Wang J, Liu M, Wu Y etal <u>Medicinal herbs as a potential strategy to decrease methane</u> <u>production by rumen microbiota: a systematic evaluation with a focus on Perilla frutescens</u> seed extract. Appl Microbiol Biotechnol. 2016 Sep 22.

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, Succinivibrio, Shuttleworthis, Pseudobutyrivbrio, 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.

Xu W, Guan R, Lu Y et al <u>Therapeutic effect of polysaccharide fraction of Atractylodis</u> macrocephalae Koidz. in bovine subclinical mastitis. BMC Veterinary Research (2015) 11:165

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.

Baravalle C, Silvestrini P, Cadoche MC, Beccaria C, Andreotti CS, Renna MS, Pereyra EA, Ortega HH, Calvinho LF, Dallard BE. <u>Intramammary infusion of Panax ginseng extract in bovine mammary gland at cessation of milking induces changes in the expression of toll-like receptors, MyD88 and NF-kB during early involution.</u> Res Vet Sci. 2015 Jun;100:52-60.

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.

Mordmuang A, Shankar S, Chethanond U, Voravuthikunchai SP. <u>Effects of Rhodomyrtus</u> <u>tomentosa Leaf Extract on Staphylococcal Adhesion and Invasion in Bovine Udder Epidermal Tissue Model.</u> Nutrients. 2015 Oct 15;7(10):8503-17.

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 rhodomyrtone, and liposomal encapsulated rhodomyrtone 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.

Chen X, Shang F, Meng Y, Li L, Cui Y, Zhang M, Qi K, Xue T. <u>Ethanol extract of Sanguisorba</u> <u>officinalis L. inhibits biofilm formation of methicillin-resistant Staphylococcus aureus in an icadependent manner.</u> J Dairy Sci. 2015 Dec;98(12):8486-91

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.

Mordmuang A, Voravuthikunchai SP. Rhodomyrtus tomentosa (Aiton) Hassk. leaf extract: <u>An alternative approach for the treatment of staphylococcal bovine mastitis.</u> Res Vet Sci. 2015 Oct;102:242-6.

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.

Zhou X, Yang C, Li Y, Liu X, Wang Y. <u>Potential of berberine to enhance antimicrobial activity of commonly used antibiotics for dairy cow mastitis caused by multiple drug-resistant Staphylococcus epidermidis infection.</u> Genet Mol Res. 2015 Aug 19;14(3):9683-92.

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 multidrug resistant S. epidermidis infected mastitis in dairy cows either alone or in combination with other commonly used antibiotics, such as penicillin, lincomycin, and amoxicillin.

Budri PE, Silva NC, Bonsaglia EC etal <u>Effect of essential oils of Syzygium aromaticum and Cinnamomum zeylanicum and their major components on biofilm production in Staphylococcus aureus strains isolated from milk of cows with mastitis.</u> J Dairy Sci. 2015 Sep;98(9):5899-904.

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.

Cui D, Wang S, Wang L, Wang H, Liu Y Prophylactic strategy with herbal remedy to reduce puerperal metritis risk in dairy cows: A randomized clinical trial LivestockScience181(2015)231–235

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-firstservice interval (68.9717.7daysvs.80.5726.6 days, Po0.05) and service per conception(1.7vs.2.1, Po0.01) 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%, Po0.05) and proportion of cows that were pregnant at 305 days in milk (89.8%vs.82.0%, Po0.01) 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 N1, Das G2, Singh P1, Haque M1. <u>Anthelmintic efficacy of crude neem (Azadirachta indica) leaf powder against bovine strongylosis.</u> 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 coproexamination, 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 anthelminthics.

Ravva SV1, Korn A2. <u>Effect of Neem (Azadirachta indica) on the Survival of Escherichia coli</u> <u>O157:H7 in Dairy Manure.</u> Int J Environ Res Public Health. 2015 Jul 10;12(7):7794-803.

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 neemfree 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.

Mullen KA, Lee AR, Lyman RL, Mason SE, Washburn SP, Anderson KL. Short communication: an in vitro assessment of the antibacterial activity of plant-derived oils. J Dairy Sci. 2014 Sep;97(9):5587-91

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.

Mullen KA, Anderson KL, Washburn SP. <u>Effect of 2 herbal intra-mammary products on milk quantity and quality compared with conventional and no dry cow therapy.</u> J Dairy Sci. 2014;97(6):3509-22.

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 (penicillindihydrostreptomycin 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 \pm standard error) of quarters with new infections were 24 \pm 21% for CON, $15 \pm 7\%$ for CIN, $30 \pm 10\%$ for P-M, $32 \pm 11\%$ for PC, and $35 \pm 11\%$ for NT. The efficacy of the herbal products was similar to that of conventional therapy, and the herbal products had no apparent adverse effects.

Cui D, Li J, Wang X, Xie J, Zhang K, Wang X, Zhang J, Wang L, Qin Z, Yang Z. <u>Efficacy of herbal tincture as treatment option for retained placenta in dairy cows.</u> Anim Reprod Sci. 2014 Feb;145(1-2):23-8.

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.

Pinedo P, Karreman H, Bothe H, Velez J, Risco C. <u>Efficacy of a botanical preparation for the intramammary treatment of clinical mastitis on an organic dairy farm.</u> Can Vet J. 2013 May;54(5):479-84.

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.

Andreotti R, Garcia MV, Cunha RC, Barros JC. <u>Protective action of Tagetes minuta (Asteraceae)</u> <u>essential oil in the control of Rhipicephalus microplus (Canestrini, 1887) (Acari: Ixodidae) in a cattle pen trial.</u> Vet Parasitol. 2013 Oct 18;197(1-2):341-5.

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.

Cui D, Wang X, Wang L, Wang X, Zhang J, Qin Z, Li J, Yang Z. <u>The administration of Sheng Hua Tang immediately after delivery to reduce the incidence of retained placenta in Holstein dairy cows.</u> Theriogenology. 2014 Mar 15;81(5):645-50

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-firstservice interval (73.2 \pm 25.1 vs. 81.9 \pm 32.8 days; P < 0.01), calving-to-conception interval (93.4 \pm 38.8 vs. 114.6 \pm 42.9 days; P < 0.01), and service per conception (1.5 \pm 0.8 vs. 1.9 \pm 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.

Taga I, Lan CQ, Altosaar I. <u>Plant essential oils and mastitis disease: their potential inhibitory effects on pro-inflammatory cytokine production in response to bacteria related inflammation.</u> Nat Prod Commun. 2012 May;7(5):675-82. Review.

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.

Baravalle C, Dallard BE, Cadoche MC, Pereyra EA, Neder VE, Ortega HH, Calvinho LF. <u>Proinflammatory cytokines and CD14 expression in mammary tissue of cows following intramammary inoculation of Panax ginseng at drying off.</u> Vet Immunol Immunopathol. 2011 Nov 15;144(1-2):52-60.

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.

Ghodrati H etal. Effect of Intramammary Injection of Nigella Sativa on Somatic Cell Count and Staphylococcus Aureus Count in Holstein Cows with S. aureus Subclinical Mastitis American Journal of Animal and Veterinary Sciences 6 (1): 31-34, 2011

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 NSEtreated 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.

McPhee CS, Anderson KL, Yeatts JL, Mason SE, Barlow BM, Baynes RE. Milk and plasma disposition of thymol following intramammary administration of a phytoceutical mastitis treatment. J Dairy Sci. 2011 Apr;94(4):1738-43.

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 μ g/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 lactation performance 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 heatstress. Forty Holstein cows (75 \pm 15 d in milk, 37.5 \pm 1.8 kg of milk/d, and 1.7 \pm 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. Aver-age 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 < P0.01). 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.

Qiao G etal Effects of supplemental Chinese herbs on growth performance, blood antioxidant function and immunity status in Holstein dairy heifers fed high fibre diet Italian Journal of Animal Science 2013; volume 12:e20

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-©). 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.

Hashemzadeh-Cigari F, Khorvash M, Ghorbani GR etal. Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. J Dairy Sci. 2014 Dec;97(12):7487-97.

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 \pm 26 d in milk and 46.3 \pm 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-topropionate 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.

Schäfer M, Sharp P, Brooks VJ, Xu J, Cai J, Keuler NS, Peek SF, Godbee RG, Schultz RD, Darien BJ. Enhanced bactericidal activity against Escherichia coli in calves fed Morinda citrifolia (Noni) puree. J Vet Intern Med. 2008 Mar-Apr;22(2):499-502.

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 wellrecognized 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.

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