SARE Project GS10-094

Evaluation of Herbal Remedies as Alternatives to Antibiotic Therapy in Dairy Cattle

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**Summary**

A comparison of two herbal dry off treatments to conventional antibiotic or no treatment determined that herbal treatments are competitive with conventional therapy at dry off. The herbal treatments tested, Phyto-Mast and Cinnatube, were just as successful as conventional therapy in curing infections during the dry period but not significantly better than no treatment. Cinnatube was significantly better than no treatment at preventing infections from occurring. Milk quality was not negatively affected by the use of any treatment. These herbal treatments are recommended for use if mastitis during the dry period and antibiotic use are of concern to the farmer.

**Introduction**

Inflammation of the mammary gland, or mastitis, is one of the most costly health issues of dairy farms; it is estimated that each clinical case costs an average of $179 due to milk loss, increased mortality and treatment costs on conventional dairy farms (Bar et al., 2008). In dairy cows, mastitis is most frequently caused by a bacterial infection. Infection can occur during lactation or in the dry period (between the end of lactation and parturition) as a result of contact with contagious or environmental pathogens (National Mastitis Council, 1997). Prophylactic use of antibiotics 60 days before parturition at the time of drying off has been shown to be effective in treating current and preventing future bacterial infections (Neave et al., 1966). However for organic producers, national organic standards prohibit the use of synthetic antibiotics in organic dairy cattle except as a last resort. If an organic dairy cow receives antibiotics she must be removed from the herd (Electronic Code of Federal Regulations, 2013). Many herbal products are available to organic dairies as alternatives to antibiotic therapy but clinical testing of their efficacy is lacking, allowing for skepticism of their efficacy (Ramey, 2007). Plant-derived antimicrobials have shown promise in vitro as treatments for mastitis; trans-cinnamaldehyde (from cinnamon bark), thymol (from oregano oil) and eugenol (from clove oil) were shown to be effective in milk culture versus several major mastitis pathogens (Baskaran et al., 2009). Thymol is one of several ingredients in Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), an herbal intramammary product labeled for improving milk quality. The purpose of this project was to engage organic dairy farmers in testing the efficacy of herbal alternatives to antibiotics for mastitis treatment in dairy cattle. This study assessed Phyto-Mast and Cinnatube (New AgriTech Enterprises, Locke, NY) as dry off treatments in four commercial dairy herds and one research herd. Phyto-Mast and Cinnatube are both made of naturally-occurring herbal extracts known to stimulate the immune system or have antibacterial effects. A preliminary study comparing Phyto-Mast to antibiotic or no treatment revealed no statistically significant difference in milk yield or somatic cell score which is a measure of mammary infection level (Mullen et al., 2010). That study was in a single 180-cow herd whereas the current study expands the preliminary study to include more farms and another herbal dry treatment strategy. The goal of the current study was to engage North Carolina organic farmers in research and provide them with reliable information regarding the efficacy of available alternatives to antibiotics.

**Objectives/Performance Targets**

1. Engage dairy farmers in mastitis research by teaching them how to administer intramammary products and how to take milk samples aseptically

1a.) Provide each dairy with a milk collection kit

1. Determine the efficacy of Phyto-Mast and Cinnatube:

2a.) Assign treatments to each herd to increase the power of the experiment

2b.) Assess the presence and amount of bacteria on each participating farm prior to treatment and after freshening

2c.) Measure milk production and somatic cell count of each cow and compare between the pre-treatment lactation and post-treatment lactation production and somatic cell count

**Materials and Methods**

1.) Four commercial dairy farms and one research herd were visited in late 2010-early 2011 for collection of the initial set of milk samples at dry off using aseptic techniques (National Mastitis Council, 2004). During the visit, farm owners and employees in the milk parlor were trained how to collect milk samples aseptically. Briefly, the teats and udder were cleaned of dirt and debris, teats were premilked to remove foremilk and stimulate milk letdown, teats were rinsed with a disinfectant (30 seconds) and dried with individual towels, teat ends were scrubbed (15 seconds) with 70% alcohol until the cotton ball was clean after contacting the teat, a milk sample was taken while being careful not to touch the teat end to anything in order to maintain sterility, and samples were stored immediately on ice or in refrigeration. These samples were used to assess bacterial content of the milk. An additional set of samples from each teat were taken to be shipped to the Dairy Herd Information Association (DHIA) laboratory to assess somatic cell count.

1a.) Each dairy was provided with a milk collection kit including sterile milk collection vials, 70% alcohol, cotton balls, freezer packs, racks for holding tubes, paper towels and permanent markers. Farmers were encouraged to pre-label vials before milk collection for ease of labeling and sample handling.

2a.) Four different treatments were assessed on the four commercial herds enrolled in the study: no dry off treatment (NOT), Phyto-Mast (PHY), Cinnatube (CIN), Phyto-Mast and Cinnatube (PC). The Center for Environmental Farming Systems (CEFS) herd in Goldsboro, NC had five treatment groups, with one group for conventional treatment with an antibiotic and an internal teat sealant (penicillin-dihydrostreptomycin plus bismuth subnitrate; CON). At CEFS, cows were pasture-based and split into two management groups, one of which received organic health treatments and the other received conventional treatments. All cows received a conventional concentrate supplement but were primarily fed on pasture. Every herd was divided into treatment groups balanced by breed, calving date, and lactation number. Four hundred and fifty cows were eligible for the experiment, which would give over 80% power to detect differences between all treatments for cure rate, new infection rate, and somatic cell score difference according to preliminary data collected in 2009-2010 at CEFS.

2b.) Duplicate milk samples were taken from all four quarters of each cow at CEFS at dry off immediately preceding administration of treatment and 3 to 5 days after calving (at freshening) using the aseptic techniques recommended by the National Mastitis Council (National Mastitis Council, 2004). A single set of milk samples were taken from cows at the commercial herds at dry off and again 3 to 5 days after calving. Samples were processed at the Milk Quality and Mastitis Laboratory at the College of Veterinary Medicine at North Carolina State University. If one quarter sample was contaminated and a duplicate was available, the duplicate was also cultured. The protocol for milk culture was, briefly: vortexed milk samples were plated (0.01 ml) on trypticase soy agar + 5% sheep blood plates. Any unusual appearance or smell of the milk sample was recorded. Plates were incubated at 36°C for 48 hours and observed for growth at 24 hours and 48 hours. Number of colonies and color of colonies were recorded. Individual colonies were selected for identification and Gram stained for initial screening. Streptococci were distinguished from enterococci using the CAMP test, esculin hydrolysis and growth on bile esculin agar with azide (Hardy Diagnostics, Santa Maria, CA). Coagulase-negative staphylococci were distinguished from *Staphylococcus aureus* by mannitol fermentation and coagulase testing. Gram negative rods were identified using morphology on MacConkey agar (Hardy Diagnostics, Santa Maria, CA) and oxidase testing. If required, the API20Strep and the API 20E identification systems (bioMérieux, Durham, NC), were used for further differentiation.

2c.) The research herd at CEFS was the only herd on DHIA test for the duration of this study, making it impossible to obtain milk production records for individual cows from the commercial herds. However, somatic cell counts (SCC) were obtained for the majority of quarters of every cow sampled.

Statistical analysis was performed at the quarter level using SAS (version 9.2, Cary, NC). Prior to model selection, diagnostic tests were run to test for normality and outliers. Following this examination, all SCC variables were transformed to linear somatic cell score (SCS) to more closely approximate the Normal distribution; this transformation was performed using the formula  to obtain the base 2 logarithmic transformation as recommended by Shook (1982). The generalized linear mixed model procedure (PROC GLIMMIX) was used, incorporating farm and cow as random effects. Model selection involved univariate analysis on all measured variables to qualify for insertion into the model (*P* < 0.20), then addition of main-effects interactions and finally backward stepwise selection, eliminating non-significant variables (*P* > 0.05) while keeping treatment forced into the model.

**Results and Discussion/Milestones**

A total of 4,665 quarter samples (2514 dry, 2151 fresh) were collected from 441 cows enrolled in this study between August of 2010 and March of 2012. Only 1,044 paired quarter samples (230 CIN, 241 NOT, 255 PHY, 214 PC, 104 CON) were available for analysis due to blind quarters, missed samples at either dry off or freshening, or culled cows.

**Effect of Treatment on Difference in SCS**

There were no significant differences between treatments for the difference in SCS between freshening and dry off. Mean SCS difference was -0.06 ± 0.36 for CIN, -0.20 ± 0.39 for NOT, 0.14 ± 0.39 for PHY, 0.41 ± 0.41 for PC, and 0.79 ± 0.49 for CON. There were no significant differences among treatments for SCS difference. A retrospective power analysis on the study design yielded over 98% power to detect differences between treatments for the difference in SCS between freshening and dry off. Of note is that SCS at dry off was different among treatments (Table 1). However, this does not explain the lack of difference between treatments for the change in SCS. There was also a strong interaction between treatment and breed for the SCS difference across the dry period; these data are presented in Table 2. Some essential oils have documented cytotoxic activity (Bakkali et al., 2008), but the concentrations present in these herbal treatments did not seem to have cytotoxic effects on the milk-producing cells in treated cows.

**Effect of Treatment on Cure Rate**

There were no significant differences among treatments for risk of cure during the dry period. The risk of cure was 30 ± 8% for CIN, 39 ± 9% for NOT, 45 ± 8% for PHY, 38 ± 8% for PC, and 74 ± 25% for CON. There was strong power (>98%) to detect differences between CON and all other treatments. However, there was comparatively low power to detect the difference between NOT and CIN (49%), NOT and PHY (24%), and NOT and PC (5%). To increase the power to 80% for these comparisons, 459, 1,094, and 37,367 quarter samples would be necessary for each group, respectively. Because of the strong power to detect differences between CON and the other treatments, it can be concluded that no treatment was inferior to conventional antibiotic therapy for curing infections during the dry period. It cannot, however, be concluded that the herbal treatments are superior to no treatment, because of the low power to detect differences between the herbal treatments and no treatment. The observed cure rates for conventional therapy for this study were lower than other studies examining either Quartermaster alone (88.9%; Arruda et al., 2013) or an antibiotic and teat sealant together (90%, Woolford et al., 1998; 91.3%, Godden et al., 2003). At the same time, those studies had a much higher rate of infection at dry off in the antibiotic-treated group (31%, Godden et al., 2003; 22%, Arruda et al., 2013;) than the 4% infection rate in the current study, except for the study by Woolford et al. (1998), with a 4.5% infection rate at dry off. Conventional therapy cured 3 of the 4 infections present at dry off (Table 3): 1 *Enterococcus* spp., 1 *Staphylococcus aureus,* and 1 *Streptococcus* spp. other than *agalactiae*. Conventional therapy did not cure 1 *Staphylococcus aureus* infection. No treatment and the herbal treatments mostly cured coagulase-negative *Staphylococcus* infections, followed by *Corynebacterium* spp. (Table 3). Coagulase-negative staphylococci and *Corynebacterium* spp. are considered minor pathogens, and generally do not cause acute clinical mastitis but can cause increases in SCC (National Mastitis Council, 1996). *Staphylococcus aureus* can cause clinical mastitis, subclinical mastitis, and increased SCC and is considered a contagious pathogen. Environmental pathogens, including *Escherichia coli, Klebsiella* spp., *Enterococcus* spp., and *Streptococcus* spp. other than *agalactiae* and *dysgalactiae*, are associated with clinical mastitis and increased SCC (National Mastitis Council, 1996).

**Effect of Treatment on New Infection Rate**

The risk of new infections for each treatment were 14 ± 7% for CIN, 35 ± 12% for NOT, 31 ± 11% for PHY, 33 ± 12% for PC, and 23 ± 20% for CON. Quarters treated with CIN were significantly less likely to experience a new infection than quarters treated with NOT (*P* = 0.02) or PC (*P* = 0.05). The power of the comparisons between the CON treatment and all other treatments, according to a retrospective power analysis, were above 35%, with the most power being attributed to the comparison between CON and NOT (65%). Cinnatube appeared to have a protective effect versus new infections compared with no treatment. Older cows (lactation 3+) had a slightly higher risk (*P =* 0.06) of new infection in the next lactation than first-lactation cows (32% vs. 20%).

**Impacts and Results/Outcomes**

This research has helped dairy producers in the Southern Region understand that alternatives to antibiotics exist for treating mammary infections in dairy cattle. The herbal alternatives evaluated in this trial are as effective at eliminating current infections as conventional therapy is. One of the herbal treatments, Cinnatube, was also effective at preventing future infections. These herbal treatments do not negatively affect cow health, as evidenced by the lack of difference in SCS change between all treatments tested. There is potential for both organic and conventional dairy producers to use these alternatives. This, in turn, benefits consumers by eliminating the risk of antibiotic residues in milk/meat and by lowering the contribution of the dairy industry to the development of antibiotic-resistant bacteria. The evidence for preventing future infections is not as strong as the evidence for curing infections, which is of consideration especially for organic producers. Organic dairy farmers are not permitted to use treatments in the absence of disease except for vaccinations, and so using herbal treatments for preventing mastitis may require review and approval by the National Organic Standards Board.

**Economic Analysis**

Treatment cost will vary by source; all treatments used in this study except for Orbeseal require a prescription from a veterinarian. Economic analysis by the farmer must include availability of these products, retail price from a reasonably local source, and value of the treatments for the herd.

**Publications/Outreach**

The results of this study were first distributed to the farmers who participated in the study. The analysis they received was specific to those cows enrolled in the study on their own farms. A preliminary analysis of the microbiology results from this study was presented at the North Carolina Graduate Student Research Symposium on March 20, 2012; the presenting author (K. Mullen) received second place in the poster competition (<http://www.ncsu.edu/grad/research/docs/grad-symposium-winners-2012.pdf>). The research was also presented at the annual American Dairy Science Association Annual Meeting in Phoenix, Arizona in July of 2012, the annual meeting of the American Holistic Veterinary Medical Association in Birmingham, Alabama in September 2012, and at the Journée d’information scientifique en production animale in Montreal, Quebec on February 27, 2013.

**Farmer Adoption**

Farmers involved in this study are currently using one or more of the herbal treatments as treatments at dry off. Through the outreach that has been done to share information about this project, 20 dairy farmers have learned about the results and potential applications of this project. For organic dairy farmers, who can only treat existing disease, this project suggests that use of an herbal remedy at dry off will be approximately as good as not using treatment for curing existing infections. However, for other farmers looking to try an alternative to antibiotics, herbal treatment seems to work to prevent infections from occurring. In either case, use of one of these two herbal treatments will not have negative implications on milk quality as measured by somatic cell score.

**Areas Needing Additional Study**

The effect of these treatments should be further studied in other regions of the country and with groups of cattle that are not managed in a pasture-based system so that the results can be generalized to a greater portion of the dairy industry. Further studies are necessary to determine the persistence of these treatments in milk and meat and for approval of the Food and Drug Administration to treat mastitis.

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**Appendix**

Table 1: Dry off somatic cell score for each treatment

|  |  |
| --- | --- |
| Treatment | Dry SCS |
| Cinnatube | 3.94 ± 0.11a |
| No Treatment | 3.27 ± 0.10b |
| Phyto-Mast | 3.49 ± 0.10bc |
| Phyto-Mast and Cinnatube | 3.71 ± 0.11ac |
| Quartermaster and Orbeseal | 3.15 ± 0.16b |

These are the somatic cell scores (mean ± standard error) at dry off for all cows assigned to each of the treatments. Different superscripts following the standard error indicate significant differences between treatments. There were some differences in SCS, which may have affected the change in SCS from dry off to freshening.

Table 2: Least squares means of SCS difference between freshening and dry off, by treatment

|  |  |  |
| --- | --- | --- |
| Treatment | Breed1 | Estimate2 ± Std. Error |
| Cinnatube | HO | -0.05 ± 0.37 |
| Cinnatube | JE | -0.11 ± 0.55 |
| Cinnatube | XX | -0.03 ± 0.41 |
| No Treatment | HO | 0.14 ± 0.36 |
| No Treatment | JE | -1.41 ± 0.72 |
| No Treatment | XX | 0.66 ± 0.38 |
| Phyto-Mast | HO | 0.08 ± 0.35 |
| Phyto-Mast | JE | 0.18 ± 0.75 |
| Phyto-Mast | XX | 0.16 ± 0.39 |
| Phyto-Mast and Cinnatube | HO | 0.25 ± 0.38 |
| Phyto-Mast and Cinnatube | JE | 0.17 ± 0.83 |
| Phyto-Mast and Cinnatube | XX | 0.82 ± 0.38 |
| Quartermaster and Orbeseal | HO | -0.43 ± 0.76 |
| Quartermaster and Orbeseal | JE | 2.00 ± 0.82 |
| Quartermaster and Orbeseal | XX | 0.81 ± 0.43 |

These are the somatic cell score differences during the dry period (fresh SCS – dry SCS) for each combination of treatment and breed enrolled in the study. A negative value is more desirable, as it indicates that SCS was reduced at freshening compared to dry off. There are no significant differences between these estimates.

1Breed: HO = Holstein; JE = Jersey, XX = crossbred.

2Estimates are given as least squares means ± standard error.

Table 3. Infections present at dry off and cured at freshening1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Organism | CIN | NOT | PHY | PC | CON |
| Coagulase-negative staphylococci | 9 (52.4%) | 11 (57.9%) | 13 (46.4%) | 11 (55.0%) | 0 (0%) |
| Coagulase-negative staphylococci and *Corynebacterium* spp. | 0 (0%) | 0 (0%) | 1 (3.6%) | 1 (5.0%) | 0 (0%) |
| Coagulase-negative staphylococci and *Enterococcus* spp. | 0 (0%) | 0 (0%) | 1 (3.6%) | 0 (0%) | 0 (0%) |
| Coagulase-negative staphylococci and coliform | 1 (5.9%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Coagulase-negative staphylococci and *Streptococcus* spp. (not *agalactiae*) | 1 (5.9%) | 0 (0%) | 1 (3.6%) | 1 (5.0%) | 0 (0%) |
| Coagulase-negative staphylococci and *Streptococcus uberis* | 0 (0%) | 1 (5.3%) | 0 (0%) | 0 (0%) | 0 (0%) |
| *Corynebacterium* spp. | 3 (17.7%) | 5 (26.3%) | 10 (35.7%) | 2 (10.0%) | 0 (0%) |
| *Enterococcus* spp. | 0 (0%) | 0 (0%) | 0 (0%) | 1 (5.0%) | 1 (33.3%) |
| *Nocardia* spp. | 0 (0%) | 0 (0%) | 1 (3.6%) | 0 (0%) | 0 (0%) |
| *Staphylococcus aureus* | 1 (5.9%) | 1 (5.3%) | 0 (0%) | 1 (5.0%) | 1 (33.3%) |
| *Streptococcus* spp. (not *agalactiae*) | 1 (5.9%) | 1 (5.3%) | 0 (0%) | 3 (15.0%) | 1 (33.3%) |
| *Streptococcus uberis* | 1 (5.9%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Yeast | 0 (0%) | 0 (0%) | 1 (3.6%) | 0 (0%) | 0 (0%) |
| Total | 17 | 19 | 28 | 20 | 3 |

This table shows the number of infections that were cured during the dry period, by treatment and by infective organism. Percentages are given as the percentage of all quarters cured by that treatment. Most cured organisms for all treatments except conventional were coagulase-negative staphylococci, which are considered minor pathogens but still can have negative effects on milk quality.

1CIN = Cinnatube (New AgriTech Enterprises, Locke, NY); NOT = No treatment; PHY = Phyto-Mast (Penn Dutch Cow Care, Narvon, PA); PC = Phyto-Mast and Cinnatube; CON = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Kalamazoo, MI) and Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI)