C, vitamin E,
and herbs on stress responses and pork quality\(^1\) Journal of Animal Science 2006 84: 7: 1827-1838

W. Windisch, K. Schedle, C. Plitzner and A. Kroismayr Use of phytogenic products as feed additives for swine
and poultry\(^1\) Journal of Animal Science 2008 86: 14_suppl: E140-E148

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pigs experimentally infected with a pathogenic Escherichia coli\(^1\), Journal of Animal Science 2013 91: 11: 5294-
5306

membranaceus and lipopolysaccharide challenge on performance, immunological, adrenal, and somatotropic
responses of weanling pigs\(^1\) Journal of Animal Science 2005 83: 12: 2775-2782

K. Maenner, W. Vahjen and O. Simon Studies on the effects of essential-oil-based feed additives on
performance, ileal nutrient digestibility, and selected bacterial groups in the gastrointestinal tract of piglets\(^1\)
Journal of Animal Science 2011 89: 7: 2106-2112

Y. Liu, T. M. Che, M. Song et al Dietary plant extracts improve immune responses and growth efficiency of pigs
experimentally infected with porcine reproductive and respiratory syndrome virus\(^1\) Journal of Animal
Science 2013 91: 12: 5668-5679
Appendix X Documentation of Trends in the Public Acceptance of Botanical Therapies: Consumer Buying Patterns, Patterns of Use, and Industry Correlates.
Introduction:

The American College of Veterinary Botanical Medicine (ACVBM) is petitioning the American Board of Veterinary Specialties (AVBS) to be established as a specialty College under the criteria established by the American Veterinary Medical Association (AVMA) for the advancement of knowledge and education in the field of veterinary botanical medicine.

The purpose of this White Paper is to provide objective data to support the establishment of this College based on increased public acceptance and use of veterinary herbal medicine, as measured by consumer and industry trends over the past 17 years.

The information contained in this document was obtained from several sources:

1. National Animal Supplement Council¹ data enumerating the trends in administration of the top 25 herbal ingredients in products for dogs, cats and horses.

2. A peer-reviewed paper² submitted for publication containing a survey of members of the American Association of Bovine Practitioners (AABP) documenting an increase in the organic dairy industry and the consequent needs for this sector to have non-pharmaceutical options for production and veterinary care of problems like mastitis, metritis, etc.

3. Market research report³ of the trending increase in consumer interest in herbal therapies for pets recently published by the oldest and most reputable association for herbal education and research, the American Botanical Council.

4. A National Pet Owners Survey from the American Pet Products Association (APPA) 2015-2016⁴, documenting the trending increase in consumer interest in dietary supplements for their pets. By definition, the APPA includes herbal remedies under the general category of dietary supplements.

1. The National Animal Supplement Council

Established in 2001, the NASC is a non-profit trade organization dedicated to protecting and enhancing the health of companion animals and horses throughout the United States. As an all-industry association of stakeholders concerned with the issues surrounding the supply of health supplements for animals which are not intended for human consumption, NASC members include manufacturers of animal health supplements, raw material suppliers, distributors, veterinarians, retailers, and pet professionals.
The NASC works directly with the FDA-CVM and with state regulatory agencies to establish a regulatory environment that is fair, reasonable, responsible and nationally consistent.

By working with the FDA-CVM to establish a unique category of health supplements specifically for companion animals not in the human food chain known as “Unapproved Drugs”, the NASC has allowed the health supplement industry to continue to grow with this federal sanction. Under this framework, the FDA-CVM exercises enforcement discretion with the caveat that companies act “responsibly”. An important function of the NASC is to educate companies regarding FDA-CVM compliance.

The NASC established a Scientific Advisory Board, which, if the College of Veterinary Botanical Medicine had been established in 2001, would have contributed significantly to the work of this advisory board. This board reviewed over 900 ingredients found on the labels of animal supplements and assigned to each supplement one of four levels of risk, “ranging from generally recognized as safe” to “potentially toxic”. Additionally, the NASC established an adverse event reporting website, and has recorded millions of administrations of health supplements and has documentation regarding the percentage of adverse events associated with those supplements in dogs, cats and horses.

It is this administration data which is presented below, that has been mined specifically to generate this numerical report and to chart the trends over the past 17 years of the number of administrations of the top 25 selling herbal ingredients for dogs, cats, and horses.

Mr. Bill Bookout, President and Founder of the NASC, in providing this confidential and proprietary information writes:

“We are providing the attached data and usage trends for the top 25 herbal ingredients used in supplements for Dogs, Horses and Cats.

The NASC Adverse Event Reporting Database NAERS™ tracks over 2000 individual ingredients contained in over 7000 products which are entered by NASC member companies. Our organization is the leading non-profit trade association for these types of products representing over 90% of sales in the US. Total bytes of data in our system exceeds 100 Billion and these data may be utilized by regulatory agencies in North America when questions arise about products and/or individual ingredients.

In an effort to assist the American College of Veterinary Botanical Medicine in its documentation for its petition for membership in the ABVS, we are pleased to provide the trends for products (similar to human dietary supplements) that contain the top 25 herbal ingredients. Please note the following:
• These data reflect the following botanical ingredients: Alfalfa, Barley grass, Boswellia, Cayenne, Celery seed, Chamomile, Cinnamon, Cranberry extract, Devil’s Claw, Echinacea, Garlic, Ginger, Gingko Biloba, Grape Seed extract, Kelp, Marshmallow root, Milk thistle, Nettles, Oregon Grape root, Parsley, Slippery Elm, Spirulina, Turmeric, Valerian root, Yucca.

• Administrations are calculated and grouped from all NASC Member companies, tracked by the target species indicated on the product label.

• Data reflects units shipped to the first distribution point for products purchased and does not necessarily indicate all administrations were 100% consumed by the animals.

• Data reflects purchases in all market channels.

• All data is the property of The National Animal Supplement Council and may be used only for the purposes described in this document. Any other use requires written permission from the President of the NASC.

• This information is confidential and proprietary and should not be provided to anyone for any purpose other than to support the objectives of the ACVBM.”

Discussion of Trends from NASC Data:

**Canine Data:** Total administrations of the top 25 herbal ingredients for dogs was 42,087,369 in 1999, the first year of tabulating this data. The number of administrations grew over the 17 years recorded to 244,797,878 administrations, estimated for the year 2015. This is 500% growth.

**Equine Data:** Total administrations of the top 25 herbal ingredients for horses was 8,385,566 for 1999, the first year of recording this data. This value increased in 17 years of data recording to 42,476,440 administrations estimated by the end of 2015. This is also a 500% growth in number of administrations, just as with the canine data.

**Feline Data:** Total administrations of the top 25 herbal ingredients for 1999 for cats was 5,638,172. This value it is estimated will increase to 81,495,270 by the end of 2015. Although cats are experiencing a down turn in number of visits to their veterinarians, the number of administrations of the top 25 herbal ingredients increased 14.5 times during this 17 year period being measured!

**Combined Data:** Total administrations of the top 25 herbal ingredients for Dogs, Cats and Horses combined for 1999 was 56,111,107. This value increased over 17 years to an estimated 368,769,588 by the end of 2015. This is a 650% increase in the administrations of these top 25 herbal ingredients, which implies a similar increase in use of these herbal supplements in all species measured.

**ANALYSIS:** The National Animal Supplement Council was established from the need that arose as a result of the rapid growth in the animal supplement industry, including the increased use of herbal remedies by veterinarians and consumers.
With the growth of interest in herbal medicine among veterinarians and consumers, most animal supplement companies are in need of scientifically-derived information regarding the safety, herb-drug interactions, and clinical applications regarding herbal therapies in veterinary species, such as the establishment of a College of Veterinary Botanical Medicine would facilitate.

Additionally, as the job of FDA-CVM oversight of product safety and compliance with FDA labeling guidelines becomes more difficult due to the significantly increased number of companies selling herbal products, the value of this non-profit trade organization has become indispensable.

**TABLES and GRAPHS of NASC DATA can be found at the end of this report.**

2. **The Growth in Interest Of Bovine Practitioners Toward Complementary And Alternative Medicine Based on Surveys with AABP Veterinarians in 2006 and 2010**

**Goal:** To investigate whether bovine veterinarians are interested in complementary and alternative medicine, and which diseases the use of alternative therapies would be of most interest to them.

**Methods:** Members of the American Association of Bovine Practitioners were invited by mail (2006) or email (2010) to participate. The survey was anonymous and included six closed-ended and two open-ended questions. It was published

**Questions:** Focused on the practitioner’s perceptions of complementary and alternative therapies and products, as well as how many organic clients they had.

**Results:** 181 veterinarians in 2006 and 185 veterinarians completed the survey. In both years, approximately 80% of the veterinarians were interested evidence-based alternative therapies, and in particular for the treatment of mastitis. From 2006 to 2010 interest increased significantly (p<0.01) in alternative treatment approaches for calf diarrhea, metritis, infertility, pneumonia and digital dermatitis/foot rot. In general veterinarians with organic clients were more interested in these alternative non-drug therapies than those veterinarians without organic clients.

**Conclusions:** The majority of bovine veterinarians were interested in evidence-based alternative or complementary therapies for bovine disease and the interest in alternative therapies for common cattle diseases increased between 2006 and 2010.

**ANALYSIS:** The majority of evidence-based complementary and alternative therapies are based on botanical remedies. This survey of the growth of bovine veterinarians’ interest in CAVM between 2006 and 2010 supports the need for the establishment of a College of Veterinary Botanical Medicine as a resource for these veterinarians to better establish the science that underlies these therapies. For industry, the graduation of Board-certified veterinarians with scientific and clinical expertise in botanical medicine and phytopharmacology will be invaluable as these companies develop and bring evidence-based botanical products to the marketplace to address the needs of these bovine practitioners.

3. **Market Research Reports**
A. The American Botanical Council:

Founded in 1988, The ABC is the leading non-profit association in the United States that provides educational material using science-based and traditional information to promote the responsible use of herbal medicine—serving the public, researchers, educators, healthcare professionals, industry and media.

Recently, this past November 2015, the results of a market research study, performed by one of the national leaders that follow the pet industry, SPINS, located in Chicago, Ill, was published in an article in the journal of the American Botanical Council. The data used by SPINS did not include sales information from Whole Foods Market or direct sales from businesses that sell solely on the internet. These data do include products sold in the natural specialty/gourmet and mass-market channels in the United States.

For a 52 week period, ending August 9, 2015 SPINS recorded aggregate sales of $43,044,385 for herbal supplements which provide a solid dosage format such as tablets or capsules or liquids, and for treats and snacks, which provide the botanical or blend of botanicals in a baked biscuit, cookie or other tasty format. This market grew by 25% between the years of 2013-2014, but for 2014-2015 a 25% decrease in this market was noted.

This marketplace is growing, in spite of year to year variation, overall sales continue to climb for botanical products, both for the consumer, and also for healthcare professionals such as veterinarians, trainers, and rehabilitation experts. This year marked the second annual industry trade show, The Petfood and Animal Nutrition Conference, which had a heavy representation of companies offering botanical raw materials and finished products.

Several large national supplement companies are now offering pet specific supplements to meet the increased consumer demand for these types of products.

Types of conditions for which consumers are buying botanical products was summarized in this article based on a ranking of individual botanical sales by the SPINS data. Supplements are being purchased to address immune system function, support of the digestive system and oral health. Omega three oils are being sourced from Flax, Chia or Hemp seed and their oils. Chamomile and parsley were cited as two very popular botanicals. Parsley is commonly used for improving gastrointestinal and urinary symptoms, including joint disorders such as gout and arthritis. Chamomile can be used topically as a salve for insect bites, allergies, bacterial or fungal infections, and orally, provides a gentle degree of calming and is a mild tonic for the digestion.
The article in Herbalgram closes with a discussion of consumer concerns regarding the quality and purity of these animal products, and how the National Animal Supplement Council has effectively addressed those concerns with its efforts to secure a reliable and consistent supply chain for manufacturers as well as direct oversight by third party inspections of manufacturing facilities and random product analysis of member products to assure they match label claims of botanical content and concentration.


The American Pet Products Association is the leading not-for-profit trade association serving the interests of the pet products industry since 1958. APPA’s membership consists of over 1000 pet product manufacturers, importers, manufacturer’s representatives and livestock suppliers worldwide.

The APPA was established to promote, develop and advance responsible pet ownership and the pet products industry. To this end, APPA supports industry-related market research, monitors and responds to industry legislation and regulation, and sponsors educational seminars.

Every two years the APPA distributes a survey questionnaire amongst pet owners to determine current consumer trends. The survey methodology was changed in 2012 to an on-line survey. Despite changes in methodology for this survey since 2012, the results indicate a faithful mapping of consumer trends based on the recurrent patterns in pet ownership based on geographic region, market size, family structure (children versus no children), household size, household composition, home ownership and marital status when compared to the results of each prior study performed by the APPA since 1990.

The APPA survey (a 2” thick, 5 pound publication) was mined for data regarding the trends in the use of herbal supplements by pet owners of dogs, cats and horses. The compilation and summary of those results follows.

NOTE:

*The 2014 APPA Survey added the new category of dietary supplements as a result of the increase in the number of these products for sale, and sales in this sector since the inception of this APPA survey in 1990. Interestingly, in the survey they mis-use the word “Homeopathic” in describing this sector. The APPA erroneously defines homeopathic as: “…alternative remedies including holistic, herbal, floral or plant-based products.” Homeopathy is a separate system of medicine using very dilute substances. This term is commonly misused by individuals with no understanding of complementary and alternative therapies. With the establishment of the College of Veterinary Botanical Medicine, members of this College will be engaged by industry to better describe these products.*
General Information About Pet Ownership Summarized from APPA Survey 2015-2016

By Household.

Significant growth in household pet ownership has been noted since the survey began in 1990. Currently 79.7 million US households own a pet, compared to 52.6 million households in 1990. That is greater than a 50% gain in pet-owning households.

By Generation.

The Baby Boomers (BB) have been an integral part of the growth of pet ownership over the past 20 years.

Baby boomers represent 37% of the Survey sample. BB are the largest segment of horse owners (44%)

By Pet Species Owned.

77.8 million dogs are estimated to be owned in the US. That averages to 1.43 dogs per dog-owning household and are estimated to cost their owners an average of $551/year.

85.8 million cats are estimated to be owned in the US. That averages to 2.0 cats per cat-owning household and are estimated to cost their owners an average of $398/year.

7.5 million horses are estimated to be owned in the US. That averages to 3.0 horses per household, and are estimated to cost their owners $416, which does not include the cost of food, which annually averages $2121 per horse per year.

Trends in Consumer Use of Herbal Therapies for Pets

The percentage of dogs and cats given medications of any kind increased to 77% over the past 12 months. 10 years ago this was 52%, indicating a trend toward better acceptance of administration of medications, and better palatability strategies. More than 90% of horses have been administered medication or supplements this past year.

The percentage of pets receiving dietary supplements excluding vitamins was 12% for dogs (9.3 million dogs), 6% for cats (5.15 million cats), and 5% for horses (375,000 horses).

Consumers source their dietary supplements from the following outlets: (Multiple response question, therefore total may exceed 100%)

1. Dogs: from Veterinarian (28%), Internet (22%), Pet chain superstore (16%), Pet store independent (13%), Discount/Mass marketing (16%) Hardware store (6%), Other (6%)
2. **Cats:** from Veterinarian (17%); Pet chain super store (25%); Internet (17%); Grocery store (17%); Discount Mass marketing (25%)

3. **Horses:** from Internet (33%); Veterinarian (25%); Feed store (17%) Tack shop (8%) Other (17%)

**Consumers source their information about dietary supplements** from the following outlets: (Multiple response question, therefore total may exceed 100%)

1. **Dogs:** from Veterinarian (65%), Internet (44%), Friends & Relatives (28%); past experience (32%); Pet store personnel (16%), Television (12%); Groomer (15%)

2. **Cats:** from Veterinarian (47%); Internet (42%); Friends & Relatives (33%); past experience (35%); Pet store personnel (13%); Television (10%); Other (8%)

3. **Horses:** from Veterinarian (73%); Internet (48%); past experience (60%); Breed club and societies (17%); Feed store personnel (25%), Farrier/Trainer (50%) Other

**ANALYSIS OF SURVEY DATA**

1. Veterinarians are the major source of supply to pet owners for herbal supplements
2. Veterinarians are the major source of information about using herbal supplements to pet owners
3. The market share that herbal supplements have, in comparison to more commonly used products like food, bedding, tack, collars is relatively small (average 11% for dogs, cats and horses).
4. The market share for herbal remedies and other alternative therapies has been growing sufficiently over the years since 1990 that this survey has been conducted, such that the APPA has now created a specific category in this survey to measure the trends in this growing segment.

**CONCLUSIONS DRAWN FROM THE ABOVE FOUR SOURCES OF DATA**


Since 1990 there has been a steady growth in consumer demand for dietary supplements that contain herbal ingredients. Most consumers consult with their veterinarian regarding supplements, and most consumers purchase their dietary supplements from their veterinarian.

Thus, veterinarians are in a unique position of providing evidence-based information to their clients about products they believe, based on the best information available, will augment their existing clinical protocols.
It is known that herbs can interact adversely with pharmaceutical therapies, and that not all herbs are safe or effective. Currently we lack an adequate body of evidence-based information, or Board certified veterinarians, to guide the use of herbal therapies concurrent with conventional therapies. In some cases, herbal therapies can serve as complete substitutes, where appropriate, for conventional therapies. Without these safeguards and without a College of Veterinary Botanical Medicine to graduate Board-certified specialists in herbal therapies, the consumer is left without adequate protections that would provide safe and effective options for the use of the botanical therapies that they are requesting and are currently using anyway.

The consumer, the marketplace and the Veterinary profession are ready for the establishment of the College of Veterinary Botanical Medicine for all of the reasons stated above.

REFERENCES

1. www.NASC.cc

2. Sorge US, Bastan A, Karreman H. Interest of Bovine Practitioners in Complementary and Alternative Veterinary Medicine in 2006 and 2010. (Dr. Sorge is from the Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108; (Dr. Bastan is on the Faculty of the Veterinary Medicine Department of Obstetrics and Gynecology, Ankara University, 006110, Diskapi, Ankara, Turkey; Dr. Karreman is a large animal veterinarian, Red Hill Road, Narvon, PA 17555.)


Trends of Top 25 Herbal Ingredients in Animal Health Supplements

All information is the property of The National Animal Supplement Council and may be used only for the purposes described in the cover letter accompanying this document.

Total Products Containing Top 25 Herbal Ingredients: 2,179

Canine Data

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*2015 estimated

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*2015 estimated
Trends of Top 25 Herbal Ingredients in Animal Health Supplements

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Total Products Containing Top 25 Herbal Ingredients: 2,179

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<td>2000</td>
<td>17,708,052</td>
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</tr>
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<td>2001</td>
<td>26,085,510</td>
<td>108,013,570</td>
</tr>
<tr>
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<td>31,838,672</td>
<td>140,917,713</td>
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<tr>
<td>2003</td>
<td>38,187,536</td>
<td>169,325,701</td>
</tr>
<tr>
<td>2004</td>
<td>41,903,163</td>
<td>202,600,622</td>
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<td>2005</td>
<td>42,487,220</td>
<td>224,716,172</td>
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<td>48,014,498</td>
<td>244,080,420</td>
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<tr>
<td>2007</td>
<td>49,712,281</td>
<td>226,214,777</td>
</tr>
<tr>
<td>2008</td>
<td>52,042,402</td>
<td>285,587,467</td>
</tr>
<tr>
<td>2009</td>
<td>58,798,209</td>
<td>304,755,627</td>
</tr>
<tr>
<td>2010</td>
<td>65,076,637</td>
<td>317,084,175</td>
</tr>
<tr>
<td>2011</td>
<td>69,587,467</td>
<td>333,071,656</td>
</tr>
<tr>
<td>2012</td>
<td>77,755,627</td>
<td>340,583,650</td>
</tr>
<tr>
<td>2013</td>
<td>81,084,175</td>
<td>331,717,656</td>
</tr>
<tr>
<td>2014</td>
<td>81,495,270</td>
<td>333,070,009</td>
</tr>
<tr>
<td>*2015</td>
<td>81,495,270</td>
<td>368,769,588</td>
</tr>
</tbody>
</table>

*2015 estimated
Dear Dr. Silver,

I am providing the attached data and usage trends for the top 25 Herbal Ingredients used in supplements for Dogs, Horses and Cats.

The NASC Adverse Event Reporting Data Base, NAERS™ tracks over 2,000 individual ingredients contained in over 7,000 products which are entered by NASC Member Companies. Our organization the leading non-profit industry trade association for these types of products representing over 90% of sales in the US. Total bytes of data in our system exceeds 100 billion and these data may be utilized by regulatory agencies in North America when questions arise about products and / or individual ingredients.

In an effort to assist the Veterinary Botanical Medical Association (VBMA) in working with the American Veterinary Medical Association (AVMA) we are pleased to provide the trends for products (similar to human dietary supplements) that contain the top 25 herbal ingredients. Please note the following:

- These Data Reflect the following Botanical / Herbal Ingredients: Alfalfa, Barley Grass, Boswellia, Cayenne, Celery Seed, Chamomile, Cinnamon, Cranberry Extract, Devil's Claw, Echinacea, Garlic, Ginger, Ginkgo Biloba, Grape Seed Extract, Kelp, Marshmallow Root, Milk Thistle, Nettle, Oregon Grape Root, Parsley, Slippery Elm, Spirulina, Turmeric, Valerian Root, Yucca.

- Administrations are calculated and grouped from all NASC Member Companies, tracked by the target species indicated on the product label.

- Data reflects units shipped to the first distribution point for products purchased and does not necessarily indicate all administrations were 100% consumed by the animals.

- Data reflects purchases in all market channels.

- All data is the property of The National Animal Supplement Council and may be used only for the purposes described in this document. Any other use requires written permission from the President of NASC.

This information is confidential and proprietary and should not be provided to anyone for any purpose other than to support the objectives of VBMA.

If you have any questions please do not hesitate to contact me directly and we hope you find this information helpful.

Thank you for supporting NASC and our members’ products.

Date: November 3, 2015
To: Robert J Silver DVM, MS, CVA
From: Bill Bookout, President
RE: Data to Support Use of Botanical Ingredients in Dogs, Horses and Cats
Bovine Practitioners Survey:

Interest of Bovine Practitioners in Complementary and Alternative Veterinary Medicine in 2006 and 2010.

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ABSTRACT

Objectives: The aim of this survey was to investigate whether bovine veterinarians are interested in complementary and alternative medicines and for which diseases alternative therapies would be of interest to them.

Methods: Members of the American Association of Bovine Practitioners were invited by mail (2006) or email (2010) to participate. The survey was anonymous and included six closed and two open-ended questions. The questions focused on the practitioner's perception of complementary and alternative veterinary medicines (CAVM), for which diseases they would be interested in alternative therapies and products, as well as how many organic clients they had.

Results: In the end, 181 veterinarians and 185 veterinarians completed the survey in 2006 and 2010, respectively. In both years, approximately 80% of veterinarians were interested in evidence-based alternative therapies and in particular for the treatment of mastitis. Interest in alternative treatment approaches for calf diarrhea, metritis, infertility, pneumonia, and digital dermatitis/foot rot significantly increased over the years. Veterinarians with organic clients were more interested in CAVM than those without.

In conclusion, the majority of veterinarians were interested in evidence-based alternative or complementary therapies for bovine diseases and the interest in alternative therapies for common cattle diseases increased between 2006 and 2010.

Keywords: alternative, bovine, complementary, therapy, veterinarian

INTRODUCTION

Antibiotics and other synthetically produced drugs are commonly used for the treatment of diseases in people and livestock (McEwen and Fedorka-Cray, 2002; Laxminarayan et al., 2013). However, the use of antibiotics on livestock operations has become under increasing public scrutiny due to concerns that it may add to the creation of multidrug resistant bacteria (Laxminarayan et al., 2013; PCAST, 2014). Whether this is indeed the main culprit for increases in multidrug resistant infections in humans remains to be seen (Laxminarayan et al., 2013). Nevertheless, changes have already been introduced in some sectors of the agricultural industry. The furthest reaching change would be the National Organic Program (NOP) of the United States (USDA, 2015) which prohibits the use of antibiotics or hormones on organically certified livestock operations. Animals treated with such therapies need to leave the farm and neither they nor their products must ever be sold as organic again. Instead, organic producers are encouraged to use preventive practices or non-synthetic treatments that do not violate guidelines of the Food and Drug Administration (FDA), such as Pasteurized Milk Ordinance (FDA, 2007) or Animal Medicinal Drug Use Clarification Act (FDA, 1994). Unfortunately, data on dosage, efficacy or even withhold times of alternative therapies (e.g. herbal) are sparse and formally FDA approved alternative therapies are virtually not existent. Yet, FDA prohibits the use of unapproved animal drugs to prevent harm to the animal and minimize the risk of an adulteration of the food supply (FDA, 1994 & 2007). This provides a potential challenge for veterinarians. We hypothesize that veterinarians would be interested in alternative therapies as long as data exists regarding their efficacy and withhold times.

Therefore, the aim of this survey was to investigate whether bovine veterinarians are interested in complementary and alternative medicines and for which diseases alternative therapies would be of interest.

MATERIAL AND METHODS

In the late fall of 2005, veterinary practitioners who were listed in the directory of the American Association of Bovine Practitioners as “dairy” or “mostly dairy” practitioners and located within
the North East, Upper Midwest and Western United States were invited by mail to participate (n = 750). The questionnaire was sent out on a pre-stamped postcard. The survey was anonymous and included six closed and two open-ended questions. The questions focused on the practitioner’s perception of complementary and alternative veterinary medicines (CAVM), for which diseases they would be interested in alternative therapies and products, as well as how many organic clients they had. The disease options given were mastitis, metritis, infertility, pneumonia, calf diarrhea, foot rot/digital dermatitis and the open ended choice of “other” diseases.

The same questionnaire was applied again as online survey (SurveyMonkey) in the spring 2010. Only the wording of the last question was changed from “Would you be interested in receiving information for a few specific products” to “Would you be interested in receiving more information about products allowed for use in organic herds?”. This time, bovine veterinarians who subscribed to the list-serve of the American Association of Bovine Practitioners (AABP-L) were invited via email. Veterinarians could provide their email address at the end of the survey, if they were interested in receiving more information regarding specific alternative therapies. The data was analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2010-2012). The significance level was set at \( \alpha = 0.05 \). The data was first summarized with frequency statistics and then comparisons between the 2 surveys were completed with Fisher’s exact statistics. Likewise, comparisons between practitioners that had organic clients and those without were also done with a Fisher’s exact test within years of the survey.

RESULTS

In the end, 181 (response rate: 24.1%) and 185 veterinarians completed the survey in 2006 and 2010, respectively. While the response rate was 24.1% in 2006, the response rate for 2010 could not be calculated as the exact number of subscribers from the United States was not available.

Approximately half of the participating veterinarians had organic dairy clients (Table 1). Of these veterinarians most (64%) had less than 3 organic clients, but one practitioner had over 100 organic clients (range 1-111 herds); the most commonly reported average herd size for organic dairy herds was less than 100 milking cows.

Many veterinarians were interested in using CAVM, but fewer showed interest in the use of CAVM or information about specific products in the second survey. However, in both years approximately 80% of veterinarians were interested in CAVM, if pharmacologic data were available for the alternative products or approaches. Veterinarians with organic clients were more interested in CAVM (interest yes: 68%, no: 5%) and specific alternative products (94% vs 78%, \( P < 0.01 \)) compared to those with no organic clients (interest yes: 43%, no: 10%; \( P < 0.01 \)). However, while in 2006 almost two-thirds of veterinarians thought that their conventional clients would be interested in CAVM, only 25% of veterinarians responded correspondingly in 2010. Yet in general few (16%) thought that none of their conventional clients would be interested in CAVM. This response was independent of them having organic clients or not (\( P = 0.74 \)).

Overall, most veterinarians indicated interest in alternative treatment approaches for all listed diseases and the interest in CAVM for all listed diseases increased significantly between the years (\( P < 0.01 \)). In both years the majority of practitioners were interested in CAVM approaches for mastitis, calf diarrhea and metritis (Table 2). The interest in CAVM for infertility, pneumonia and digital dermatitis or foot rot increased even by 23 to 24 percent points between surveys (\( P < 0.01 \)). In addition to the listed diseases, veterinarians also expressed interest in alternative treatments for coccidiosis (n=2), adult enteric diseases (n=1), cystic ovaries (n=1) and
acupuncture for downer cows (n=1) in 2006. Again, veterinarians with organic clients were more interested in CAVM for the different diseases than veterinarians with conventional clients only (P <0.01).

Further comments from veterinarians in 2006 and 2010 are listed in Table 3. Comments in 2006 mostly focused on interest in more scientific information (n=5) regarding efficacy, side effects or interactions as well as residue avoidance, that some of their clients considering to transition to organic (n=2) and the suggestion to use oregano for mastitis (n=1). The comments in 2010 were different. Two veterinarians strongly expressed their opinion opposed to organic farming (“Organic’ is somewhat of a lie [...]” and “The organic movement is crap [...]”) and their concern about the regulations, residues avoidance as well as animal welfare. Another comment also focused on their confusion regarding allowable substances under NOP regulations, while the other two comments broached the issues of management for disease prevention and acupuncture against pain on organic farms.

**DISCUSSION**

Although other studies have speculated about the interest of veterinary practitioners in alternative treatment approaches and their need for accompanying pharmacological data (Arlt and Heuwiser, 2014; Mathias 2004 & 2007), this is the first survey to quantify bovine veterinarians’ interest in CAVM over multiple years. The response rate was reasonable and probably fairly consistent between surveys as probably approximately the same number of people was initially invited through AABP-L. Both surveys were anonymous and so no direct comparison between answers of respondents was possible. Although the organic dairy industry is a niche market and increased only from 1 to 3% of the dairy industry between 2005 and 2010, (ERS, 2012) about half of the participants had at least one organic client. Therefore, there may have been an overrepresentation of participants with organic clients. However, this bias may be negligible as most veterinarians had less than 3 organic clients and the distribution of organic herd sizes of the clients mirrored the distribution of dairy herd sizes in the United States: most herds were milking less than 100 cows and approximately 6% of the organic clients’ herds had over 500 milking cows (NASS, 2007).

As expected, the majority of veterinarians were interested in using evidence-based CAVM in both years. However, several things are noteworthy. First, it is evident that the data supporting the efficacy of CAVM became more important to practitioners over the years: The number of positive respondents regarding the use of CAVM without mentioning specific efficacy data dropped significantly, most comments 2006 already focus on the need for more data on alternative practices and in 2010 most comments reflected the challenges veterinarians may face when trying to balance both NOP as well as FDA guidelines. Interestingly, 4% of respondents were absolutely against CAVM – even if data to support its use would be provided. The reason for this is unknown. One might speculate that those respondents were possibly still concerned about violating FDA regulations such as PMO (FDA, 2007) and AMDUCA (FDA, 1994), which prohibit the use of unapproved veterinary drugs on livestock operations. Similarly while veterinarians provided positive comments regarding herds transitioning to organic dairying in 2006, several comments in 2010 were rather opposed to organic farming, which, as aforementioned, may be the result of the veterinarians’ frustration trying to interpret and align several regulations and balance the wishes of clients with their professional ethics (Rollins, 2006; Ludbrook, 2007). Second, veterinarians with organic clients were more interested in alternative treatment approaches compared to those without. Veterinarians with organic clients probably want to remain a resource for animal health concerns to their clients while accommodating the different needs and management approaches of those organic clients before having to use NOP prohibited antibiotics or hormones.
Last, the interest in CAVM for the diseases listed in the survey increased over the years. It is not surprising that the need for alternative therapies for mastitis was indicated by over 80% of respondents. After all, mastitis therapy accounts for most used antibiotics on dairy farms in the United States (USDA, 2010). Furthermore, not all bacterial causes are susceptible to available antibiotics (Schukken et al., 2013) and various approaches to reduce antibiotic usage for mastitis have been discussed for years (Royster and Wagner, 2014). Similarly, the efficacy of antibiotics against calf diarrhea (Smith, 2014) and respiratory diseases of cattle (Fulton et al., 2009) is not guaranteed and alternative approaches are needed. In particular pneumonia is a cause of losses on dairy farms. It is the most common disease (5.4%) and cause of death for weaned heifers – almost half of all weaned heifer deaths are due to respiratory disease (46.5%) (USDA, 2009). Similarly, although, on average, only 3.3% of mature cows experience pneumonia, it is the 4th most common cause of mature cow losses on dairy farms (USDA, 2009) and veterinarians may want alternative therapy approaches to save those cows.

In conclusion, most veterinarians, but in particular veterinarians with organic clients, are interested in CAVM. The interest in alternative therapeutic approaches for common cattle diseases increased between the survey years, while the need for evidence-based approaches remained high in both years of the survey.

REFERENCES


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Rollins BE. Veterinary Medical Ethics. Can Vet J 2006; 47: 1064


USDA. National Organic Program. URL: http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&sid=3f34f4c22f91aa8e6d9864c2683cc02&tpl=/ecfrbrowse/Title07/7cfr205_main_02.tpl. 2015. Last Accessed: June 24, 2015


Table 1. Responses of veterinarians regarding their client base as well as their interest in complementary and alternative veterinary medicine (CAVM). Results are presented as frequencies of overall, 2006 and 2010 answers. The reported P-value is based on Fisher’s exact statistics and compares frequencies of 2006 and 2010 responses.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Overall N</th>
<th>Overall %</th>
<th>2006 N</th>
<th>2006 %</th>
<th>2010 N</th>
<th>2010 %</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you work with organic or transitioning-to-organic dairy herds?</td>
<td>No</td>
<td>174</td>
<td>48</td>
<td>81</td>
<td>45</td>
<td>93</td>
<td>50</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>192</td>
<td>52</td>
<td>100</td>
<td>55</td>
<td>92</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Average number of milking cows of organic clients</td>
<td>1-50</td>
<td>91</td>
<td>49</td>
<td>49</td>
<td>52</td>
<td>42</td>
<td>46</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>51-100</td>
<td>51</td>
<td>28</td>
<td>25</td>
<td>27</td>
<td>26</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>101-500</td>
<td>33</td>
<td>18</td>
<td>16</td>
<td>17</td>
<td>17</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1000</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td></td>
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<tr>
<td>Would you or your clients be interested in the use of CAVM?</td>
<td>No</td>
<td>26</td>
<td>7</td>
<td>9</td>
<td>5</td>
<td>17</td>
<td>9</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>192</td>
<td>52</td>
<td>100</td>
<td>55</td>
<td>92</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Would you be interested in CAVM products, if they are presented from a rational, pharmacologic basis?</td>
<td>No</td>
<td>14</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>291</td>
<td>80</td>
<td>144</td>
<td>80</td>
<td>147</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Would you feel that any of your conventional clients/herds would be interested in CAVM?</td>
<td>No</td>
<td>61</td>
<td>16</td>
<td>35</td>
<td>19</td>
<td>26</td>
<td>14</td>
<td>&lt;0.01</td>
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<td></td>
<td>Yes</td>
<td>161</td>
<td>44</td>
<td>115</td>
<td>64</td>
<td>147</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Would you be interested in receiving information for a few specific products (2006)/products allowed for use in organic herds (2010)?</td>
<td>No</td>
<td>51</td>
<td>13</td>
<td>8</td>
<td>4</td>
<td>43</td>
<td>23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>314</td>
<td>86</td>
<td>173</td>
<td>96</td>
<td>141</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Interest in complementary and alternative veterinary medication by bovine veterinarians for specific cattle diseases reported as overall, 2006 and 2010 responses as well as by veterinarians with or without organic clients. The reported P-value is based on Fisher’s exact statistics and compares frequencies of responses of 2006 and 2010 as well as those from veterinarians with and without organic clients.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Overall N</th>
<th>Overall %</th>
<th>2006 N</th>
<th>2006 %</th>
<th>2010 N</th>
<th>2010 %</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastitis</td>
<td>No 73</td>
<td>19</td>
<td>25</td>
<td>19</td>
<td>38</td>
<td>21</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Yes 293</td>
<td>80</td>
<td>146</td>
<td>81</td>
<td>147</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Calf Diarrhea</td>
<td>No 117</td>
<td>32</td>
<td>75</td>
<td>42</td>
<td>42</td>
<td>23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Yes 248</td>
<td>67</td>
<td>105</td>
<td>58</td>
<td>143</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Metritis</td>
<td>No 129</td>
<td>35</td>
<td>80</td>
<td>44</td>
<td>49</td>
<td>26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Yes 237</td>
<td>64</td>
<td>101</td>
<td>56</td>
<td>136</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Infertility</td>
<td>No 143</td>
<td>39</td>
<td>93</td>
<td>51</td>
<td>50</td>
<td>27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Yes 223</td>
<td>60</td>
<td>88</td>
<td>49</td>
<td>135</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>No 148</td>
<td>40</td>
<td>95</td>
<td>52</td>
<td>53</td>
<td>28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Yes 218</td>
<td>59</td>
<td>86</td>
<td>48</td>
<td>132</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Digital Dermatitis/Foot Rot</td>
<td>No 159</td>
<td>43</td>
<td>99</td>
<td>55</td>
<td>60</td>
<td>32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Yes 267</td>
<td>56</td>
<td>82</td>
<td>45</td>
<td>125</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Comments from veterinarians at the end of the survey either written in the margins of the postcard (2006 survey) or in the comment section of the “Thank you for participation” page in Survey Monkey.

<table>
<thead>
<tr>
<th>Additional Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Survey 2006</strong></td>
</tr>
<tr>
<td>A 1000 cow dairy is considering!</td>
</tr>
<tr>
<td>2 are considering (120 cows each)</td>
</tr>
<tr>
<td>Main interest would be residues (concern slaughter)</td>
</tr>
<tr>
<td>No testimonials as scientific evidence, please</td>
</tr>
<tr>
<td>Interested in side effects and interactions (with conventional meds) of CAVM products</td>
</tr>
<tr>
<td>Need more info to rationally reply</td>
</tr>
<tr>
<td>More scientific proves</td>
</tr>
<tr>
<td>Possibly oregano for mastitis</td>
</tr>
<tr>
<td><strong>Survey 2010</strong></td>
</tr>
<tr>
<td>“Organic” is somewhat of a lie. Exemptions exist, allowing deworming in “emergency” situations, but not for routine health maintenance. Unproven, and often ineffective, products are promoted as superior. The consumer gets screwed, the farms go broke, and the cows suffer. The organic movement is crap and it is set up to use government money to declare one citizen’s products as superior to another’s. This is not free market and is wrong. I have been working with just a few organic herds. As I’ve do more reading, I sometimes become more confused. For instance, I read that if a cow has a follicle, and you want her to show a heat, you can treat her with Folliculin. A quick internet search of Folliculin reveals that it is a synthetic estrogen. I feel like that shouldn’t even be legal for conventional herds. These are the kinds of questions I would appreciate clarification about. Thanks. The point is of production medicine is prevention. The diseases you list are all management induced. We shouldn’t have to treat. That is where our interest lies. This stimulates a question regarding how much is acupuncture used for pain management in these animals?</td>
</tr>
</tbody>
</table>
Flax for Fido and Seaweed for Kitty

The Growing Market for Herbal Pet Care in the United States

Health-conscious consumers are not only using botanicals in record numbers for their own well-being but, increasingly, they also are turning to herbal supplements for their pets. "What's good for the goose is good for the gander," and many herbs that show up on the supplement aisle, such as chlorophyll/chlorella (Chlorella vulgaris, Chlorellaceae) and flax (Linum usitatissimum, Linaceae), are being marketed for companion animals, mainly cats, dogs, and horses.

According to the labeling used by Chicago-based marketing firm SPINS, the market for "pet supplements" comprises two broad categories: supplements, which deliver the botanicals in what is usually a solid dosage form, and treats and snacks, which include the botanical or blend of botanicals as a part of a baked biscuit, cookie, or other tasty reward. The market so far shows uneven growth: for a 52-week period that ended August 9, 2015, SPINS recorded total aggregate sales of $43,044,385 across both categories, a 15% decrease from the same time period in 2014. However, the market in 2014 grew by 12% over 2013. These data include products sold in the natural, specialty/gourmet, and mass-market channels in the United States. They do not take into account sales from Whole Foods Market, which does not report its sales to marketing firms, or direct sales from businesses that sell solely on the Internet.

Natural Medicine Practice for Pets

While complementary and integrative therapies for animals, including herbal supplements, are gaining popularity and visibility in the United States, they are not new practices. Traditional Chinese veterinary medicine (TCVM), along with traditional Chinese Medicine for humans, has been practiced for thousands of years, using both herbal remedies and practices such as acupuncture, nutritional therapy, and therapeutic massage.

"Far and away, I used Chinese herbal formulations most often because that is where most of my training lies," wrote Clay Bernard, DVM, head veterinarian at Even Flow Veterinary Herbal and Acupuncture in Austin, Texas (email, October 28, 2015). "I see great results from them…. Many pet owners come to me looking for another option for treatment when conventional options have either been exhausted (or) unsuccessful.

The United States has several professional societies and training programs for the practice of integrative veterinary medicine, including the American Holistic Veterinary Medical Association, Veterinary Botanical Medicine Association, and the American College of Veterinary Botanical Medicine. "I think there are still a few skeptics among clinicians and pet guardians," Dr. Bernard admitted, "but the number seems to be shrinking. I think most people have seen results first-hand, or know someone whose animal has benefited from acupuncture, herbs, food therapy, chiropractic, or other means of natural healing."

The market is responding. In October 2015, the second annual Petfood and Animal Nutrition Conference was held in Chicago, Illinois. Exhibitors and speakers from all aspects of the animal nutrition industry, including natural supplements, were represented. In 2015, natural supplement brand NOW® Foods released its own line of pet supplements. In 2009, former HerbalGram Managing Editor Courtney Cavaliere examined the pet supplement market from a regulatory perspective in issue 82.1

According to Bill Bookout, president and chair of the board of directors of the National Animal Supplement Council (NASC), an industry trade organization, little change has been made regarding the regulatory status of animal supplements (oral communication, November 3, 2015). The Dietary Supplement Health and Education Act of 1994 (DSHEA) applies only to products intended for human consumption, and no parallel law creating a "dietary supplement" category for animal products exists. Therefore, products for animals are classified as either a "food" or a "drug." Legally, most animal supplements are food additives, said Bookout. Since they are not dietary supplements, they cannot be labeled with or advertise a structure/function claim.

Long-term solutions pertaining to the regulation of supplements for animals have been debated since the passing of DSHEA, and still are being sought at the state and federal levels. However, "the industry has been able to operate very successfully under a framework of enforcement discretion, provided companies act responsibly," said Bookout.

The Herbal Pet Supplement Market in the United States

For the past three years, chlorophyll/chlorella supplements have taken the first spot in both the supplement and treats/snacks categories (Table 1, Table 2). These ingredients primarily are used to support immune...
system function in humans, and have similar benefits for animals, including support of the digestive tract and oral health. Other ingredients that are popular across both categories include flax seed and/or oil, parsley (Petroselinum crispum, Apiaceae), and chamomile (Matricaria recutita, Asteraceae).

### Table 1: The 20 Top-Selling Herbal Supplements for Animals in the United States, August 2014 – August 2015 (per SPINS)*

<table>
<thead>
<tr>
<th>Botanical</th>
<th>Latin Binomial</th>
<th>Total Sales</th>
<th>% Change from 2014**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Chlorophyll/Chlorella</td>
<td>NA/Chlorella vulgaris</td>
<td>$4,548,754</td>
<td>-64.80%</td>
</tr>
<tr>
<td>2 Parsley</td>
<td>Petroselinum crispum</td>
<td>$3,246,821</td>
<td>5.89%</td>
</tr>
<tr>
<td>3 Peppermint and Other Mints</td>
<td>Mentha spp.</td>
<td>$1,281,459</td>
<td>7.94%</td>
</tr>
<tr>
<td>4 Chamomile</td>
<td>Matricaria recutita</td>
<td>$725,928</td>
<td>-15.29%</td>
</tr>
<tr>
<td>5 Garlic</td>
<td>Allium sativum</td>
<td>$495,399</td>
<td>-2.01%</td>
</tr>
<tr>
<td>6 Alfalfa</td>
<td>Medicago sativa</td>
<td>$285,237</td>
<td>2.79%</td>
</tr>
<tr>
<td>7 Flax seed and/or oil</td>
<td>Linum usitatissimum</td>
<td>$208,423</td>
<td>-24.17%</td>
</tr>
<tr>
<td>8 Lavender</td>
<td>Lavandula angustifolia</td>
<td>$134,844</td>
<td>-55.58%</td>
</tr>
<tr>
<td>9 Grass (Wheat or Barley)</td>
<td>Triticum aestivum or Hordeum vulgare</td>
<td>$75,906</td>
<td>-25.63%</td>
</tr>
<tr>
<td>10 Valerian</td>
<td>Valeriana officinalis</td>
<td>$51,419</td>
<td>22.78%</td>
</tr>
<tr>
<td>11 Mullein</td>
<td>Verbascum thapsus</td>
<td>$33,309</td>
<td>-0.37%</td>
</tr>
<tr>
<td>12 Cranberry</td>
<td>Vaccinium macrocarpon</td>
<td>$29,273</td>
<td>5.09%</td>
</tr>
<tr>
<td>13 Cayenne</td>
<td>Capsicum annuum</td>
<td>$21,572</td>
<td>-88.14%</td>
</tr>
<tr>
<td>14 Yucca</td>
<td>Yucca spp.</td>
<td>$12,828</td>
<td>-54.82%</td>
</tr>
<tr>
<td>15 Red Clover</td>
<td>Trifolium pretense</td>
<td>$4,733</td>
<td>1897.05%</td>
</tr>
<tr>
<td>16 Barberry</td>
<td>Berberis vulgaris</td>
<td>$3,735</td>
<td>-35.11%</td>
</tr>
<tr>
<td>17 St. John's Wort</td>
<td>Hypericum perforatum</td>
<td>$3,414</td>
<td>61.88%</td>
</tr>
<tr>
<td>18 Goldenseal</td>
<td>Hydrastis canadensis</td>
<td>$3,179</td>
<td>2272.39%</td>
</tr>
<tr>
<td>19 Skullcap</td>
<td>Scutellaria lateriflora</td>
<td>$2,887</td>
<td>N/A</td>
</tr>
<tr>
<td>20 Boswellin or Boswellia</td>
<td>Boswellia glabra</td>
<td>$2,885</td>
<td>-13.13%</td>
</tr>
<tr>
<td>All Other Herbs Total</td>
<td></td>
<td>$11,172</td>
<td></td>
</tr>
<tr>
<td>Total Sales</td>
<td></td>
<td>$11,183,177</td>
<td>-43.67%</td>
</tr>
</tbody>
</table>

*Source: SPINSscan Natural, SPINSscan Specialty Gourmet, and SPINSscan Conventional Multi-Outlet powered by IRI, 52 weeks ending August 9, 2015.
†Herb coded as primary ingredient.
**52 weeks ending August 10, 2014.

Flax seed and its oil contain alpha-linolenic acid, linoleic acid, and omega-3 fatty acids, which aid in the development and maintenance of the brain, liver, and heart. Animal models have shown that these are vital to the healthy development of young animals and may also improve their skin, coat, and nails.¹² Flax can be administered either in supplement form or the ground seeds and oil can be added directly to the animal’s food.¹¹ The consumption of parsley can improve gastrointestinal and urinary symptoms, as well as joint disorders, such as gout and arthritis. Chamomile can be used in the diet as a tea or tincture, or applied as a salve or ointment for a multitude of benefits. Given orally, chamomile acts as a mild sedative and gentle digestive tonic. Applied topically, chamomile preparations can relieve mild inflammation due to insect bites, allergies, or bacterial or fungal infections.¹³

SPINS data identified new or more robust sales for several ingredients in the supplement and treat market in 2015. The latest botanicals considered beneficial for pets include slippery elm bark (Ulmus rubra, Ulmaceae) and skullcap (Scutellaria lateriflora, Lamiaceae) supplements, and cranberry (Vaccinium macrocarpon, Ericaceae) has been added to treats. Slippery elm is commonly used in pets for its mucilaginous and anti-inflammatory properties, and can be administered in cases of gastrointestinal distress, such as diarrhea and constipation.¹⁴ Skullcap, considered a nervine tonic, can benefit animals as an analgesic and anti-spasmodic for
jittery conditions. Cranberry is a source of proanthocyanidins, antioxidants that give cranberry its dark red color. This makes it a useful addition to pet treats, which generally are given daily and can help maintain urinary tract health.

Table 2. The 15 Top-Selling Herbal Treats and Snacks for Animals in the United States, August 2014 – August 2015 (per SPINS)*

<table>
<thead>
<tr>
<th>Botanical†</th>
<th>Latin Binomial</th>
<th>Total Sales</th>
<th>% Change from 2014**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Chlorophyll/Chlorella</td>
<td>NA/Chlorella vulgaris</td>
<td>$20,623,873</td>
<td>1.02%</td>
</tr>
<tr>
<td>2 Flax seed and/or oil</td>
<td>Linum usitatissimum</td>
<td>$6,415,345</td>
<td>-44.64%</td>
</tr>
<tr>
<td>3 Parsley</td>
<td>Petroselinum crispum</td>
<td>$3,167,581</td>
<td>-12.89%</td>
</tr>
<tr>
<td>4 Alfalfa</td>
<td>Medicago sativa</td>
<td>$1,451,904</td>
<td>-0.07%</td>
</tr>
<tr>
<td>5 Chamomile</td>
<td>Matricaria recutita</td>
<td>$93,124</td>
<td>-70.01%</td>
</tr>
<tr>
<td>6 Chia seed or oil</td>
<td>Salvia hispanica</td>
<td>$24,320</td>
<td>309.77%</td>
</tr>
<tr>
<td>7 Turmeric</td>
<td>Curcuma longa</td>
<td>$22,937</td>
<td>-2.23%</td>
</tr>
<tr>
<td>8 Clove</td>
<td>Syzygium aromaticum</td>
<td>$20,092</td>
<td>136.96%</td>
</tr>
<tr>
<td>9 Cherry fruit</td>
<td>Prunus avium</td>
<td>$17,147</td>
<td>-15.11%</td>
</tr>
<tr>
<td>10 Ginkgo</td>
<td>Ginkgo biloba</td>
<td>$8,676</td>
<td>39.71%</td>
</tr>
<tr>
<td>11 Kelp</td>
<td>Laminaria digitate</td>
<td>$8,169</td>
<td>34.76%</td>
</tr>
<tr>
<td>12 Cranberry</td>
<td>Vaccinium macrocarpon</td>
<td>$2,729</td>
<td>N/A</td>
</tr>
<tr>
<td>13 Lemon balm</td>
<td>Melissa officinalis</td>
<td>$1,558</td>
<td>360.95%</td>
</tr>
<tr>
<td>14 Grass (Wheat or Barley)</td>
<td>Triticum aestivum or Hordeum vulgare</td>
<td>$1,522</td>
<td>-39.22%</td>
</tr>
<tr>
<td>15 Thyme</td>
<td>Thymus vulgaris</td>
<td>$873</td>
<td>-4.69%</td>
</tr>
<tr>
<td>All Other Herbs Total</td>
<td></td>
<td>$1,358</td>
<td></td>
</tr>
<tr>
<td><strong>Total Sales</strong></td>
<td></td>
<td><strong>$31,861,208</strong></td>
<td><strong>-15.11%</strong></td>
</tr>
</tbody>
</table>

*Source: SPINSscan Natural, SPINSscan Specialty Gourmet, and SPINSscan Conventional Multi-Outlet powered by IRI, 52 weeks ending August 9, 2015.
†Herb coded as primary ingredient.
**52 weeks ending August 10, 2014.

As with the human supplement market, consumers have concerns regarding the quality and purity of the products given to their animals. Bookout acknowledges the need for vigilant oversight and accountability to ensure consumer confidence. He called product quality “not a destination, but a journey,” and said that the NASC “supports a philosophy of continuous improvement.”
In an effort to ensure a trustworthy supply chain for manufacturers, the NASC has recently instituted its "Preferred Supplier Program," in which interested parties must provide the NASC with an ingredient profile sheet for each botanical, vitamin, or other product they produce. The standards are stringent, but manufacturers who qualify for the Preferred Supplier Program will be available for viewing to NASC members, along with their ingredient profiles, testing data, and regulatory inspection audits. Bookout said that the NASC consults with leaders in the supplement industry to determine what tests are necessary to maintain high quality and purity standards.

Off-Market Considerations: Cannabis

With a growing number of states legalizing medical and/or recreational marijuana (Cannabis sativa, Cannabaceae) use, it follows, of course, that products containing marijuana are becoming an increasingly popular — and dubiously legal — option for pet owners. Even in states with legal medical marijuana use, however, veterinarians cannot by law prescribe or recommend cannabis for animals (though in Nevada, that may soon change: a law, SB372, which was introduced to the legislature in March 2015, has a provision called "pot for pets"). Another option for the cannabis-minded pet owner is hemp and hemp products. Industrial hemp farming has been legal in Canada since 1998, and to date, 22 American states have legalized hemp production, as well. Unlike marijuana, hemp contains a negligible amount of the psychoactive compound tetrahydrocannabinol (THC) and cannot be used to produce psychoactive effects. Hemp can be processed as a source of fibers for rope, cloth, and paper, and its seed is a nutritious food product that contains protein, vitamin E, and the essential fatty acids omega-3 and omega-6.

Because of marijuana’s strict Schedule I classification in the United States, research into its efficacy for humans is minimal, and efficacy for animals even more so. However, anecdotal evidence from owners who used marijuana or marijuana products to ease their animal's end-of-life care, joint pain, or degenerative condition (including cancer) indicates the possibilities for future research. "I get asked about it at least weekly, so the demand is there," Dr. Bernard noted. "I’m all for ‘pot for pets’ and anything natural that can [facilitate] healing and eliminate/minimize pain or discomfort. I think there is still much to learn, however, about its use in the animal world…. To not explore that further would be a shame." But pet owner beware: cannabis pet products have no regulatory oversight from the US Food and Drug Administration (FDA), and marijuana does not always present a safe, non-toxic treatment option. Though no lethal human overdoses have been recorded, marijuana ingestion can be injurious or fatal to animals. States with legalized medical marijuana have seen increasing reports of marijuana toxicosis in pets. According to one study, it was responsible for the death of two dogs.

Conclusion

The practice of herbal medicine for animals has a millennia-long history, especially in the Chinese tradition. In the United States, the market for herbal pet products is so far uneven, but pointing towards a trend of growth overall. Pet owners increasingly are seeking out alternative therapies for their companion animals, embracing holistic practitioners and natural medicines — including medicinal marijuana. The growing mainstream interest and introduction of new products indicate that the US market for animal-oriented herbal remedies, though unstable at the moment, may be at the start of an impressive upswing.

—Hannah Bauman

References


American Pet Products Association 2015-2016 Pet Owners Survey

About APPA

The American Pet Products Association (APPA) is the leading U.S. not-for-profit trade association serving the interests of the pet products industry. Founded in 1958 with 35 member firms, APPA’s membership currently includes over 1,000 pet product manufacturers, importers, manufacturers’ representatives and livestock suppliers representing both large corporations and growing enterprises worldwide.

APPA was established to promote, develop and advance responsible pet ownership and the pet products industry. To this end, APPA supports industry-related market research, monitors and responds to industry legislation and regulation, and sponsors educational seminars, networking and PR opportunities, giving members the tools they need to make important business decisions. APPA also works closely with other major organizations dedicated to similar goals to accomplish these and other important objectives.

Each year, APPA hosts Global Pet Expo, the largest annual pet products trade show in the world. Global Pet Expo is the premier event in the pet products industry and enables APPA members to showcase their latest pet product lines.

2015 – 2016 APPA National Pet Owners Survey
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www.americanpetproducts.org

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Appendix X1 Industry Support:
Academics
Diplomates
Practitioners
Veterinary Students
Public
TO: American Board of Veterinary Specialties (ABVS)  
FROM: Cynthia A. Daley, Ph.D., California State University Chico, College of Agriculture

Dear ABVS members,

As a member of the consortium of University’s involved in organic dairy research and education, I am wholly supportive of the efforts of the American College of Veterinary Botanical Medicine (ACVBM) to increase the proficiency and competence of health professionals in field of ethno-veterinary medicine.

The need for well trained health professionals within the field of phyto-medicine has never been greater. The organic dairy industry has been one of the fastest growing segments of the $35 billion-dollar organic industry, according to USDA ARS, and is a prime candidate for the use of phyto-medicine as an approved approach within certified herds. University support for the transition within the dairy industry has been slow. The availability of trained veterinary professionals who understand alternative medicine as a means to support dairyman in the paradigm shift to organic has also been in short supply.

Even so, the herbal supplement industry has blossomed over the course of the last three decades into a multinational, multibillion dollar industry that includes both professional and trade organizations, national and international practice and research conferences. We now have specialized integrated medicine practices and clinics in pain management and adjunctive cancer therapy. Many conventional medical colleges have introduced CAM degree-level education programs which is also quite encouraging. Finally, there are now credible sources of funding through the U.S. National Institutes of Health (NIH) National Center for Complementary and Alternative Medicine (NCCAM; http://nccam.nih.gov/), and the Australian National Institute of Complementary Medicine (NICM; http://www.nicm.edu.au), so that researchers interested in pursuing careers in TCM or CAM can do so with the knowledge that funding will be available to support their work.

The field of ethno-veterinary medicine is at a critical stage in its evolution here in the US. There currently is no recognized group of experts with in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine. For that reason, I strongly urge the AVMA to create the charter for diplomats of the ACVBM, to represent veterinarians who are recognized specialists in herbal medicine. These experts are to be consulted regularly by veterinarians and representatives of industry for information regarding appropriate use of botanicals in animals. I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,
Cynthia A. Daley, Ph.D.,
Director of the Organic Dairy Education & Research Program
College of Agriculture
California State University Chico
Date: Friday, December 18, 2015  
TO: American Board of veterinary Specialist (ABVS)  
From: Auburn University College of Veterinary Medicine

Dear ABVS,
Thank you for the opportunity to write to you in support of botanical medicine within veterinary medicine. My name is Barbara Kemppainen, professor, Auburn University College of Veterinary Medicine (AUCVM) started working with botanical medicines in 2007. My interest in veterinary botanicals is primarily studying their effectiveness in treating diseased food animals that are being raised organically. Organic food is the fastest growing area of agriculture, and organic food animal products are the fastest growing aspect of organic food. In 2010 and 2011, I developed an elective course for our veterinary students entitled “Complementary and Alternative Veterinary Medicine”, [CAVN]. One of the junior veterinary students who completed the course wrote me a thank you note saying the course was her favorite of the courses she had completed at her I have been active in obtaining funds to support research on the botanical medicines that potentially reduce the effect of poisons on poultry. I have attached Appendix 1, which is an abstract entitled “Investigation of the sparing effects of American skullcap, Scutellaria lateriflora on aflatoxin-contaminated feed in broiler chickens”. The reason skullcap was chosen to test its ability to reduce aflatoxin’s adverse effects is that aflatoxin causes its toxic effects by anti-oxidants and anti-inflammatory effects. When the anti-oxidant effects of skullcap were measured and compared to about 30 different anti-oxidants, skullcap was among the 5 strongest anti-oxidant effects. This abstract was published at the Annual Conference of the American Council for Medicinal Plants, July 2011, Huntsville, AL.

I was also the Chair person for the session entitled “Bioactives – Animal Health Benefits”, at the meeting “the Annual Conference of the American Council for Medicinal Plants, July 2011, Huntsville, AL.”
In the last several months, I’ve had several journal editors send me manuscripts to review. John Richards, Associate Editor of “Journal of Veterinary Science & Animal Husbandry (JVSAH) asked me to review the manuscript entitled “Comparative Efficacy of Neem Leaves, Pineapple Leaves, and Levamisole Against Gastrointestinal Nematodiasis in Sheep.”.  
[Continuation from page 1]
In addition, the Editor of BioMed Central Complementary and Alternative Medicine, Dr. Vivek K. Bajpai “Pharmacokinetics and Bioavailability of Orthosiphon Ethanolic Extract and Nano Ethanolic Extract and its Nano Liposomes in Sprague Dawley Rats”.

Please contact me if you have any questions or need further information.
Sincerely,

Barbara Kemppainen

Dr. Barbara Kemppainen
Professor, AUCVM
Auburn AL 36832
Phone 334 844 5415, cell 334 750-2152
Appendix 1

Investigation of the sparing effects of a native medicinal plant (American skullcap, *Scutellaria lateriflora*) on aflatoxin-contaminated feed in broiler chickens. M. Lohani¹, B. T. Akingbemi¹, E. G. Welles¹, J. Right¹, F. Hoerr¹, K. S. Joiner¹, F. W. Van Ginkel¹, J. Hess², W. B. Berry², A. Similien², D. A. Shannon², G. E. Rottinghaus³, D. R. Ledoux⁴ and B. W. Kemppainen¹, ¹College of Veterinary Medicine, ²College of Agriculture, Auburn University, Auburn, AL, ³Veterinary Medical Diagnostic Lab, and ⁴College of Agriculture, Food, and Natural Resources, University of Missouri, MO, USA, 65205

Poultry are very sensitive to the toxic effects (poor performance, immunosuppression, and liver disease characterized by diffuse fatty changes) caused by chronic consumption of feed contaminated with low levels of aflatoxin (AF), a naturally occurring fungal toxin. American skullcap’s flavonoids (bioactive compounds) have potent anti-oxidant and anti-inflammatory actions that oppose oxidative damage and inflammation caused by AF. The objective of this research was to determine if American skullcap reduces damage caused by AF fed to chickens. Three different groups of chicken were given feed amended with a range of doses of dried American skullcap (50, 250 and 1250 mg/kg, BW) for six weeks; 3 treatment groups consumed feed amended with the same doses of American skullcap for 1 week, followed by five weeks of feed amended with the same doses of American skullcap and 1.4 PPM aflatoxin. Negative and positive control groups received basal diet and basal diet amended with AF. Results showed that highest dose of American skullcap (1250 mg/kg BW) was associated with toxic effect [lower (p<0.05) body weight after 42 days of exposure]. However, forty-two days of exposure to AF resulted in severe liver damage [glutamate dehydrogenase (GLDH), 9.8 IU/L ±1.99] that was not observed in chicks consuming AF and the highest dose of American skullcap (4.16±0.73). Additionally, the highest dose of American skullcap partially protected chickens from AF-induced liver weight (g liver/100 g BW); and as the dose of skullcap increased, there was a linear (R²=0.85) decrease in the AF-induced hepatic lipidosis. In conclusion, the highest dose of skullcap was associated with the adverse effect of lower body weight and beneficial effect of partially protecting chickens from AF-induced liver damage. The protective effect of skullcap on AF-induced liver damage could be due in part to skullcap’s flavonoids decreasing AF metabolism to its toxic form by the liver.

**Key words:** American skullcap, Aflatoxin, *Scutellaria lateriflora*, Broiler chickens.
DATE: January 4, 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: Meg M Sleeper VMD, DACVIM (cardiology)

Dear ABVS members,

I am writing to support the use of botanical medicine within veterinary medicine. I spent my sabbatical a few years ago learning about Traditional Chinese Veterinary Medicine (TCVM), specifically acupuncture and herbal treatment. I was very interested to learn how these modalities are being used in an integrative approach for an enhanced therapeutic effect. It was particularly interesting for me as someone coming from training in western veterinary medicine that many owners decline western medicine in favor of these other approaches. I firmly believe it is important to have veterinarians properly educated in the use of botanical medicines to promote safety and efficacy, avoid side effects of herbal-drug combinations and to be capable of communicating with owners about the strengths and weakness of all the treatment options that are available. Clearly TCVM and western veterinary medicine both have limitations and an integrative approach is probably the ideal.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession. With the rise of herbal medicines used in veterinary practice worldwide, there is a need for a recognized group of experts with in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research on herbal veterinary medicine, and for assisting pet owners in the responsible use of herbs for their pets. The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine and I support their effort to become a boarded specialty.

Sincerely,

Meg M. Sleeper VMD DACVIM (cardiology)
Dear Members of the American Board of Veterinary Specialties:

Please accept my endorsement of the proposed college of veterinary botanical medicine, which as proposed stands to recognize and elevate the scientific study of plant-based medicines and their derivatives. The prevalence of ethnopharmacologic interventions is well established in the scientific literature, and plants and their derivatives have served as the foundation of a number of important contributions to the veterinary medical pharmacopeia. The continued investigation and utilization of such substances requires a body of dedicated veterinarians with academic and experiential knowledge in the traditional uses of plants for veterinary and human health, as well as the scientific validation of their principles and therapeutic effects. The current College of Veterinary Clinical Pharmacology and the Board of Veterinary Toxicology may have some impact in these areas, but the lack of focus on the clinical utilization of these supplements and derivatives would stand in contrast to the proposed college described by the organizing committee of this particular current effort.

The petitioned college structure would allow for a repository of knowledge and qualified veterinarians which would inform the public, practicing veterinarians, and the human health field on the potential promise and toxicities of ethnopharmacologic agents. There are a number of studies which have highlighted the safety, efficacy, and adverse reactions from such substances in clinical patients. Surveys suggest that due to the unregulated nature of plant-based supplements, many of which are known to have drug-like effects, such products are often used by owners without the advisement of a veterinarian. Although the lack of appropriate queries as to whether these products are being administered by owners is likely one reason for this trend, there is a lack of suitable information or resources for veterinarians to consult. Therefore, one key advantage of the college would be an ability to disseminate and expand on the knowledge which is necessary to ensure patient safety and to identify areas where plant-based agents could influence the course of particular diseases.

A specialty in botanical medicine would easily draw on supporting knowledge from nutrition, toxicology, pharmacology, integrative medicine, and physiology. Training programs would therefore be academically rigorous in their foundation and in the application of clinical techniques. Undoubtedly, the organizing committee will submit a detailed list of charter members who can facilitate a critical mass of scientific study and training to support the goals of the college. The public would be served by an
increased number of specialists trained to offer *evidence-based* guidelines for the use of products, which are already given for a number of conditions. Ethnopharmacology holds additional promise of identifying ways to ensure the standardization or purification of products designed for animal use. Such products would be expected to be safer, their clinical effects more reliable, and the rate of contamination lower. As such compounds are also used in animal production, additional study would benefit human health as the impact of residues of such compounds in milk or meat remains largely unknown.

I strongly recommend the proposed college for approval given that it stands to provide more informed veterinary recommendations to the public, to serve a unique need in providing an organizational structure for botanical researchers and practitioners to advance scientific study, and to be distinct from the missions of the other approved specialties. The qualified founding members would be adequate to support a clear and scientifically-based training and education program in this area.

Please do not hesitate to contact me with any additional questions about my support of this endeavor.

Warm regards,

Justin Shmalberg DVM DACVN DACVSMR CVA CVFT CVCH
Clinical Assistant Professor of Integrative Medicine
Medical Director, Small Animal Hospital
College of Veterinary Medicine
University of Florida
shmalberg@ufl.edu
352.392.2235
DATE: November 23, 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: Robert B Hillman, Retired from Cornell University College of Veterinary Medicine

Dear ABVS members,

I am writing to support the use of botanical medicine within the veterinary medicine. Botanical medicine can be used to treat most conditions recognized by conventional medicine and many that are not. Botanical medicines are often prescribed to treat conditions for which there is no diagnosis or treatment available or in cases where conventional medical treatment has failed or is contraindicated.

Botanical medicines are especially helpful in the treatment of organ failure, chronic and geriatric diseases and cancer. They are often used to relieve pain, help protect and restore internal organ function, strengthen and support the immune system and reduce the dosage and frequency of conventional medications and to reduce their side effects.

Often a botanical and conventional medical prescription will be used in an integrative approach for an enhanced therapeutic effect. It is important to have veterinarians properly educated in the use of botanical medicines to promote safety and efficacy, avoid side effects of herbal-drug combinations and promote better treatment outcomes.

Organic producers are requesting botanical medications in lieu of antibiotics due to public demand for antibiotic free meat, milk and eggs. Also, to prevent the development of antibiotic resistant organisms from antibiotic overuse.

According to surveys done by the National Center for Complementary and Alternative Medicine (NCCAM), the use of botanical medications is steadily rising in the human sector and by extension more people are requesting botanical mediations to treat their pets in addition to themselves.

Due to these demands, it is incumbent upon the veterinary profession to educate veterinarians in the proper use of botanical medications, in order to safely prescribe them as a primary therapy or in combination with conventional drugs, reduce the incidence of adverse effects, and to educate clients and the veterinary profession as a whole on the safe use of botanical medicines.

Sincerely Yours,

Robert B. Hillman

Dr. Robert B. Hillman, BS, MS, DVM, DACT, CVA
Emeritus Sr Clinician
TO: American Board of Veterinary Specialties (ABVS)

Dear ABVS members,

Herbal medicine is of increasing interest to pet owners, pet professionals, and researchers. The American Botanical Council reported in September 2015 that sales of herbal supplements rose for the 11th consecutive year. As the ultimate authority in the care of animals, veterinarians must become proficient in the use of botanicals where clients request it, but more importantly, to help develop alternatives in an era of antibiotic resistance and other emerging medical challenges.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession.

The American Veterinary Medical Association’s policy on complementary, alternative, and integrative veterinary medicine (of which herbal medicine is considered a part) states that “veterinarians should have the requisite knowledge and skills for every treatment modality they consider using.” The largest corporate veterinary hospital chain in the US acknowledges that herbal medicine is a part of veterinary practice, but presents outdated information from a defunct website on the VCA practice website. Standard textbooks such as Ettinger’s Internal Medicine as well as the Merck Manual offer a chapter on herbal medicine, acknowledging the interest in use of herbs by the profession, yet provide little practical guidance on actual clinical uses of plant medicine.

The field of ethnoveterinary medicine, which brings scientific scrutiny to traditional practices, is gaining credence. Traditional herbal medicine has an established place in the management of livestock and food animals in third world countries, as reviewed by the Food and Agriculture Organization of the United Nations. A Medline search on the word “ethnoveterinary” yields 50 publications from 2013-2015 as compared to 30 from the period 2010-2012, and 24 from the period 2007 to 2009.

With the rise of herbal medicines used in veterinary practice worldwide, advanced training is now being offered. The College of Integrative Veterinary Medicine (online C.E. based in Australia) and the Chi Institute (Florida, USA) both offer Masters level training in specialty herb practice. Still, there is no recognized group of experts with such in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. Experts in this specialty are consulted regularly by veterinarians and representatives of industry and government for information regarding appropriate use of botanicals in animals. I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

Debra L. Zoran, DVM, PhD, DACVIM-SAIM
Professor of SA Internal Medicine
Medical Operations Supervisor, Texas A&M VET
College of Veterinary Medicine and Biomedical Sciences
Texas A&M University
References


August 20, 2016

To Whom It May Concern:

My name is Amanda Fulmer and I am a veterinary medical oncologist currently practicing in Greenville, SC. I am currently completing my final assignments to receive a graduate diploma in Veterinary Chinese Herbal Therapy. I have been a student of the course provided by the College of Integrative Veterinary Therapies for the past two years. My coursework has consisted of reviewing lectures, course notes, and reading published scientific research articles in the field of herbal medicine. This very thorough training has provided an excellent background in this ancient form of Eastern medicine, and has prepared me for adding this valuable treatment modality to my oncology practice.

I was trained conventionally in Western veterinary medicine and knew early in my veterinary career that I wanted to pursue internship and residency training. I achieved Diplomate status in 2007 and have practiced in large multi-specialty practices since that time. Throughout my time in practice, I have received questions from owners regarding alternative forms of therapy. Owners were interested in using herbal medicine either in addition to conventional chemotherapy or radiation or as an alternative to these forms of treatment. Over the years, I have found that owners were asking about herbal medicine more often and my patients were more frequently receiving herbal supplements that owners had discovered through online research or through recommendations from friends.

I became interested in training in herbal medicine for two primary reasons. I wanted to be able to answer questions about herbal therapies and supplements intelligently for owners, and also to learn whether they were truly safe for my patients. I also wanted to have alternative treatment options available for my patients. Conventional cancer therapy often results in limited outcomes, so the opportunity to provide treatment that could improve these outcomes is appealing. There are also many owners who, for various reasons, have no interest in pursuing chemotherapy or radiation therapy for their pets, but are willing to pursue more natural forms of treatment.

Since beginning the practice of herbal medicine, I have found that herbs have not only affected cancers directly, but also lessen chemotherapy side effects and alleviate anxiety in more anxious pets. I am also providing herbal therapy for patients throughout the hospital and have had significant success in managing chronic renal failure, severe skin disease, degenerative joint disease and immune-mediated disease. My training has also provided information regarding dietary and nutritional changes that have helped many of these patients experience improved quality of life. I have several patients receiving herbs as their sole therapy who have survived significantly longer than the expected average of a patient who receives no treatment for a particular disease.
Herbal medicine provides a safe, effective treatment option either in conjunction with or instead of conventional therapies. Herbs have very few side effects and are cost effective for most owners. I would fully support the movement to make Chinese Herbal Medicine a specialty under the AVMA. There is no other discipline that exploits the properties of entire plants and other organic materials to the benefit of people and animals. Herbalism allows practitioners to take advantage of the antioxidant, anti-inflammatory, anti-neoplastic, and other properties of these natural substances that have been used for thousands of years to treat and prevent disease.

My course faculty promotes responsible, conscientious methods of practice, including responsibly sourcing our herbs. We as students are taught the importance of data collection and moving towards case collaboration and scientific publication to provide evidence that herbal medicine is a safe and effective practice. At no time are we encouraged to eschew Western veterinary practices, but to work in tandem to provide an even more desirable outcome. As alternative medicine practices become more common for people, owners will continue to utilize herbal medicine and other alternative treatments for their pets. It is important for our profession to embrace alternative therapies as valid and to promote research and collaboration between general practitioners, specialists, and practitioners of alternative medicine. Veterinarians will appreciate having more management options for certain cases, while owners will benefit from the availability of multiple treatment choices.

If you need additional information, please feel free to contact me by email at oncovet@live.com or by phone at (571) 232-1520. I appreciate your consideration and can’t say enough about the value of the education I have received by pursuing this course of study.

With Regards,

Amanda Fulmer

Amanda Fulmer, DVM, DACVIM (Oncology)
Friday, January 22, 2016

TO: American Board of Veterinary Specialties (ABVS)

FROM: BC Phytoceutical Corporation

Dear ABVS members,

I am a veterinary researcher in Canada. I hold a PhD in veterinary science and a post doc in veterinary population medicine. Recently, I have received a significant grant from the veterinary phytoceutical industry to undertake research in Veterinary Herbal Medicine due to the high demand among Canadian veterinary clinics for evidence-based findings on the therapeutic use of herbal products.

In light of this growing interest in the industry, I am writing to support the mission of the American College of Veterinary Botanical Medicine (ACVBM) “to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine”. ACVBM proposes to fulfill a much-needed role for the profession.

The American Veterinary Medical Association’s policy on complementary, alternative, and integrative veterinary medicine (of which herbal medicine is considered a part) states that “veterinarians should have the requisite knowledge and skills for every treatment modality they consider using.” The largest corporate veterinary hospital chain in the US acknowledges that herbal medicine is a part of veterinary practice, but presents outdated information from a defunct website on the VCA practice website. Standard textbooks such as Ettinger’s Internal Medicine as well as the venerable Merck Manual offer a chapter on herbal medicine, acknowledging the interest in use of herbs by the profession, yet provide little practical guidance on actual clinical uses of plant medicine.

The field of ethnoveterinary medicine, which brings scientific scrutiny to traditional practices, is gaining credence. Traditional herbal medicine has an established place in the management of livestock and food animals in third world countries, as reviewed by the Food and Agriculture Organization of the United Nations. A Medline search on the word “ethnoveterinary” yields 50 publications from 2013-2015 as compared to 30 from the period 2010-2012, and 24 from the period 2007 to 2009.

In British Columbia, Canada, the number of veterinary practices offering herbal medicine is growing exponentially. Further, with the rise of herbal medicines used in veterinary practice worldwide, advanced training is now being offered. The College of Integrative Veterinary Medicine (online C.E. based in Australia) and the Chi Institute (Florida, USA) both offer Masters level training in specialty herb practice. Still, there is no recognized group of experts with such in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry and government for information regarding appropriate use of botanicals in animals. I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

Nadine Gourkow, PhD (Vet Sciences)

References


November 22, 2015

American Board of Veterinary Specialties (ABVS)

Donna M. Raditic DVM, DACVN, CVA
Nutrition and Integrative Medicine
Stamford, CT

Dear ABVS members,

Herbal medicine is of increasing interest to pet owners, pet professionals, and researchers. The American Botanical Council reported in September 2015 that sales of herbal supplements rose for the 14th consecutive year\(^1\). As the ultimate authority in the care of animals, veterinarians must become proficient in the use of botanicals where clients request it, but more importantly, to help develop alternatives in an era of antibiotic resistance and other emerging medical challenges.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession.

The American Veterinary Medical Association’s policy on complementary, alternative, and integrative veterinary medicine (of which herbal medicine is considered a part) states that “veterinarians should have the requisite knowledge and skills for every treatment modality they consider using.” The largest corporate veterinary hospital chain in the US acknowledges that herbal medicine is a part of veterinary practice, but presents outdated information from a defunct website on the VCA practice website\(^2\). Standard textbooks such as Ettinger’s Internal Medicine as well as the venerable Merck Manual offer a chapter on herbal medicine, acknowledging the interest in use of herbs by the profession, yet provide little practical guidance on actual clinical uses of plant medicine\(^3\).

The field of ethnoveterinary medicine, which brings scientific scrutiny to traditional practices, is gaining credence. Traditional herbal medicine has an established place in the management of livestock and food animals in third world countries, as reviewed by the Food and Agriculture Organization of the United Nations\(^4\). A Medline search on the word “ethnoveterinary” yields 50 publications from 2013-2015 as compared to 30 from the period 2010-2012, and 24 from the period 2007 to 2009.

With the rise of herbal medicines used in veterinary practice worldwide, advanced training is now being offered. The College of Integrative Veterinary Medicine (online C.E. based in Australia) and the Chi Institute (Florida, USA) both offer Masters level training in specialty herb practice. Still, there is no recognized group of experts with such in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry and government for information regarding appropriate use of botanicals in animals. I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

[Signature]

Donna M. Raditic DVM, DACVN, CVA
References


DATE: 12/31/15

TO: American Board of Veterinary Specialties (ABVS)

FROM: Oakland Veterinary Referral Services

Dear ABVS members,

I am writing to support the use of botanical medicine within veterinary medicine. Botanical medicine can be used to treat most conditions recognized by conventional medicine and many that are not. Botanical medicines are often prescribed to treat conditions for which there is no diagnosis or treatment available or in cases where conventional medical treatment has failed or is contraindicated.

Botanical medicines are especially helpful in the treatment of organ failure, chronic and geriatric diseases and cancer. They are often used to relieve pain, help protect and restore internal organ function, strengthen and support the immune system and reduce the dosage and frequency of conventional medications and to reduce their side effects.

Botanical medicines provide effective avenues of treatment for pets who may have no other safe or effective conventional options. For example, with the use of botanical medicines patients who are poor candidates for conventional cancer therapies routinely experience improved quality of life, disease stabilization and, in some cases, even tumor regression.

Additionally, for some botanical medicines there are no comparable pharmaceutical drugs. This is the case for botanicals such as San Qi (pseudoginseng), which is effective at controlling cancer related hemorrhage. Botanical medicines are also a promising addition to the antiangiogenic treatment approach for cancer, many of which have demonstrated antiangiogenic properties and immune modulating properties known to be important in tumor progression and metastasis.

Often a botanical and conventional medical prescription will be used in an integrative approach for an enhanced therapeutic effect. It is important to have veterinarians properly educated in the use of botanical medicines to promote safety and efficacy, avoid side effects of herbal-drug combinations and promote better treatment outcomes.

According to surveys done by the National Center for Complementary and Alternative Medicine (NCCAM), the use of botanical medications is steadily rising in the human sector and by extension more people are requesting botanical medications to treat their pets in addition to themselves.

This trend is observable in my clinical experience, where client interest and demand for integrative therapies and botanical medicines in cancer treatment has steadily increased over the past 10 years. I routinely see clients who would, without the option of consultation to advise on botanical medicine, administer these therapies to their pet without training or veterinary supervision.

Sincerely Yours,

-Page 1-
Due to these demands, it is incumbent upon the veterinary profession to educate veterinarians in the proper use of botanical medications, in order to safely prescribe them as a primary therapy or in combination with conventional drugs, reduce the incidence of adverse effects, and to educate clients and the veterinary profession as a whole on the safe use of botanical medicines.

Sincerely Yours,

Dr. Erin Bannink, DVM, DACVIM (oncology), GDVCHM, CVA
To: American Board of Veterinary Specialties (ABVS)
From: Margot Mercer, fourth year veterinary student, Oregon State University

Dear ABVS members,

Herbal medicine is of increasing interest to pet owners, pet professionals, and researchers. The American Botanical Council reported in September 2015 that sales of herbal supplements rise for the 11th consecutive year (ABC). As the ultimate authority in the care of animals, veterinarians must become proficient in the use of botanicals where clients request it, but more importantly, to develop alternatives in an era of antibiotic resistance and other emerging medical challenges.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession. As a veterinary student, and part of the future of our profession, I believe that organizations like the ACVBM will play a vital role in ensuring that veterinary practitioners stay well informed in a changing landscape of client desires.

The American Veterinary Medical Association’s policy on complementary, alternative, and integrative veterinary medicine (of which herbal medicine is considered a part) states that “veterinarians should have the requisite knowledge and skills for every treatment modality they consider using.” The largest corporate veterinary hospital chain in the US acknowledges that herbal medicine is a part of veterinary practice, but presents outdated information from a defunct website (altvetmed) on the VCA VCA 2015 practice website. Standard textbooks such as Ettinger’s Internal Medicine as well as the venerable Merck Manual offer a chapter on herbal medicine, acknowledging the interest in use of herbs by the profession, yet provide no practical guidance on actual clinical uses of plant medicine (Merck Manual online, 2015).

It is imperative that veterinary professionals have the appropriate knowledge base to provide advice regarding recommendations and/or prescriptions about plant-based medications. As with any prescribed therapy, herbal medicine is not benign; knowledge of the physiology of how plants interface with animal systems, herb-drug interactions, herb-disease interactions and herb-herb interactions are crucial for the safety of our patients. Without proper training and the backing of a reputable diplomat organization it is difficult to ensure that veterinarians are providing the appropriate advice and care.

The field of ethnoveterinary medicine, which brings scientific scrutiny to traditional practices, is gaining credence. Traditional herbal medicine has an established place in the management of livestock and food animals in third world countries, as reviewed by the Food and Agriculture Organization of the United Nations (FAO). A Medline search on the word “ethnoveterinary” yields 50 publications from 2013-2015 as compared to 30 from the period 2010-2012, and 24 from the period 2007 to 2009. Many organic livestock producers often turn to botanical medical treatments, and again it would behoove the veterinary community to not provide confident, evidence-based guidance under the umbrella of an organization such as the ACVBM to ensure the safety of those animals and our food chain.

With the rise of herbal medicines used in veterinary practice worldwide, advanced training is now being offered. The College of Integrative Veterinary Medicine (online C.E. based in Australia) and the Chi Institute (Florida, USA) both offer Masters-level training in specialty herb practice. Still, there is no recognized group of experts with such in-depth knowledge to whom the profession
and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry for information regarding appropriate use of botanicals in animals (Wynn). I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA and as a student on the cusp of joining the profession I look forward to the day where I may one day join the ACVBM as well.

Sincere thanks for your consideration,
Margot Mercer

References


Dear ABVS members,

With Dr. Google at a client’s fingertips, there are a ton of resources, both informational and misinformational available. As a veterinary student, almost veterinarian, I hope to have resources that I can turn to when I am unfamiliar with specific treatments that clients are either already using or inquiring about, or become an expert and well-versed in such treatments. The VBMA (Veterinary Botanical Medical Association) is an incredible resource that offers science and education should I choose to incorporate botanical medicine into my future practice. Most pharmaceuticals have been derived from plant materials. Having formal training in the safety, efficacy, indications, and contraindications for these plants would enable me to provide the best quality of care to my patients as possible. Rather than the stigma of “being a quack”, a specialty in Botanical Veterinary Medicine will empower me to have the most scientific evidence behind herbal practice when other standard treatments yield unsatisfying results.

Herbal medicine is of increasing interest to pet owners, pet professionals, and researchers. The American Botanical Council reported in September 2015 that sales of herbal supplements rise for the 11th consecutive year (ABC). As the ultimate authority in the care of animals, veterinarians must become proficient in the use of botanicals where clients request it, but more importantly, to develop alternatives in an era of antibiotic resistance and other emerging medical challenges.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession.

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The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry for information regarding appropriate use of botanicals in animals (Wynn). I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

Jodie Joseph  
CUCVM DVM Candidate 2017

References


DATE: 04. December. 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: VetImage West, Incorporated

Dear ABVS members,

Herbal medicine is of increasing interest to pet owners, pet professionals, and researchers. The American Botanical Council reported in September 2015 that sales of herbal supplements rose for the 11th consecutive year. As the ultimate authority in the care of animals, veterinarians must become proficient in the use of botanicals where clients request it, but more importantly, to help develop alternatives in an era of antibiotic resistance and other emerging medical challenges.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to Diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession.

The American Veterinary Medical Association’s policy on complementary, alternative, and integrative veterinary medicine (of which herbal medicine is considered a part) states that “veterinarians should have the requisite knowledge and skills for every treatment modality they consider using.” The largest corporate veterinary hospital chain in the US acknowledges that herbal medicine is a part of veterinary practice, but presents outdated information from a defunct website on the VCA practice website. Standard textbooks such as Ettinger’s Internal Medicine as well as the venerable Merck Manual offer a chapter on herbal medicine, acknowledging the interest in use of herbs by the profession, yet provides little practical guidance on actual clinical uses of plant medicine.

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With the rise of herbal medicines used in veterinary practice worldwide, advanced training is now being offered. The College of Integrative Veterinary Medicine (online C.E. based in Australia) and the Chi Institute (Florida, USA) both offer Masters level training in specialty herb practice. Still,
there is no recognized group of experts with such in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

I have personally been monitoring a feline patient with hypertrophic cardiomyopathy (HCM) in conjunction with Dr. Richard Palmquist over the past 18 months. Based upon my numerous measurements on this patient’s cardiac parameters, the HCM has significantly improved with herbal therapies only. This is very encouraging in my opinion knowing that feline HCM can improve significantly without the use of traditional Western medications. To date there has been no significant complications or unwanted side effects from the herbal therapies. I encourage the ABVS to seriously consider bringing the ACVBM into the specialty family of our profession.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry and government for information regarding appropriate use of botanicals in animals. I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

Sandy Sanford, DVM, DABVP (Canine/Feline)
President, VetImage West, Incorporated

References


DATE: December 9, 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: Veterinary Cancer Group

Dear ABVS members,

I am writing to you in support of the need for recognition of an additional subspecialty under the umbrella of the ABVS. As an oncologist, I am faced daily with the pet owning public expecting guidance from veterinarians regarding the use of botanicals and other complementary methodologies. When we are deficient in providing this expertise, pet owners will use every and any sources to guide them.

As a board certified doctor, this troubles me. Pet owners are more and more educated regarding the existence of specialists in veterinary medicine. Yet when it comes to the use of botanicals, there currently does not exist board certified specialists to guide them. Currently, any veterinarian can hang out a “shingle” announcing they are a holistic veterinarian, without any advanced training to guarantee a standardized proficiency.

The supplement industry is a multi-billion dollar industry and growing. We cannot lose sight of the fact these products are drugs. There is no regulation regarding the safety and appropriate use of these products. When pet owners are unable to get guidance, they purchase these botanicals and use them, sometimes to the detriment to their pet’s health. Some pet owners may be reluctant to share they are self medicating, leading to the potential for dangerous interactions of supplements and pharmaceuticals. We know the use of high doses of antioxidants will negate the effects of chemotherapy and radiation, for instance. Specialists in botanical medicine would help the pet owning public to choose manufacturers that produce pure, high quality products and use them in the safest, most appropriate ways.

Our Veterinary Oath is based on safeguarding animal health and welfare. To this end, we need to advance our scientific knowledge regarding the use of botanicals for supportive care and treatment of animals. The best way to do this is to recognize the College of Veterinary Botanical Medicine.

Please do not hesitate to contact me if you have additional questions.

Sincerely,

Mona Rosenberg, DVM, DACVIM (Oncology)
Chief of Staff
Veterinary Cancer Group
www.vetcancergroup.com
TO: American Board of Veterinary Specialties (ABVS)

FROM: Animal Vision Center  February 23, 2016

Dear ABVS members,

I am writing to support the use of botanical medicine within veterinary medicine. Botanical medicine can be used to treat most conditions recognized by conventional medicine and many that are not. Botanical medicines are often prescribed to treat conditions for which there is no diagnosis or treatment available or in cases where conventional medical treatment has failed or is contraindicated.

Botanical medicines are especially helpful in the treatment of organ failure, chronic and geriatric diseases and cancer. They are often used with the goal to relieve pain, help protect and restore internal organ function, strengthen and support the immune system and reduce the dosage and frequency of conventional medications and to reduce their side effects.

Often a botanical and conventional medical prescription will be used in an integrative approach for an enhanced therapeutic effect. It is important to have veterinarians properly educated in the use of botanical medicines to promote safety and efficacy, avoid side effects of herbal-drug combinations and promote better treatment outcomes.

Organic producers are requesting botanical medications in lieu of antibiotics due to public demand for antibiotic free meat, milk and eggs and to prevent the development of antibiotic-resistant organisms from antibiotic overuse.

According to surveys done by the National Center for Complementary and Alternative Medicine (NCCAM), the use of botanical medications is steadily rising in the human sector and by extension more people are requesting botanical medications to treat their pets in addition to themselves.

Due to these demands, it is incumbent upon the veterinary profession to educate veterinarians in the proper use of botanical medications, in order to safely prescribe them as a primary therapy or in combination with conventional drugs, reduce the incidence of adverse effects, and to educate clients and the veterinary profession as a whole on the safe use of botanical medicines.

Sincerely,

[Signature]
April 30, 2016

To: American Board of Veterinary Specialties (ABVS)
From: Dr. Lori Siemens

Dear ABVS Members,

As a veterinary cardiologist, I am always both grateful and relieved when I hear that a patient that I am treating for heart disease is also seeing an integrative veterinarian. The additional supportive care that they receive with herbal therapy, supplements, acupuncture, and diet clearly helps my patients' quality, and in my opinion, quantity of life. My clients benefit greatly too since they can see their pets thriving despite sometimes having advanced cardiac disease.

I am disheartened when I think about how little I know about herbal medicine; I received zero training in veterinary school and do not feel comfortable attempting to educate myself. Not only should veterinarians be given opportunities to attain a deeper understanding in this area of medicine but there should be specialists in botanical medicine to standardize and coordinate the dissemination of trustworthy educational material.

I would love to be able to become more familiar with the botanical therapy that my patients receive so I can feel more competent discussing it with my clients and their pets' other doctors, learn more about their benefits, and tailor my conventional therapy to better complement plant-derived treatments. I strongly support adding the American College of Veterinary Botanical Medicine to the list of specialties recognized by the AVMA.

Sincerely,

Lori M. Siemens, DVM
DACVIM (Cardiology)
May 22, 2016

Cynthia Lankenau, DVM
ACVBM

Dear Dr Lankenau,

I am a veterinary ophthalmologist in Jupiter, Florida. My patients include dogs, cats, horses, as well as many marine mammals including pinnipeds and cetaceans, as well as a few birds.

I have been using herbal extracts in the form of nutraceuticals to complement traditional therapy for over 15 years. I have found that these products have improved the healing of my patients, they have stabilized certain diseases including Progressive Retinal Atrophy and diabetic cataracts, when used consistently, and they have contributed to overall wellness in many other patients.

When I was part of the faculty of The Ohio State University’s College of Veterinary Medicine, my laboratory did funded research on grapeseed extract and lutein. The grapeseed extract work has been published in the American Journal of Veterinary Research. We also studied resveratrol.

I am such a believer in these ingredients and products that I co-created a veterinary and a human vision supplement called OcuGLO™. In addition to OcuGLO™, my associates and we also co-created veterinary products for diabetes, dementia, and weight loss.

If I can be of more help or be a part of your College, please let me know! I would be honored to be a part of your group!

Warmly,

Carmen MH Colitz, DVM, PhD
Diplomate, American College of Veterinary Ophthalmologists
February 16, 2016
American Board of Veterinary Specialties (ABVS)

Dear ABVS members;

I support the specialty of botanical medicine within the AVMA for a number of reasons.

After a number of years in general and emergency small animal medicine, I have been in a specialized field for 21 further years (anesthesia and analgesia) with a faculty position, scientific training, and clinical application of high quality medicine. I understand the scientific process and that herbal therapy can fit well into this process.

Client use of herbal therapy for themselves and their animals is commonplace and they are finding resources to self medicate. Laypeople are also starting to fill the gap of need by advising and providing herbal therapy to animal owners. This is inappropriate to good medicine and should be in the realm of veterinary oversight.

I now provide mostly holistic care and medicine for both small animals and equine. In this setting, herbal use is predominant, along with acupuncture and chiropractic. I see tremendous change clinically in patients that not only could be treated conventionally but mostly in those that cannot be such as in those with chronic degenerative or inflammatory diseases. These results are in the anecdotal realm but therapy would benefit and grow immensely with the guidance of specialty use and research. The AHVMA and its foundation has started some terrific research already as the basis for specialty medicine.

I have post-DVM training in all of these areas. I have taught many classes and workshops to veterinary students (and in post DVM certification programs), and see how readily the scientific training can be not only accepted but applied by burgeoning veterinarians in the basis of good scientific principles.

Just to emphasise, I daily use herbs, and successfully in my practice. I fully advocate more rigorous study and training in this already incredibly successful field for all of the above reasons.

Sincerely Yours,
Shauna Cantwell, DVM, MVSc, Diplomate ACVAA
www.shaunacantwell.com

Shauna Cantwell, DVM, MVSc, DACVAA
November 25, 2015

Re: Letter of Support For The American College of Veterinary Botanical Medicine

Dear ABVS members,

Herbal medicine is a massive field that bridges ancient and modern times. The American Botanical Council reported in 2015 that sales of herbal supplements continue to rise.¹ At the same time research is growing exponentially.² Because veterinarians have a legal and ethical position responsible for the overall care of animals, it is vital that our profession embrace and recognize the importance of botanical medicine. Our profession faces rising difficulties in managing antibiotic resistant infections and a host of chronic diseases for which herbal medicine is being used daily to relieve suffering and even bring about resolution.³ Research is booming in this area as it becomes a recognized gold mine of potential health solutions. The time has come for those using these agents to organize and associate in such ways that veterinary research is done and utilized to improve the quality of care available to animals in our country and the world.

I did not enter this field as a supporter. In fact, my first exposure to herbal medicine was seeing resolution in a case of biopsied, chronic, active hepatitis after conventional therapy failed. I contacted my veterinary school and was told that no evidence existed for herbal medicine in this case. It was embarrassing, to say the least, to advise clients that herbal medicine was "primitive" only to see the patient recover quickly once given milk thistle, a treatment we now recognize as effective. Since that time, I have developed an interest in botanical medicine and hold a graduate diploma in Chinese herbal medicine. Because of my training, many people travel long distances and lament that such professionals are not available in their area. I share their concern for this shortcoming in our profession.

The American College of Veterinary Botanical Medicine (ACVBM) formed to increase the competence and proficiency of veterinary health care professionals in the use of medicinal plants. This organization fully intends to develop into full-fledge diplomat status for the field. I support its efforts and see it filling a much needed function in the field of veterinary medicine. The college is dedicated in support of the American Veterinary Medical Association (AVMA) policy on complementary and alternative medicine, which states, "veterinarians should have requisite knowledge and skills in every treatment modality they consider using." An organization such as ACVBM is well equipped to offer quality, science-based continuing education, assist veterinarians in developing competence and understanding the use and abuse of botanicals in practice. Furthermore, ACVBM is serious about expanding properly done, high quality scientific research needed in the practice of modern veterinary medicine.

I am available to discuss this further as your group may need. Please support this application and encourage the group to fulfill its important mission in our profession.

Yours truly,
Richard Palmquist, DVM GDVCHM

References:


DATE: December 9, 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: Veterinary Cancer Group

Dear ABVS members,

I am writing to you in support of the need for recognition of an additional subspecialty under the umbrella of the ABVS. As an oncologist, I am faced daily with the pet owning public expecting guidance from veterinarians regarding the use of botanicals and other complementary methodologies. When we are deficient in providing this expertise, pet owners will use every and any sources to guide them.

As a board certified doctor, this troubles me. Pet owners are more and more educated regarding the existence of specialists in veterinary medicine. Yet when it comes to the use of botanicals, there currently does not exist board certified specialists to guide them. Currently, any veterinarian can hang out a “shingle” announcing they are a holistic veterinarian, without any advanced training to guarantee a standardized proficiency.

The supplement industry is a multi-billion dollar industry and growing. We cannot lose sight of the fact these products are drugs. There is no regulation regarding the safety and appropriate use of these products. When pet owners are unable to get guidance, they purchase these botanicals and use them, sometimes to the detriment to their pet’s health. Some pet owners may be reluctant to share they are self medicating, leading to the potential for dangerous interactions of supplements and pharmaceuticals. We know the use of high doses of antioxidants will negate the effects of chemotherapy and radiation, for instance. Specialists in botanical medicine would help the pet owning public to choose manufacturers that produce pure, high quality products and use them in the safest, most appropriate ways.

Our Veterinary Oath is based on safeguarding animal health and welfare. To this end, we need to advance our scientific knowledge regarding the use of botanicals for supportive care and treatment of animals. The best way to do this is to recognize the College of Veterinary Botanical Medicine.

Please do not hesitate to contact me if you have additional questions.

Sincerely,

Mona Rosenberg, DVM, DACVIM (Oncology)
Chief of Staff
Veterinary Cancer Group
www.vetcancergroup.com
DATE: November 23, 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: Chris Bessent, DVM, MSOM, Dipl.O.M, L.Ac.
CEO HerbsmithRX

Dear ABVS members,

I support the petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Herbal medicine is of increasing interest to pet owners, pet professionals, and researchers. The veterinary or pet markets strongly follow the lead of the human supplement markets. Just as humans are seeking "natural - less side effect"options for themselves they are also seeking similar options for their pets. Many herbal formulas and herbs are sold in veterinary practices, over the counter and via the internet and veterinarians need to be versed in herbs to safely guide pet owners. As the ultimate authority in the care of animals, veterinarians must become proficient in the use of botanicals where clients request it, but more importantly, to help develop alternatives in an era of antibiotic resistance and other emerging medical challenges

I am a Veterinarian, have a Masters degree in Oriental Medicine, am a Diplomat of Oriental Medicine (Herbal medicine and Acupuncture) for use in humans by the NCCAOM, (National Certification Commission for Acupuncture and Oriental Medicine) and Licensed Acupuncturist. The NCCAOM - Herbal Medicine in humans recognizes the standard of care and the importance of training and the diplomat status. I recommend the ABVS do the same via the American College of Veterinary Botanical Medicine (ACVBM) diplomat program.

The American Veterinary Medical Association's policy on complementary, alternative, and integrative veterinary medicine (of which herbal medicine is considered a part) states that "veterinarians should have the requisite knowledge and skills for every treatment modality they consider using." The mission of the ACVBM is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomat status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill a much-needed role for our profession.

With the rise of herbal medicines used in veterinary practice worldwide, advanced training is now being offered. The College of Integrative Veterinary Medicine (online C.E. based in Australia) and the Chi Institute (Florida, USA) both offer Masters level training in specialty herb practice. Still, there is no recognized group of experts with such in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry and government for information regarding appropriate use of botanicals in animals. I support their petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

Chris Bessent, D.V.M., M.S.O.M., Dipl. O.M., L.A.C
DATE: December 21, 2015

TO: American Board of Veterinary Specialties (ABVS)

Dear ABVS members,

I am writing to support the need for quality, practitioner-level botanical medicine education for veterinarians. Herbs are increasingly being used by pet owners, trainers and breeders. Veterinarians must be educated to understand both the benefits and concerns of these practices.

As an owner of an herbal products company that makes its products for humans, we are constantly being asked questions by pet owners, professionals and vets. The need for high quality, clinically based information is critical.

Those involved with animals are already using herbs. They want to understand the alternatives; the veterinary practice must keep pace with these changes. Herbal medicine can offer excellent support in normalizing body systems, especially in the area of digestion, discomfort, stress, skin health, urinary tract & kidney health, liver health, cardiovascular system and Immune system.

Herbal medicine is critically important in treating feedstock animals raised organically. Livestock owners without access to appropriate herbal medicine put their investment and right livelihood at risk.

Many vets have been learning about Asian herbal formulas through their acupuncture education, but they need a deeper understanding of how the components of these formulas work. When they truly understand how these formulas work, they are able to know when to add in additional support to increase effectiveness for an individual’s case.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. As such, the ACVBM proposes to fulfill this much-needed role for veterinarians.

Sincerely,

Elizabeth K. Lambert
CEO – Herbalist & Alchemist, Inc.
March 15, 2016

TO: American Board of Veterinary Specialties (ABVS)

Dear ABVS members,

We write to you today to ask that you consider recognition of botanical medicine as a specialty organization of the AVMA.

Our personal experience with our 10 year old Briard who was diagnosed with osteosarcoma, in a very difficult anatomical site, led us to the creation of a "dream team" comprised of extraordinarily gifted and caring practitioners from both Western and Eastern disciplines. This team of oncology radiology and nutritional specialties along with a master herbalist and acupuncture practitioner forged a care plan that allowed for the best possible quality of life, reduction in pain, and no loss of hair for our beloved family member.

So strong is our hope that others can benefit from this individualized and integrative approach, that we have endowed both The DeeDee Arrison Holistic and Integrative Wellness Seminar Series at Cornell University and The May Arrison Fund for Acupuncture.

Our goal is to combine the best of both worlds of medicine, to truly allow for complementary, integrative, and individualized treatment options for all veterinary clients and to increase the size of the tool box from which practicing veterinarians can draw to create the best outcomes for each of their patients.

The mission of the American College of Veterinary Botanical Medicine (ACVBM) is to increase the proficiency and competence of veterinarians in the use of medicinal plants, ultimately leading to diplomate status in the specialty of veterinary botanical medicine. This would be a great step forward and a real help for animals now and in the future.

Sincerely yours,

35 Lincoln Parkway   Buffalo, NY 14222   716-881-1761   karrison@mac.com
Dear American Board of Veterinary Specialties Board:

I am writing to support the recognition of the American College of Veterinary Botanical Medicine (ACVBM) as a specialty organization of the American Board of Veterinary Specialties.

Professional recognition is imperative to maintain highest standards in today’s climate for veterinarian services as so many unfounded claims for pet care exist on the Internet. Unsuspecting consumers often read the “testimonials” of these websites and fall prey to unsupported claims. This occurs at the expense of the pet owner, but more so, it ultimately costs the health and life of their pet family members.

Academic centers of excellence provide high quality, innovative academic-based care and advising that will enable vets and consumers to make sound decisions regarding educational, personal, and professional goals for the treatment of animals. The ACVBM is one organization diligently working towards making the goal of becoming an academic center for the advanced study of plant medicines a reality.

Recognition by the American Board of Veterinary Specialties will not only help veterinarians, but the animal feed and supplements industry by providing veterinarians who may be employed by such companies with better training and understanding how to utilize the safe use of herbs as feed supplements, herbal remedies to treat conditions, improve health and resistance to diseases.

I have been an internationally recognized advanced practice nurse and university professor nurse for over 35 years. Veterinary care is no different from human health care. The need for professional recognition of the ACVBM is similar to the credentialing and professional recognition required of health care professionals.

The bottom line is – do you want a quack taking care of you? The recognition of the ACVBM is no different from the professional recognition purposes for human health care. The ACVBM is seeking to assure the safety of veterinary practice and the public by:

- Standardizing training protocols and some of the treatment protocols, so that there is confidence that a veterinarian is well versed in veterinary botanical medicine, as a veterinarian treating animals and giving advice to the clients
- Assuring the referral of veterinarians and clients are made to a well-trained veterinarian in this area of medicine
- Assuring the lecturer giving a presentation is well trained in the subject being presented
Today’s “do it yourself” trends are geometrically on the rise:

- The public demand for alternative therapies has caused many to seek Dr. Google for advice which can be faulty and even dangerous
- The public demand for herbal remedies has caused many to seek the internet for advice on how to use them, many times from non-veterinarian sources

Professional recognition of the ACVBM would provide the hallmark for educated speakers to help conventional veterinarians understand how alternative therapies work and be able to adjust their treatment regiments. In the era of evidence-based medicine, there is no excuse for the replication of traditional untested treatments which may compromise the health of animals, safety and quality of veterinary care.

Furthermore, we are now at a crossroad where public knowledge of alternative medicine is increasing and conventional veterinarians often ridicule pet owners causing them to seek information elsewhere or a worse case occurs – the client does not tell their veterinarian what they are doing. This may complicate treatment plans or the unthinkable – cause damage at the expense of the animal’s life.

I strongly support the ACVBM’s diligence in working towards creating an academic center for the advanced study of plant medicines. Safe veterinary practices are the cornerstone of high-quality animal health care. Much of the work in establishing the bases for reducing adverse animal health outcomes lay in the objectives of the ACVBM:

1. Establishing requirements for post-doctoral education and experience pre-requisite to certification in the specialty of veterinary botanical medicine
2. Providing programs of required study including: phytochemistry, phytopharmacology, pharmacognasy, ethnopharmacology, ethnoverterinary medicine, traditional and cultural uses of herbal medicines, traditional/oriental medicine & western medicine herbology
3. Supporting scientific research in phytochemistry, phytopharmacology, phytopharmacodynamics, and toxicology
4. Examining and certifying veterinarians as specialists in Botanical Veterinary Medicine.

With the rise of herbal medicines used in veterinary practice worldwide, advanced training is now a societal requirement. There is no recognized group of experts with such in-depth knowledge to whom the profession and industry can turn for help in advancing the practice and research base on herbal veterinary medicine, or for assisting pet owners in the responsible use of herbs for their pets.

The charter diplomats of the ACVBM represent veterinarians who are recognized specialists in herbal medicine. These experts are consulted regularly by veterinarians and representatives of industry and government for information regarding appropriate use of botanicals in animals.

I support the ACVBM petition to obtain recognition of botanical medicine as a specialty organization of the AVMA.

Sincerely,

Alice M. Tse, PhD, RN, APRN, FAAN
Associate Professor of Nursing
TO: American Board of Veterinary Specialties (ABVS)

FROM: Department of Veterinary Medicine, College of Bioresource Sciences, NIHON UNIVERSITY

Dear ABVS members,

I am writing to support the use of botanical medicine within veterinary medicine. Botanical medicine can be used to treat most conditions recognized by conventional medicine and many that are not. Botanical medicines are often prescribed to treat conditions for which there is no diagnosis or treatment available or in cases where conventional medical treatment has failed or is contraindicated.

Botanical medicines are especially helpful in the treatment of organ failure, chronic and geriatric diseases and cancer. They are often used to relieve pain, help protect and restore internal organ function, strengthen and support the immune system and reduce the dosage and frequency of conventional medications and to reduce their side effects.

Often a botanical and conventional medical prescription will be used in an integrative approach for an enhanced therapeutic effect. It is important to have veterinarians properly educated in the use of botanical medicines to promote safety and efficacy, avoid side effects of herbal-drug combinations and promote better treatment outcomes.

Organic producers are requesting botanical medications in lieu of antibiotics due to public demand for antibiotic free meat, milk and eggs. Also, to prevent the development of antibiotic resistant organisms from antibiotic overuse.

According to surveys done by the National Center for Complementary and Alternative Medicine (NCCAM), the use of botanical medications is steadily rising in the human sector and by extension more people are requesting botanical medications to treat their pets in addition to themselves.

Due to these demands, it is incumbent upon the veterinary profession to educate veterinarians in the proper use of botanical medications, in order to safely prescribe them as a primary therapy or in combination with conventional drugs, reduce the incidence of adverse effects, and to educate clients and the veterinary profession as a whole on the safe use of botanical medicines.

Sincerely Yours,

[Signature]

Dr Hiroshi KOIE, DVM, Ph.D
DATE: December 19th, 2015

TO: American Board of Veterinary Specialties (ABVS)

FROM: Ryan Guldenpfennig, DVM

Dear ABVS members,

I am writing to support the use of botanical medicine within veterinary medicine. Botanical medicine can be used to treat most conditions recognized by conventional medicine and many that are not. Botanical medicines are often prescribed to treat conditions for which there is no diagnosis or treatment available or in cases where conventional medical treatment has failed or is contraindicated.

Botanical medicines may be helpful in the treatment of organ failure, chronic and geriatric diseases and cancer. They are often used to relieve pain, help protect and restore internal organ function, strengthen and support the immune system and reduce the dosage and frequency of conventional medications and to reduce their side effects.

Often a botanical and conventional medical prescription will be used in an integrative approach for an enhanced therapeutic effect. It is important to have veterinarians properly educated in the use of botanical medicines to promote safety and efficacy, avoid side effects of herbal-drug combinations and promote better treatment outcomes. Many veterinarians are asked about botanicals as therapeutic adjuncts but they are unfamiliar with their use. A reliable and consistent source would benefit inquiring veterinarians and their clients.

According to surveys done by the National Center for Complementary and Alternative Medicine (NCCAM), the use of botanical medications is steadily rising in the human sector and by extension more people are requesting botanical medications to treat their pets in addition to themselves.

Due to these demands, it is incumbent upon the veterinary profession to educate veterinarians in the proper use of botanical medications, in order to safely prescribe them as a primary therapy or in combination with conventional drugs, reduce the incidence of adverse effects, and to educate clients and the veterinary profession as a whole on the safe use of botanical medicines.

Sincerely Yours,

Dr. Ryan Guldenpfennig, DVM
Iowa State University, Class of 2007
To whom it may concern,

This is a letter of support for Botanical Medicine to be accepted as a valid and reliable practice in the Western Biomedical veterinary community today. This would mean recognition by the AVMA of the efficacy and usefulness of Botanical Medicine. I feel that this recognition should come soon.

Please let me share a little of my background to put this issue in perspective. I received both my Master of Science in Animal Behavior and my Doctorate of Veterinary Medicine degrees from the University of Florida. While attending the university, one of my older brothers was enrolled in the College of Pharmacy where he received his PharmD. I am a very curious person by nature, and found myself reading books from his courses quite often. I was very intrigued by one book, in particular, that was titled Pharmacognosy. For those of you unfamiliar with Pharmacognosy, it is the study medicinal drugs derived from the herbs. As many of you know, over 70% of our modern drugs are directly or indirectly derived from herbs. They are generally derived from what are known as secondary defensive compounds in plants (e.g. cardiac glycosides) which the plants have apparently evolved in their tissues to protect them against consumption by herbivores.

Why am I mentioning this now? Simply because plants MUST HAVE pharmacological activity for there to be the possibility of deriving drugs from them. And if plants DO have such pharmacological activity, then the plants in their natural or semi-processed states would seemingly be candidates for both further research and use as drug-like agents in veterinary practice. To me, at least, this seems like a completely logical conclusion from the previous brief discussion.

Are my comments just a Botanical Medicine fantasy? For the last decade I have been an adjunct senior lecturer at Murdoch University College of Veterinary and Biomedical Sciences, an AVMA-recognized school in Perth, Western Australia. I also had a clinical practice in Murdoch’s Veterinary Teaching Hospital for 7 years, in which I practiced Traditional Chinese Veterinary Medicine (TCVM). The main treatment tools in my practice were Acupuncture and Herbal Medicine. My practice was busy, successful, and accepted by my Western Biomedical-practice colleagues because I had excellent results with some of the most complex cases that we see in Veterinary Teaching hospitals such as cancer, immune-mediated diseases, chronic infections, fever of unknown origin, chronic immune deficiency, chronic arthropathies, etc. Again, for emphasis, my tools were acupuncture and botanical medicines.

Did I use plants from my backyard to treat my patients? I would like to say that I am a “down-to-earth” herbalist with my hands in the soils, but I am not. What is true is that there are large, well-regulated industries which procure, test, process, and sell herbal products for both human and veterinary practice. And Australia, which has some of the strictest biosecurity laws on the planet, has approved many of these products for importation and use in Australia.

The manufacturers of the best products test the incoming raw herbs after having trained experts check the actual materials for their botanical identity. They then use mass spectography and gas
chromatography procedures to identify “fingerprints” or biomarkers of the most important pharmacologically active ingredients to assure themselves that the raw product will be useful when processed. The processing is a highly controlled procedure done in “clean rooms” under very closely observed conditions. Samples are saved and may be used for future investigation if there is any problem with the final manufactured product. The Botanical Medicines are, in fact, treated in a similar fashion as our most prized pharmacological agents used by Western Biomedical practitioners.

All of this is to say three rather simple things. First, it should be clear to everyone involved that many plants contain compounds that have pharmacological activity. And this pharmacological activity may be taken advantage of by utilizing plant products in Veterinary practice. This use, if by properly trained veterinarians in correctly diagnosed patients with quality products, can be at least as powerful and commonly more safe than the use of western biomedical drugs. Lastly, there already exists a well-regulated and highly respected industry of Botanical product manufacturers. The Botanical product industry makes the product, the animals can benefit from use of the product, and if trained veterinarians are available and supported by the AVMA, these Botanical products will be utilized and investigated by scientists such as ourselves. If we fail to grasp this opportunity, then such use will surely fall into the hands of the poorly-trained lay practitioner.

If you have any questions or comments, please feel free to contact me.

Sincerely,

Bruce Ferguson, DVM, MS
Arbeitskreis Phytotherapie der Gesellschaft für Ganzheitliche Tiermedizin (www.ggtm.de)

Dear Mr. Banasiak,

We are writing in support of the petition to establish Veterinary Botanical Medicine as a registered Veterinary Specialty.

In 1993 the Academy for Education of veterinarians (Akademie für Tierärztliche Fortbildung, ATF) which is responsible for the regulations for postgraduate qualification of veterinarians in Germany, established an education in veterinary phytotherapy because there was growing interest in medicinal plants and herbal medicine for the treatment of animals. Today, 24 years later, the interest of German veterinarians and pet owners in herbal medicine is still growing.

Since 2000 the society for holistic medicine (GGTM, number of members: 770) has been holding an annual congress on which phytotherapy is an important part in seminars and talks.

For years we notice that there is an increase in participants because veterinarians are more often confronted with limitations of academic medicine. It is not only the wish of many pet owners to treat their animals with plants, but there are more civilization diseases where conventional medicine does not offer viable treatment strategies.

In Europe, especially in Germany, there is a discussion going on how to minimize antibiotics. Phytotherapy can also be used during this dilemma, because in some diseases we can rely on the effectiveness of herbal medicine. In severe cases it is possible to render multi-resistant bacteria vulnerable to antibiotics again when given in combination with plants.

We expect that in all parts of the world the interest in medicinal plants and herbal medicine for the treatment of animals is growing.
The founding of the networking group on “Medicinal Plants and Natural Products in Animal Healthcare and Veterinary Medicine” within the “Society for Medicinal Plant and Natural Product Research” (Gesellschaft für Arzneipflanzen- und Naturstoff-Forschung, GA, a scientific society founded 1953 and meanwhile counting more than 1'200 members in more than 90 countries of the world) in 2013 and the following inclusion of the animal health theme as one of the main topics of the annual international GA-Congresses might be one more important indicator for this.

With regard to these aspects we strongly support the petition of the American College of Veterinary Botanical Medicine. There is a need for botanical veterinary medicine and we would like to ask you to add it to the list of recognized veterinary specialities.

With Kind regards

Dr. Caecilia Brendieck-Worm
Leiterin des Arbeitskreis Phytotherapie

Dr. Heidi Kübler
Vorstandsvorsitzende GGTM e.V.

C. Brendieck-Worm  Dr. Heidi Kübler
Dear David Banasiak

The interest of Swiss veterinarians in herbal medicine (in Switzerland called “Phytotherapie”) is growing. Within the more than 750 members counting Swiss Medicinal Society for Phytotherapy (Schweizerische Medizinische Gesellschaft für Phytotherapie; SMGP, www.smgp.ch) a veterinary section (SMGPvet) was founded in 2007. In the year 2010 the section for complementary and alternative medicine (Schweizerische Tierärztliche Vereinigung für Komplementär- und Alternativmedizin; camvet.ch, www) of the Swiss Veterinary Society (Gesellschaft Schweizer Tierärztinnen und Tierärzte; GST) included phytotherapy as an own therapeutic method.

In close cooperation of the SMGP (which offers the advanced education) and GST (which is responsible for the regulations for postgraduate qualification of veterinarians in Switzerland) a regulation for the qualification title “Fähigkeitsausweis Veterinärphytotherapie GST” was established in 2012. This is to recognise the need for qualifications and specialisation in this area.

We expect that in all parts of the world the interest in medicinal plants and herbal medicine for the treatment of animals is growing. The founding of the networking group on “Medicinal Plants and Natural Products in Animal Healthcare and Veterinary Medicine” (please find some information about this group attached) within the “Society for Medicinal Plant and Natural Product Research” (Gesellschaft für Arzneipflanzen- und Naturstoff-Forschung, GA, a scientific society founded 1953 and meanwhile counting more than 1’200 members in more than 90 countries of the world) in 2013 and the following inclusion of the animal health theme as one of the main topics of the annual international GA-Congresses might be on more important indicator for this.

With regard to these aspects we strongly support the petition of the American College of Veterinary Botanical Medicine.

With Kind regards

Roger Eltbogen
President SMGP

Michael Walkenhorst
Head of the veterinary section SMGPvet
Dear Mr. Banasiak,

I am writing a letter for supporting the existing ACVBM petition in favor of creating a formal board certification in botanical medicine.

Medicinal herbs have been used as a core part of Complementary and Alternative Medicine for humans and animals for a long history. In addition, medicinal herbs are a core part of my CAM practice, College of Veterinary Medicine, University of Minnesota.

Please consider my supporting letter for ACVBM.
If you need further information, please feel free to contact me.

Sincerely,

Keum Hwa Choi
DVM, PhD, MSOMD, LAc, Associate Professor
Dipl. NCCAOM
Section chief of Complementary & Alternative Medicine
College of Veterinary Medicine
University of Minnesota
January 21, 2016

Dr. Signe Beebe
AVMA

Dear Dr. Signe Beebe,

On behalf of the Northeast Organic Dairy Producers Alliance (NODPA) and the Midwest Organic Dairy Producers Alliance (MODPA) we are writing to you to express our enthusiastic support for the creation of the American College of Veterinary Botanical Medicine. There is a great need for this specialty within the organic farming community.

NODPA is the largest organic dairy producer organization in the country with a membership of eight hundred and thirty six organic dairy producers in the Eastern US. NODPA’s mission is to “enable organic dairy family farmers, situated across an extensive area, to have informed discussion about matters critical to the well being of the organic dairy industry as a whole.” NODPA is not aligned with any one processor or cooperative and therefore is able to represent the views and needs of many different farmers.

MODPA represents organic dairy producers in WI, MN, ND, SD, IA, NE, KS, MO, IL, and MI with the mission to promote communication and networking for the betterment of all Midwest organic dairy producers and enhance a sustainable pay price.

In the experience of our organic dairy farmers learning to manage herd health issues within the confines of organic management is a very steep learning curve. Our farmers will always be grateful to the pioneering vets in this field who helped our farmers with our herd health problems and emergencies through internet lists, phone calls, and a few excellent books.

Liz Bawden, President, NY
Kirk Arnold, Vice President, NY
Steve Morrison, Secretary, ME
George Wright, Treasurer, NY
Henry Perkins, Past President ME

Craig Russell, VT
Rick Segalla CT
Steven Russell, ME
Morvan Allen, MA
Ryan Murray, NY
Ed Zimba, MI
Darlene Coehorn, WI
Bruce Drinkman, WI
Andrew Dykstra, WI

State Reps:
Siobhan Griffin, NY
Arden Landis, PA
Cindy-Lou Amey, NH
Robert Moore, NY
Bonnie and Tom Boutin, VT
Jeep Madison, VT
Aaron Bell, ME
John Gould, NY
John Stolzfus, NY
Dana Sgrecci, NY
Rodney Martin, VA
Roman Stolzfoos, PA

Policy Committee
Kathie Arnold, NY

Executive Director
Ed Maltby

Webmaster/Newsletter layout
Chris Hill

Media Editor, Membership and Event Coordinator
Nora Owens

NODPA’s Mission

To enable organic dairy family farmers, situated across an extensive area, to have informed discussion about matters critical to the well-being of the organic dairy industry as a whole.
The truth is that most of our farmers live in areas that are simply not served by veterinarians with interest or skills in botanical/holistic medicine, especially in large animal practices. When there is an animal with a health issue, our farmers call their vets to diagnose the issue, then they explain to our farmers what they would normally prescribe or suggest on a conventional dairy. Our farmers are left to decide between the conventional treatment (most often this would be using drugs that are not allowed in organic production like antibiotics or steroids), or something else. But it is usually up to the farmer to research what that “something else” is to be.

One of the greatest possible outcomes would be that the newly formed College for Veterinary Botanical Medicine and its members and graduates could serve as a real source of information and experience. They could be the consultants for veterinarians not experienced in alternative forms of treatment --- so that there is better information used in determining the best treatment for an ailing animal.

Organic dairy is certainly a growing industry, with increases in sales of between 7-9 % per year and we believe this need will only continue to expend.

We strongly support the new American College of Veterinary Botanical Medicine as a board recognized specialty.

Thank you for your consideration. Please contact Ed Maltby if you require any more information.

Sincerely

[Signature]

NODPA Board Chair and New York organic dairy farmer

[Signature]

Darlene Coehoorn, MODPA President

[Signature]

NODPA Executive Director
January 21, 2016

Dr. Signe Beebe

AVMA

Dear Dr. Signe Beebe,

On behalf of the Northeast Organic Dairy Producers Alliance (NODPA) and the Midwest Organic Dairy Producers Alliance (MODPA) we are writing to you to express our enthusiastic support for the creation of the American College of Veterinary Botanical Medicine. There is a great need for this specialty within the organic farming community.

NODPA is the largest organic dairy producer organization in the country with a membership of eight hundred and thirty six organic dairy producers in the Eastern US. NODPA’s mission is to “enable organic dairy family farmers, situated across an extensive area, to have informed discussion about matters critical to the well being of the organic dairy industry as a whole.” NODPA is not aligned with any one processor or cooperative and therefore is able to represent the views and needs of many different farmers.

MODPA represents organic dairy producers in WI, MN, ND, SD, IA, NE, KS, MO, IL, and MI with the mission to promote communication and networking for the betterment of all Midwest organic dairy producers and enhance a sustainable pay price.

In the experience of our organic dairy farmers learning to manage herd health issues within the confines of organic management is a very steep learning curve. Our farmers will always be grateful to the pioneering vets in this field who helped our farmers with our herd health problems and emergencies through internet lists, phone calls, and a few excellent books.

Liz Bawden, President, NY
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NODPA’s Mission

To enable organic dairy family farmers, situated across an extensive area, to have informed discussion about matters critical to the well-being of the organic dairy industry as a whole.
The truth is that most of our farmers live in areas that are simply not served by veterinarians with interest or skills in botanical/holistic medicine, especially in large animal practices. When there is an animal with a health issue, our farmers call their vets to diagnose the issue, then they explain to our farmers what they would normally prescribe or suggest on a conventional dairy. Our farmers are left to decide between the conventional treatment (most often this would be using drugs that are not allowed in organic production like antibiotics or steroids), or something else. But it is usually up to the farmer to research what that “something else” is to be.

One of the greatest possible outcomes would be that the newly formed College for Veterinary Botanical Medicine and its members and graduates could serve as a real source of information and experience. They could be the consultants for veterinarians not experienced in alternative forms of treatment --- so that there is better information used in determining the best treatment for an ailing animal.

Organic dairy is certainly a growing industry, with increases in sales of between 7-9 % per year and we believe this need will only continue to expend.

We strongly support the new American College of Veterinary Botanical Medicine as a board recognized specialty.

Thank you for your consideration. Please contact Ed Maltby if you require any more information.

Sincerely

Darlene Coehoorn, MODPA President
I am writing in support of the new American College of Veterinary Botanical Medicine as a board recognized specialty.

We own and operate a certified organic farm and over the last 20 years have used a variety of botanicals for the health and well-being of our livestock.* There is significant and growing need for this expertise by natural and organic-minded livestock producers, especially among conventional dairy farmers transitioning to organic certification who've long relied upon allopathic medicine and fast-acting pharmaceuticals. Complementary veterinary medicine needs to keep pace with the steadily rising demand for organic dairy and livestock products, as well as with increasing consumer awareness and expectations regarding animal welfare on all farms.

A continuing challenge that dates back to our certification in 1996 is the need for holistic veterinary care and expertise that is consistent with our natural approach to farming, and ideally is supported by evolving organic, grassfed and animal welfare standards. In the early years we were largely on our own for livestock health care, relying upon a limited selection of foreign books to help us manage our organic dairy and beef herd. Thankfully in the last decade additional veterinary texts have been published in this country and internet access has made online resources available, but none of this takes the place of veterinarians coming out to the farm to see and treat their patients. Many animals would likely have remained on our farm and in organic production if we were fortunate enough to have an "organic vet" in our region all these years.

The closest "alternative farm vets" are several hours away and don't serve our rural area. We are fortunate to have a veterinary center that is "open to alternatives" and has taken an interest in serving the organic community (due in part to the large number of certified organic dairies in their service area), but their expertise is limited. They've told us vets in their practice would attend training to broaden this understanding if education credits were available and they didn't have to travel halfway across the country to get them.

Our farm is located near Cornell's Veterinary School, where we understand there is growing interest among students in complementary approaches - but they are not taught. We’re told that students determined to learn them must seek out alternative practitioners to intern with, but that there aren’t enough such vets available. As the number of large animal veterinarians continues to decline we need to do more to nurture and meet the needs of those veterinary students whose interests extend to herbal and alternative therapies.
Botanicals are an excellent alternative to the pervasive dependance and overuse of antibiotics and other drugs in the dairy and livestock industries. A strong economic case for farmers can be made for the reduced withholding times on botanicals vs. pharmaceuticals. There will be significant public benefit if botanicals can replace more of the antibiotic use in the livestock sector. Cattle in particular respond well to herbal treatments.

Please let us know what more we can do to support the efforts of the new ACVBM.

Thank you,
Pam Moore
Moore Farms
Nichols NY

* including: St. Johnswort, Echinacea, Belladonna, Cedar, Peppermint, Cinnamon, Geranium, Thyme, Lemongrass, Rosemary, Calendula, Comfrey, Turmeric, Goldenseal, Hypericum, Aloe Vera, Garlic, Cayenne, White Willow, Chamomile, Fennel, Tea Tree, Eucalyptus, Apple, Kelp, Pulsatilla, Blue Cohosh, Wintergreen, Clove, Yucca, Black Walnut, Eye Bright
Hello,

I am writing to express my support for the American College of Veterinary Botanical Medicine. I am a herdsman at an organic dairy farm in New York, Chaseholm Farm, where use of botanicals has been integral to our treatment protocols. Because we do not have access to antibiotics, steroids, and hormones, botanicals play an important role in the health of the herd. For example, my most favorite treatment I use is yarrow and goldenseal powder to heal the tissue when a cow has an abscess. Yarrow acts to heal the tissue while goldenseal is an antiseptic to prevent the infection from spreading. Another botanical we use often is oregano oil. This is used on top of their grain when they are fighting off infections like mastitis or pneumonia because it acts as an antibiotic. This past winter, we had a pneumonia outbreak and all the cows in the barn received herbal tinctures to help stave off the disease. They received tinctures to help with their immune system as well as to provide lung support. Most importantly, when a cow has mastitis we give them an oral garlic tincture treatment and that has been the most successful treatment for mastitis we have seen. The more veterinarians in support of this, the more successful organic dairy farming can be.

Organic Dairy Farms need the support of the veterinarians involved in the American College of Veterinary Medicine because we trust their advice and their experience and it is important for these veterinarian to have support.

Please consider this email in your decision making process.

Thank You