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Effect of 2 herbal intramammary products on milk quantity and quality compared with conventional and no dry cow therapy

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ABSTRACT

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 Agri-Tech 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 5 d 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.

Key words: dry cow therapy, organic mastitis treatment, alternative to antibiotics

INTRODUCTION

Mastitis, or inflammation of the mammary gland, is a costly disease often caused by bacterial infection. A single case of clinical mastitis can cost in excess of US$100 (Bar et al., 2008; Cha et al., 2011). Dry cow therapy at the end of lactation is aimed at eliminating current and preventing future intramammary (**IMM**) bacterial infections. The benefit of dry cow therapy is that it typically reduces the rate of new infections by 67 to 82% (Smith et al., 1967c; Hillerton and Berry, 2005). Dry cow therapy is normally accomplished using IMM antibiotics labeled for treatment of gram-positive bacterial infections (US Food and Drug Administration, 2013a). However, the growing population of organic dairy producers is not allowed to use synthetic antibiotics in dairy cattle, making mastitis therapy challenging.

The organic dairy industry is growing at a rapid rate in the United States. Total sales of organic fluid milk products doubled between 2006 and 2011, representing 4% of the total fluid milk market in 2011 (USDA-AMS, 2012). Certified organic dairy farms in the United States are not permitted to use antibiotics to treat cattle. The exception is that if organic methods fail, the producer must use conventional medicine to restore a sick animal to health. If prohibited products (including antibiotics and hormones) are used, then treated cattle permanently forfeit their organic status, and no milk or meat from them can be sold as organic. However, organic farmers must not withhold treatment to preserve the organic status of any animal (USDA, 2013).

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Organic standards focus on disease prevention by allowing cattle to exercise natural behaviors by mandating pasture access and allowing the use of biologics (USDA, 2013). Administration of medication in the absence of illness is prohibited in organic dairy production in the United States, except for biologics. Provisions for use of products (including herbal products) on US organic dairies are made by the National Organic Standards Board.

Mastitis is a challenge for organic dairy farmers for 2 reasons. One is the prevalence of mastitis; organic dairy farms in the United States face the same mastitis-causing organisms as conventional farms (Pol and Ruegg, 2007; Cicconi-Hogan et al., 2013; Mullen et al., 2013), including a higher prevalence of *Streptococcus agalactiae* (Pol and Ruegg, 2007), a gram-positive organism easily controlled using antibiotics (Wilson et al., 1999). The second reason is that no alternatives to antibiotics are currently approved by the Food and Drug Administration (**FDA**) for IMM treatment of mastitis and accepted by the US National Organic Program, making it more difficult for organic dairy farmers to manage this costly and prevalent disease. Scientists have expressed concern that the inability of organic dairy farmers to use approved antibiotics for mastitis therapy could lead to increases in udder health problems and hence to decreased milk quality (Zwald et al., 2004).

When evaluating alternatives to antibiotics, it is important to consider that the mammary gland is very susceptible to irritation and that any intramammary infusions should be nonirritating (Sanderson, 1966). The mammary gland is more susceptible to infection and subsequent inflammation during the dry period when it is in a state of transition from lactation to colostrogenesis (Oliver and Sordillo, 1988). Thus, it is important to both effectively prevent infections from occurring and to use an intramammary therapy that is nonirritating. Although organic dairy farmers in the United States have been reported to use a wide variety of mastitis treatments (Ruegg, 2009), no scientific studies evaluating the efficacy of herbal products in vivo on US dairies have been published to date.

The objective of this study was to determine the effects of administration of 2 herbal IMM products on milk quantity and quality when used as dry cow therapies.

MATERIALS AND METHODS

Two trials were conducted to evaluate the safety (trial 1) and microbiological efficacy (trial 2) of 2 herbal products when used as dry cow therapy. The 5 treatments, assessed in both trials, were a synthetic IMM antibiotic (penicillin-dihydrostreptomycin; Quartermaster; Zoetis, Florham Park, NJ) plus internal teat sealant (bismuth subnitrate; Orbeseal; Zoetis); an herbal internal teat sealant (Cinnatube; New AgriTech Enterprises, Locke, NY); an herbal IMM product (Phyto-Mast; Bovinity Health LLC, Narvon, PA); a combination of Phyto-Mast and Cinnatube; and no treatment. Phyto-Mast is approved for use to improve milk quality by an accredited organic certifying agent, the Ohio Ecological Food and Farm Association (Columbus, OH), and its ingredients comply with the USDA National Organic Standards Board regulations. The components of Phyto-Mast and Cinnatube are listed in Table 1.

Regulatory Compliance

All sample collection from cows was performed in accordance with the North Carolina State University Institutional Animal Care and Use Committee (Raleigh) approved protocol 11-029-A.

Trial 1: Milk Production and Cow-Level SCS

Experimental Design. Trial 1 took place over 3 yr at the Center for Environmental Farming Systems (Goldsboro, NC) using a seasonal-calving, pasture-based herd consisting of Holstein, Jersey, and Holstein and Jersey crossbred cattle. Cattle in this herd calve between October and February each year.

In calving season 2009–2010, 120 cattle were assigned to this trial consisting of 3 initial treatments: 40 to conventional antibiotic and teat sealant (**CON**), 40 to Phyto-Mast (**P-M**), and 40 to no treatment (**NT**). In calving season 2010–2011, 116 cows were used and 2 additional treatments were included: 22 assigned to CON, 24 to Cinnatube (**CIN**), 24 to P-M, 24 to Phyto-Mast and Cinnatube (**PC**), and 23 to NT. In calving season 2011–2012, 100 cows (20 for each of 5 treatments) were enrolled in the study. Treatment assignments were balanced within year by breed, age, and projected calving date.

Data Collection. Milk production and SCS data were obtained from DHIA monthly tests. Milk data included the previous lactation 305-d mature-equivalent milk production (**PrevLact305MEM**), first test-date postcalving milk production, and 305-d mature-equivalent milk production for the lactation following treatment (**PostLact305MEM**). Somatic cell score data included previous lactation-average SCS, the last recorded SCS of the previous lactation (**PrevLactLastSCS**), and SCS of the first test-date postpartum (**TD01_SCS**).

In preparation for treatment administration, cows were milked for the last time, aseptic milk samples were collected, and then teat ends were cleaned with 70%

Table 1. Phyto-Mast[1] and Cinnatube[2] herbal oil ingredients, major chemical components, and bioactivity

Ingredient	Common name	Bioactivity	Reference (species)
Phyto-Mast			
Angelica dahuricae	Bai zhi	Antiinflammatory	Kang et al., 2008 (in vitro murine macrophages)
Angelica sinensis	Chinese angelica	Immunomodulatory	Liu et al., 2003 (rats)
Gaultheria procumbens	Wintergreen	Analgesic	Poppenga, 2002
Glycyrrhiza uralensis	Chinese licorice	Antiinflammatory	Abe et al., 2003 (in vitro murine liver cells); Kai et al., 2003 (dairy cattle), Genovese et al., 2009 (mice)
Thymus vulgaris	Thyme	Antibacterial	Helander et al., 1998; Kalemba and Kunicka, 2003; Tsai et al., 2011 (in vitro human leukemia cells)
Cinnatube			
Calendula	Marigold	Antiinflammatory	Jost, 1984; Muley et al., 2009 (rats)
Cinnamomum spp.	Cinnamon	Antibacterial	Cowan, 1999; Baskaran et al., 2009
Eucalyptus gobulus	Eucalyptus	Antibacterial	Cowan, 1999
Melaleuca alterniflora	Tea tree	Antibacterial	Carson et al., 1995; Rotblatt and Ziment, 2002; Fitzi et al., 2002 (dogs)
Beeswax			

[1]Intramammary treatment for improving milk quality (Bovinity Health LLC, Narvon, PA).
[2]Intramammary treatment for use as an internal teat sealant (New AgriTech Enterprises, Locke, NY).

isopropanol-soaked cotton balls. Conventionally treated cows were first infused with penicillin-dihydrostreptomycin in all functional quarters. Then, teat ends were cleaned once more with isopropanol before infusion of bismuth subnitrate, which was restricted to placement in the teat end by pinching the top of the teat during administration. Cows receiving the PC treatment had their teat ends cleaned with isopropanol, and then were infused with Phyto-Mast. Teat ends were cleaned once more before administration of Cinnatube, which was infused without pinching the top of the teat. Quarters were not massaged after administration of any treatment. After dry cow therapy was administered, teats were postdipped with 1.0% iodine with 10% emollient (Della Barrier, DeLaval, Kansas City, MO). Conventionally treated cows had the internal teat sealant stripped out before the first milking. Cows receiving the other treatments had no additional udder preparation before the first milking. Cows were observed during the dry period and during the first 5 d of the subsequent lactation for clinical mastitis. If clinical mastitis was detected, it was recorded and the milk from the affected quarter was cultured. No cows had clinical mastitis at the time of dry-off.

Trial 2: Quarter-Level SCS and Microbiological Efficacy

Experimental Design. Two certified organic dairy farms, 1 research farm, and 2 conventional farms participated in this trial from 2010 to 2012. All farms were located in North Carolina and had Holstein, Jersey, or crossbred cattle. The CON treatment was assessed only in the research herd because managers of all 4 organic and conventional collaborating herds wanted to maintain eligibility for organic certification relative to restricted use of antibiotics or wanted to reduce use of antibiotics (1 conventional herd). Treatments were balanced within farm by breed, lactation number, and expected calving date. Only the research herd was a seasonal-calving herd; the rest of the herds practiced year-round calving. In both trials, researchers assigned cows to treatments using herd records, considering first breed, then lactation number, and then expected calving date, balancing the treatments for each of these factors.

Data Collection. Before data collection, vials were labeled with the farm name, cow ID, quarter, and date sampled. Quarter milk samples were collected aseptically following normal premilking preparation. Normal premilking preparation included pre-dip, stripping each quarter 3 to 5 times, and wiping off pre-dip after at least 30 s of contact time. Collected milk samples were immediately stored on ice and kept cold until they were returned to the laboratory, where they were frozen at least overnight before milk culture. Each udder quarter was sampled again into 90-mL vials containing a bronopol tablet preservative for SCC analysis at the United DHIA laboratory (Blacksburg, VA). Each functional udder quarter of each cow involved in the study was sampled before treatment at dry-off and 3 to 5 d postcalving. Milk microbiology samples were taken in duplicate at the research farm because extra technicians were available to assist with taking samples.

Treatments were administered directly following the last milking at dry-off using the same protocol as described for trial 1. No cows had clinical mastitis at the time of dry-off.

Milk Microbiology. Microbiological analysis was performed in the Milk Quality and Mastitis Laboratory at the College of Veterinary Medicine (North Carolina State University, Raleigh). Microbiological

identification was performed using methods consistent with those of the NMC (1999) and outlined in Mullen et al. (2013), using 0.01 mL of milk per sample. Milk cultures containing 3 or more dissimilar colony types were considered contaminated. Quarters with contaminated samples at dry-off, freshening, or both, were not considered in the analysis. In the case of the research herd, if one sample was contaminated, the duplicate was cultured. If the duplicate was also contaminated, the quarter was recorded as contaminated. If the duplicate was not contaminated, then its result was recorded for the quarter tested.

Definitions. All 4 infection status categories (cure, new IMI, no change in IMI status, no change, still not infected) were mutually exclusive. Categories were defined as follows.

- Presence of infection: If a bacterial species (except CNS) was present at ≥100 cfu/mL, it was recorded as an IMI in the quarter cultured; for CNS, at least 200 cfu/mL had to be present to be designated as an IMI (Dohoo et al., 2011).
- Cure: Quarters were considered cured if all microbiological organisms present in dry-off milk samples were not present in the postpartum milk sample.
- New IMI: Quarters had a new IMI if either they had no microbiological growth in milk at dry-off and one or more organisms present postpartum, or had a new organism present in milk postpartum that was not present at dry-off. Quarters experiencing a cure of one organism and a new IMI with a different organism were classified as having a new IMI.
- No change in IMI status: Quarters infected with the same microorganism postpartum as at dry-off were classified as having no change in IMI status. Quarters with no microbiological growth in milk at dry-off and no growth in milk postpartum were classified as no change, still not infected.

Statistical Analyses

Statistical analyses for trial 1 were performed using mixed linear models (PROC MIXED) in SAS software (version 9.2; SAS Institute Inc., Cary, NC). The hypothesis for trial 1 was that the herbal products (CIN, P-M, and PC) had the same effect on milk quality and quantity as NT and CON. Responses modeled included the difference between PostLact305MEM and PrevLact305MEM and the difference between TD01_SCS and PrevLactLastSCS. Categorical variables offered into these models included treatment (forced), year, and breed (Holstein, Jersey, or crossbred). Quantitative variables used for modeling included lactation number, month of calving, date of last calving, first test-date milk production, previous lactation SCS average, PrevLactLastSCS (for milk difference modeling only), and TD01_SCS (for milk difference modeling only).

Trial 2 was designed as both a noninferiority trial and a negative control trial. Noninferiority trials aim to establish if one treatment is as effective as another treatment. Because proving equality of treatments is statistically impossible, a pre-experiment margin of noninferiority ($\mathbf{\Delta}$) must be defined (Piaggio et al., 2006; Schukken et al., 2013). This Δ value is established based on the range of efficacy of treatments acceptable in a clinical setting or the results of previous research (Schukken and Deluyker, 1995). The null hypothesis ($\mathbf{H_0}$) tested in this study's noninferiority trial was that herbal products are inferior to CON for curing infections during the dry period; the alternative hypothesis ($\mathbf{H_A}$) was that the herbal products are noninferior to CON by more than $-\Delta$. Rejecting H_0 results in accepting H_A—that the herbal products are noninferior to CON treatment:

$$H_0 : [P_{\text{cure}} \text{ (P-M, CIN, PC)} - P_{\text{cure}} \text{ (CON)}] \leq -\Delta \text{ and}$$

$$H_A : [P_{\text{cure}} \text{ (P-M, CIN, PC)} - P_{\text{cure}} \text{ (CON)}] > -\Delta,$$

where P_{cure} is the probability of a quarter experiencing a cure, and Δ is the margin of noninferiority. In this trial, noninferiority analysis was completed by creating a figure containing the confidence intervals for risk of bacteriological cure in quarters treated with CIN, P-M, and PC relative to the CON control and the margin of noninferiority (Piaggio et al., 2006; Arruda et al., 2013; Schukken et al., 2013).

All statistical analyses for trial 2 were performed at the quarter level using SAS software (version 9.2; SAS Institute Inc.). Sample size was calculated for trial 2, with cure rate as the outcome of interest. The minimum margin of inferiority for comparison of the herbal products with CON was set at 10%, the margin used in another dry cow treatment evaluation (Arruda et al., 2013). Data from a preliminary dry cow study on the research farm used in this trial were used for the a priori sample size calculation. To demonstrate noninferiority of one herbal product compared with CON, 236 udder quarters would be required (118 per treatment group), assuming $\alpha = 0.05$, $\beta = 0.2$, 20% loss of samples from dry-off to freshening, 19% of quarters infected at dry-off, and cure proportions of 87 and 33% for CON and an herbal product, respectively. This sample size was also sufficient to detect noninferiority of the herbal products compared with CON for proportion of quarters with new infections. The NT treatment was also

included in trial 2 to test the hypothesis that the herbal products (CIN, P-M, and PC) were no different from NT in proportion of cured quarters or proportion of quarters with new infections.

For trial 2, the generalized linear mixed model (GLIMMIX) procedure was used, incorporating farm and cow as random effects. Cure and new infection were recorded as binary outcomes for each quarter. Cow was incorporated into the model to account for the fact that treatments were assigned on a cow basis, not a quarter basis. Categorical variables offered into each model included treatment group (forced), breed of cow (Holstein, Jersey, or crossbred), lactation number (as a continuous variable), lactation group (group 1 = first lactation pre-dry off, group 2 = second lactation pre-dry off, group 3 = third and greater lactation pre-dry off), dry period length (short = <45 d, normal = 45 to 60 d, long = >60 d), quarter, organism present at dry off, organism present at freshening, and treatment result (only offered when modeling SCS). Continuous variables offered to each model included dry-off date, freshening date, number of days dry, dry-off SCS, fresh SCS, and the difference between fresh and dry-off SCS (**SCSDiff**). In trial 2, milk and component production were not available for most of the cows because 3 of the 4 collaborating herds were not on DHI test during the study.

Before model selection for both trials, diagnostic tests were run to test for normality and outliers. Following this examination, all SCC variables were transformed to linear SCS to more closely approximate the normal distribution; this transformation was performed using the formula $\log_2\left(\frac{\text{SCC}}{100,000}\right)+3$, to obtain the base 2 logarithmic transformation as recommended by Shook (1993). Model selection for both trials was performed similarly to Arruda et al. (2013), and began with univariate analysis of the aforementioned variables, using difference in milk production and SCS (trial 1) and proportion of infections cured, proportion of quarters with new infections, and SCSDiff (trial 2) as dependent variables. Variables were retained in the model if the univariate analysis yielded a P-value < 0.20. Once univariate selection was complete, all main-effect interactions with treatment were included in the model. The final model selection step involved backward elimination of any variables with $P > 0.05$ in a stepwise manner, unless forced into the model. Models were also compared using the corrected Akaike information criterion. Because some cows were enrolled in the study for multiple years, each model also included a statement to account for possible repeated records for cows.

Significance is reported at $P < 0.05$. Means are presented as least squares means with standard error. Differences between means were calculated using the Tukey-Kramer adjustment for multiple comparisons.

RESULTS

Trial 1: Milk Production and Cow-Level SCS

Data were obtained from a total of 192 unique cows (334 cow records) over the 3 yr of this experiment. Records from 5 cows were not available due to culling during the subsequent lactation (5 cows; 1 CON, 1 P-M, 3 NT), leaving a total of 329 cow records from 187 unique cows for analysis. Lactation number was similar across all groups, averaging 2.9 ± 1.8 lactations. Treatments were balanced by breed for treatments CON, P-M, and NT (Table 2). The CIN and PC treatments had fewer cows from each breed group because those treatments were not used in the first year and were added in the second and third year. Calving month was balanced in a similar way, with fewer overall cows in the CIN and PC treatments. Most cows calved in October and November. No cows required treatment with antibiotics between calving and the posttreatment sampling date. No clinical mastitis cases or signs of visible irritation to the udder occurred during the dry period or during the first 5 d of lactation.

Difference Between PostLact305MEM and PrevLact305MEM. Raw mean milk yield difference (PostLact305MEM − PrevLact305MEM; ±SD) was 523 ± 1,895 kg for CON cows, 82 ± 1,758 kg for CIN cows, −108 ± 1,639 kg for P-M cows, 40 ± 1,424 kg for PC cows, and 84 ± 1,298 kg for NT cows. The final model used to predict milk yield difference included treatment, breed, lactation number, first test-date postpartum milk production, year, last calving date, TD01_SCS, PrevLactLastSCS, and the interaction of treatment and breed. Least squares means are given in Table 3. With the large standard errors within treatment groups for changes in production from one lactation to the next, we detected no significant differences among treatments for milk yield difference. Similarly, we found no significant differences in previous or post-lactation mature-equivalent milk production among breed within treatment groups (data not shown).

Only lactation number ($P < 0.002$), first test-date postpartum milk production ($P < 0.001$), the interaction of treatment and breed ($P = 0.013$), and year ($P = 0.009$) had significant contributions to the model. As lactation number increased, the difference in milk yield between freshening and dry off decreased. As first

Table 2. Numbers of cow records and lactation number by breed in a study comparing conventional, herbal, and no dry cow therapy conducted over 3 yr on a research herd in North Carolina (trial 1)

	Breed of cow						
	Holstein		Jersey		Crossbred		
Treatment[1]	No.	Lactation	No.	Lactation	No.	Lactation	Total
CON	12	3.3 ± 1.5	13	4.3 ± 1.8	54	3.8 ± 2.0	79
CIN	5	4.8 ± 1.9	5	5.3 ± 1.7	34	3.9 ± 1.6	44
P-M	11	5.1 ± 1.4	11	4.5 ± 1.3	61	3.4 ± 1.6	83
PC	3	5.0 ± 1.4	5	2.0 ± 0	35	3.8 ± 1.6	43
NT	13	5.2 ± 2.5	11	4.6 ± 1.6	56	3.5 ± 1.3	80
Total	44		45		240		329

[1]Treatments: CON = conventional intramammary dry cow therapy including Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis); CIN = herbal internal teat sealant (Cinnatube; New AgriTech Enterprises, Locke, NY); P-M = herbal intramammary treatment for improving milk quality (Phyto-Mast; Bovinity Health LLC, Narvon, PA); PC = treatment with Phyto-Mast and Cinnatube; NT = no treatment. The CIN and PC treatments were only assessed for 2 of the 3 yr.

test-date postpartum, milk production increased as did the milk yield difference. The interaction of treatment and breed can be explained by the fact that crossbreds, Holsteins, and Jerseys had milk yield differences (665 to 2,802 kg) significantly greater than zero in treatments CON, NT, and PC, respectively. In contrast, milk yield differences for other breed and treatment combinations were not different from zero. Cows that dried off in 2009 had a lower milk difference (−605 ± 1,017 kg) than cows that dried off in 2010 (−29 ± 288 kg) or 2011 (1,948 ± 979 kg). Treatment, breed, last calving date, TD01_SCS, and PrevLactLastSCS were not significant but were included in the model.

Difference Between TD01_SCS and PrevLactLastSCS. We found no significant differences among treatments for the change in SCS from dry off to freshening (TD01_SCS – PrevLactLastSCS). Mean SCS change is presented in Table 3. The final model for SCS difference included treatment ($P < 0.03$), calving month ($P < 0.02$), previous lactation SCS average ($P < 0.001$), and the interaction between PostLact305MEM and treatment ($P < 0.04$). Treatment with P-M resulted in a significant reduction ($P = 0.009$) in SCS from dry off to freshening. No other treatments had an SCS change significantly different from zero. Cows that calved in January or November had a significant ($P < 0.005$) reduction in SCS from dry off to freshening, whereas the difference in SCS was not different from zero for cows calving in September, October, December, or February. Previous lactation SCS average had a negative relationship with the change in SCS from dry off to freshening; as the previous lactation SCS average increased, the SCS change from dry off to freshening decreased. The interaction between Post-

Table 3. Effect of Phyto-Mast, Cinnatube, Phyto-Mast and Cinnatube, no treatment, and conventional dry cow therapy on milk production and SCS of cows in a pasture-based research herd in North Carolina over 3 yr (trial 1)[1]

Item[2]	CON (n = 79)	CIN (n = 44)	P-M (n = 83)	PC (n = 43)	NT (n = 80)
PrevLact305MEM	7,584 ± 278	6,249 ± 516	7,426 ± 290	6,638 ± 516	6,726 ± 277
PostLact305MEM	7,339 ± 269	7,012 ± 499	7,600 ± 281	7,957 ± 499	7,293 ± 269
Milk difference*	−245 ± 348	763 ± 646	175 ± 363	1,322 ± 645	570 ± 347
PrevLactLastSCS	3.29 ± 0.26	2.96 ± 0.37	3.34 ± 0.28	3.86 ± 0.39	3.43 ± 0.26
TD01_SCS	3.07 ± 0.32	3.70 ± 0.56	2.91 ± 0.31	3.29 ± 0.54	3.20 ± 0.34
SCS difference*	−0.71 ± 0.42	0.54 ± 0.67	−1.20 ± 0.45	−0.37 ± 0.66	−0.40 ± 0.44

[1]Treatments: CON = conventional intramammary dry cow therapy including Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis); CIN = herbal internal teat sealant (Cinnatube; New AgriTech Enterprises, Locke, NY); P-M = herbal intramammary treatment for improving milk quality (Phyto-Mast; Bovinity Health LLC, Narvon, PA); PC = treatment with Phyto-Mast and Cinnatube; NT = no treatment. The CIN and PC treatments were only assessed for 2 of the 3 yr.

[2]PrevLact305MEM = previous lactation 305-d mature-equivalent milk production (LSM ± SE, in kg); PostLact305MEM = posttreatment lactation 305-d mature equivalent milk production (LSM ± SE, in kg); PrevLactLastSCS = last recorded SCS of the lactation before treatment; TD01_SCS = SCS of the first test date postpartum.

*Although numerically variable, treatments did not differ significantly for the difference in mature-equivalent milk from previous lactation to the next lactation or for the differences among treatments in SCS from the last test-day of the previous lactation to the first test-day in the subsequent lactation.

Lact305MEM and treatment was significant because cows producing between 4,536 and 9,072 kg (10,000 to 20,000 lb) of milk and receiving P-M treatment, as well as cows producing over 9,072 kg (20,000 lb) and receiving CON treatment, had an SCSDiff significantly lower than zero. Breed and the interaction between treatment and breed were also included in the model for improving the fit of the model and for calculation of least squares means presented in Table 3. Previous lactation last SCS and TD01_SCS least squares means are also presented in Table 3.

Trial 2: Quarter-Level SCS and Microbiological Efficacy

A total of 4,373 quarter samples (2,327 dry and 2,046 fresh) were collected from 441 cows enrolled in this study between August 2010 and March 2012. Of those samples collected, 3,048 (1,566 dry and 1,482 fresh) were duplicate samples taken from the research herd. Due to contaminated samples, missed samples at either dry off or freshening, or culled cows, only 1,044 paired quarter samples were available for analysis (104 CON, 230 CIN, 255 P-M, 214 PC, 241 NT). Contamination rates ranged from 5.1% in the research herd to 13.3% in 1 commercial herd, with an overall contamination rate of 6.1%. Of those 1,044 paired quarter samples, 466 were from Holstein cows, 60 were from Jersey cows, and 518 were from crossbred cattle. Lactation number at the start of the trial was not different among treatments (2.8 ± 1.7). Duration of the dry period was also not different among treatments (78 ± 38 d). Somatic cell score at dry off was not different among the treatment groups, but freshening SCS of CON, CIN, and P-C were significantly higher than freshening SCS of NT (Table 4). No incidences of clinical mastitis or noticeable udder irritation during the dry period were noted by the dairy managers participating in this trial.

SCS Difference. Mixed model regression of SCSDiff revealed no significant differences among treatments. The results of the mixed model regression are shown in Table 5. Dry periods <45 d had an average SCSDiff of −0.13, dry periods 45 to 60 d long had an average SCSDiff of 1.00, and dry periods >60 d had an average SCSDiff of 0.15. Of all possible results of treatment, only new IMI had an SCSDiff significantly different from zero. Cows beginning their second lactation after treatment and receiving the CON treatment were more likely to have a higher (1.38 ± 0.56) SCSDiff. Of the interactions between treatment and breed, only NT Jerseys and CON Jerseys were significantly different from each other ($P = 0.04$) with the difference in favor of no treatment. Only 3 treatment and breed combinations had SCSDiff significantly different from zero: CON Jerseys and PC crossbreds had SCSDiff >0 and NT Jerseys had SCSDiff <0.

IMI at Dry Off. The majority (839 or 80.4%) of samples had no infection present at dry off. The most prevalent organism present in the dry-off samples was CNS, present in 93 of the 205 quarters with IMI (45.4%). *Corynebacterium* spp. were present in 21% of infected quarters (43 quarters), followed by *Staphylococcus aureus* (14.6% or 30 quarters) and *Streptococcus* spp. other than *Strep. agalactiae* (6.3% or 13 quarters). The remaining IMI were caused by mixed infections (8.3% or 17 quarters), gram-positive organisms (3.4% or 7 quarters), gram-negative organisms (0.5% or 1 quarter), and yeasts (0.5% or 1 quarter). Frequencies of IMI present at dry off for each treatment are given in Table 6.

IMI at 3 to 5 Days Postcalving. Most samples (804 or 77.0%) had no infection present 3 to 5 d postcalving. The most prevalent organism in the postcalving sample was CNS, present in 82 of the 240 quarters with IMI (34.2%). *Streptococcus* spp. other than *Strep. agalactiae* were present in 21.7% of samples (52 quarters), followed by *Corynebacterium* spp. (16.7% or 40 quarters) and *Staph. aureus* (11.7% or 28 quarters). The remaining IMI were caused by mixed infections (7.1% or 17 quarters), gram-positive organisms (5.8% or 14 quarters), gram-negative organisms (2.5% or 6 quarters), and yeasts (0.3% or 1 quarter). Frequencies of IMI present postcalving for each treatment are given in Table 6.

Table 4. Quarter-level SCS for cows treated with conventional, herbal, or no dry cow therapy from 5 dairies in North Carolina (trial 2)

Treatment[1]	Dry SCS	Fresh SCS
Quartermaster and Orbeseal	3.82 ± 0.31[a]	4.67 ± 0.33[a]
Cinnatube	4.35 ± 0.22[a]	4.35 ± 0.23[ab]
Phyto-Mast	4.34 ± 0.24[a]	4.51 ± 0.26[a]
Phyto-Mast and Cinnatube	4.47 ± 0.26[a]	4.89 ± 0.27[a]
No treatment	3.97 ± 0.23[a]	3.83 ± 0.25[b]

[a,b]Estimates with different letters within a column are significantly different ($P < 0.05$).

[1]Treatments: Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis); Cinnatube (New AgriTech Enterprises, Locke, NY); Phyto-Mast (Bovinity Health LLC, Narvon, PA). Estimates given are LSM ± SE.

Table 5. Mixed model regression results for SCS difference of cows treated with conventional, herbal, or no dry cow therapy from 5 dairies in North Carolina (trial 2)

Effect	Description	Estimate	SE	*P*-value	95% CI
Random effects	Farm	0.49	0.36		
	Cow	2.82	0.18		
Treatment[1]	Conventional	0.79	0.49	0.11	−0.16–1.75
	Cinnatube	−0.06	0.36		−0.77–0.64
	Phyto-Mast	0.14	0.39		−0.63–0.90
	Phyto-Mast and Cinnatube	0.41	0.41		−0.39–1.22
	No treatment	−0.20	0.39		−0.96–0.56
Days dry				0.04	
Treatment result	No change	0.07	0.40	<0.01	−0.70–0.85
	No change, still not infected	0.005	0.33		−0.65–0.66
	Cure	−0.40	0.39		−1.18–0.37
	New infection	1.18	0.36		0.47–1.89
Treatment × lactation group	Conventional × first lactation	1.38	0.56	0.02	0.27–2.49
Treatment × breed	No treatment × Jersey	−1.41	0.72	0.04	−2.82–0.01
	Phyto-Mast and Cinnatube × crossbred	0.82	0.38		0.07–1.56
	Conventional × Jersey	2.00	0.82		0.38–3.61

[1]Treatments: Conventional = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis); Cinnatube (New AgriTech Enterprises, Locke, NY); Phyto-Mast (Bovinity Health LLC, Narvon, PA).

Table 6. Frequency of IMI[1] in quarters infected at dry off and at 3 to 5 DIM in the subsequent lactation in a comparison of conventional, herbal, and no dry cow therapy on 5 dairies in North Carolina (trial 2)[2]

	IMI present at dry off					IMI present 3 to 5 DIM				
Organism	CON	CIN	P-M	PC	NT	CON	CIN	P-M	PC	NT
Gram-positive										
CNS	0	19	27	29	18	2	12	20	24	24
	(0)	(8.26)	(10.59)	(13.55)	(7.47)	(1.92)	(5.22)	(7.84)	(11.22)	(9.96)
Corynebacterium spp.	0	9	15	8	11	0	12	10	12	6
	(0)	(3.91)	(5.89)	(3.74)	(4.56)	(0)	(5.22)	(3.92)	(5.61)	(2.49)
Enterococcus spp.	1	0	0	1	2	1	3	1	2	2
	(0.96)	(0)	(0)	(0.47)	(0.83)	(0.96)	(1.30)	(0.39)	(0.93)	(0.83)
Staphylococcus aureus	2	7	7	9	5	0	5	10	8	5
	(1.92)	(3.04)	(2.75)	(4.21)	(2.07)	(0)	(2.17)	(3.92)	(3.74)	(2.07)
Streptococcus spp.	1	2	1	3	6	1	11	17	8 (3.74)	15
(not *agalactiae*)	(0.96)	(0.87)	(0.39)	(1.40)	(2.49)	(0.96)	(4.78)	(6.67)		(6.22)
Other gram-positives[3]	0	1	1	0	0	0	3	0	2	0
	(0)	(0.43)	(0.39)	(0)	(0)	(0)	(1.30)	(0)	(0.93)	(0)
Total gram-positives	4	38	51	50	42	4	46	58	56	52
	(3.85)	(16.5)	(20.00)	(23.36)	(17.43)	(3.84)	(20.00)	(22.75)	(26.17)	(21.58)
Gram-negative										
Total gram-negatives[4]	0	0	1	0	1	0	1	4	0	1
	(0)	(0)	(0.39)	(0)	(0.41)	(0)	(0.43)	(1.57)	(0)	(0.41)
Other										
Yeast	0	0	1	0	0	0	0	0	1	0
	(0)	(0)	(0.39)	(0)	(0)	(0)	(0)	(0)	(0.47)	(0)
Mixed infections	0	5	8	3	1	0	1	6	3	7
	(0)	(2.17)	(3.14)	(1.40)	(0.41)	(0)	(0.43)	(2.35)	(1.40)	(2.90)
Total infected quarters	4	43	61	53	44	4	48	68	60	60
	(3.85)	(18.70)	(23.92)	(24.77)	(18.26)	(3.85)	(20.87)	(26.67)	(28.04)	(24.90)
Uninfected quarters	100	187	194	161	197	100	182	187	154	181
Total of all quarters	104	230	255	214	241	104	230	255	214	241

[1]Percentages (shown in parentheses) are percentage of all observed quarters receiving that specific treatment that were infected with the specific organism.

[2]CON = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis, Florham Park, NJ); CIN = Cinnatube (New AgriTech Enterprises, Locke, NY), an herbal internal teat sealant; P-M = Phyto-Mast (Bovinity Health LLC, Narvon, PA), an herbal intramammary product for improving milk quality; PC = Phyto-Mast and Cinnatube; NT = no treatment.

[3]Other gram-positives include *Bacillus* spp., *Nocardia* spp., and *Trueperella pyogenes.*

[4]Gram-negatives include *Enterobacter aerogenes*, *Escherichia coli*, and *Klebsiella* spp.

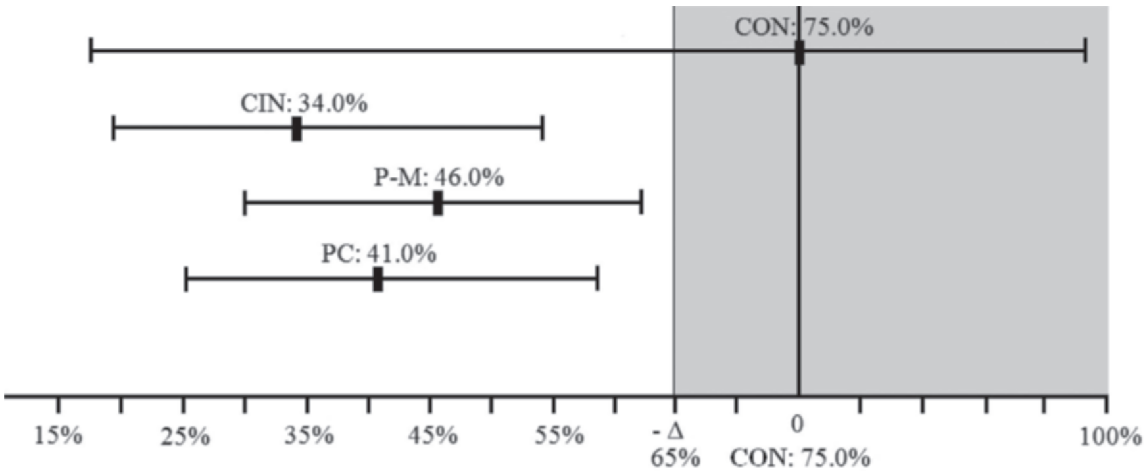

Figure 1. Noninferiority analysis of risk of cure for quarters from cows treated with Cinnatube (CIN; New AgriTech Enterprises, Locke, NY; LSM = 0.34; 95% CI: 0.19–0.53), Phyto-Mast (P-M; Bovinity Health LLC, Narvon, PA; LSM = 0.46; 95% CI: 0.30–0.62), or Phyto-Mast and Cinnatube (PC; LSM = 0.41; 95% CI: 0.26–0.60) compared with cows treated conventionally (CON) with Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis) (LSM = 0.75; 95% CI: 0.18–0.98). The error bars indicate the bounds of the 95% CI and the shaded area indicates the region of noninferiority, with Δ as the predetermined margin of noninferiority (10%).

Effect of Treatment on Probability of Cure. Mixed model logistic regression of proportion of quarters cured during the dry period resulted in no significant differences among treatments. The results of the noninferiority analysis are shown in Figure 1. Although none of the herbal products reached the zone of noninferiority, the 95% CI for CON overlapped the intervals for all of the herbal products. Results of the regression are shown in Table 7. Increased SCC at dry off was associated with lower chance of cure. An SCSDiff <0 was associated with a higher chance of cure.

The CNS accounted for 50.5% of all IMI cured during the dry period, followed by *Corynebacterium* spp., representing 23.0% of all IMI cured during the dry period. Analysis comparing the efficacy of the treatments at curing IMI with specific pathogens was not possible because few infections were present in CON cows at dry off (Table 6). However, analysis comparing the other 4 treatments showed no differences in cure rate for CNS or *Corynebacterium* spp. Numbers of quarters cured by pathogen are listed in Table 8. The research herd had a lower initial infection rate (11.2%) than the commercial herds (34.0%). In the research herd alone, CON cured 75% of infections (3 of 4), CIN cured 28.6% of infections (2 of 7), P-M cured 25% of infections (3 of 12), PC cured 52.4% of infections (11 of 21), whereas 47.4% of infections were cured in the absence of treatment (NT; 9 of 19). We found no significant differences in proportion of quarters cured among all treatments within the research herd.

When IMI were grouped into gram-positive, gram-negative, other, and mixed infections, there were still no significant differences among all treatments for ability to cure any of these categories of infections.

Effect of Treatment on Probability of a New Infection Postcalving. Mixed model logistic regression results of the proportion of quarters with new infections postpartum are shown in Table 7. The results of the noninferiority analysis are presented in Figure 2. The mean probability of new infection for each of the herbal products was within the zone of noninferiority. Quarters treated with CIN were significantly less likely to experience a new infection than quarters treated with NT ($P = 0.03$). New infection rates among the other treatments did not differ significantly. Higher postcalving SCC was associated with higher probability of new infection. Jerseys were the most likely to have a new infection postcalving (35 ± 15%), followed by Holsteins (29 ± 10%) and crossbreds (17 ± 8%). Jerseys were more likely ($P = 0.08$) than crossbreds to have a new infection present postcalving. The interaction of dry period length with treatment was significant because every treatment except for CON had a higher probability of new infections when the dry period was >60 d. Length of the dry period was also included in the model although it was not significant.

New infection rates for specific organisms could not be calculated because of the low number of new infections, especially in CON cows. When IMI were grouped into gram-positive, gram-negative, other, and mixed infections, we found no significant differences among

Table 7. Mixed model logistic regression results for cure risk and new infection risk models for cows treated with conventional, herbal, or no dry cow therapy from 5 dairies in North Carolina (trial 2)

Effect	Descript vcvc ion	Estimate	SE	*P*-value	95% CI
Proportion of quarters cured					
Random effects	Farm	0.13	0.23		
	Cow	1.00	0.11		
Treatment[1]	Conventional	0.75	0.25	0.70	0.18–0.98
	Cinnatube	0.34	0.09		0.19–0.53
	Phyto-Mast	0.46	0.09		0.30–0.62
	Phyto-Mast and Cinnatube	0.41	0.09		0.26–0.59
	No treatment	0.41	0.09		0.25–0.60
SCS at dry off				<0.01	
SCS difference[2]				<0.01	
Proportion of quarters with new IMI					
Random effects	Farm	0.96	0.74		
	Cow	1.02	0.05		
Treatment	Conventional	0.24	0.21	0.02	0.03–0.75
	Cinnatube	0.15	0.07		0.06–0.33
	Phyto-Mast	0.30	0.10		0.14–0.53
	Phyto-Mast and Cinnatube	0.32	0.11		0.15–0.55
	No treatment	0.35	0.11		0.17–0.58
Postpartum SCC				<0.01	
Dry period duration × treatment				0.01	
Breed	Holstein	0.29	0.11	0.57	0.14–0.52
	Jersey	0.35	0.15		0.13–0.65
	Crossbred	0.17	0.08		0.07–0.37

[1]Conventional = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis); Cinnatube (New AgriTech Enterprises, Locke, NY; Phyto-Mast (Bovinity Health LLC, Narvon, PA).
[2]SCS 3 to 5 d postpartum − SCS at dry off.

treatments for probability of new infections in any of these groups.

DISCUSSION

Scientific evaluation of alternatives to antibiotics is essential to ensure that such treatments are safe and effective. This is especially important in the case of organic dairy farmers, who are prohibited from using antibiotics to treat mastitis and need viable alternatives. This study examined the effects of 2 herbal IMM products on milk production and milk quality when administered as a dry cow therapy. To the authors'

Table 8. Proportion of IMI cured and proportion of new IMI by control, herbal, or no dry cow therapy during the dry period in 5 dairies in North Carolina[1,2]

	Proportion of IMI cured					New IMI during the dry period				
Organism	CON[3]	CIN	P-M	PC	NT	CON[3]	CIN	P-M	PC	NT
Gram-positive										
Bacillus spp.	0/0	0/0	0/0	0/0	0/0	0	1	0	1	0
CNS	0/0	9/19	13/27	11/29	11/18	2	5	12	10	19
Corynebacterium spp.	0/0	3/9	10/15	2/8	5/11	0	4	4	7	3
Enterococcus spp.	1/1	0/0	0/0	1/1	0/2	1	3	1	2	1
Nocardia spp.	0/0	0/1	1/1	0/0	0/0	0	1	0	0	0
Staphylococcus aureus	1/2	1/7	0/7	1/9	1/5	0	0	4	1	2
Streptococcus spp. (not *agalactiae*)	1/1	1/2	0/1	3/3	1/6	1	11	12	8	14
Trueperella pyogenes	0/0	0/0	0/0	0/0	0/0	0	0	0	1	0
Gram-negative[4]	0/0	0/0	0/1	0/0	0/1	0	1	4	0	0
Other										
Yeast	0/0	0/0	1/1	0/0	0/0	0	0	0	1	0
Mixed infections	0/0	2/5	3/8	2/3	1/1	0	1	6	3	7
Total, quarters cured/infected (%)	3/4 (75.0)	17/43 (39.5)	28/61 (45.9)	20/53 (37.7)	19/44 (43.2)	4/104 (3.8)	27/230 (11.7)	43/255 (16.9)	34/214 (15.9)	46/241 (19.1)

[1]Infections are recorded as number of quarters cured/number of quarters infected at dry off.
[2]CON = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis); CIN = Cinnatube (New AgriTech Enterprises, Locke, NY); P-M = Phyto-Mast (Bovinity Health LLC, Narvon, PA); PC = Phyto-Mast and Cinnatube; NT = no treatment.
[3]The conventional treatment was only assessed on the research herd due to antibiotic use limitations on the other dairies.
[4]Gram-negative infections present included *Enterobacter aerogenes*, *Escherichia coli*, and *Klebsiella* spp.

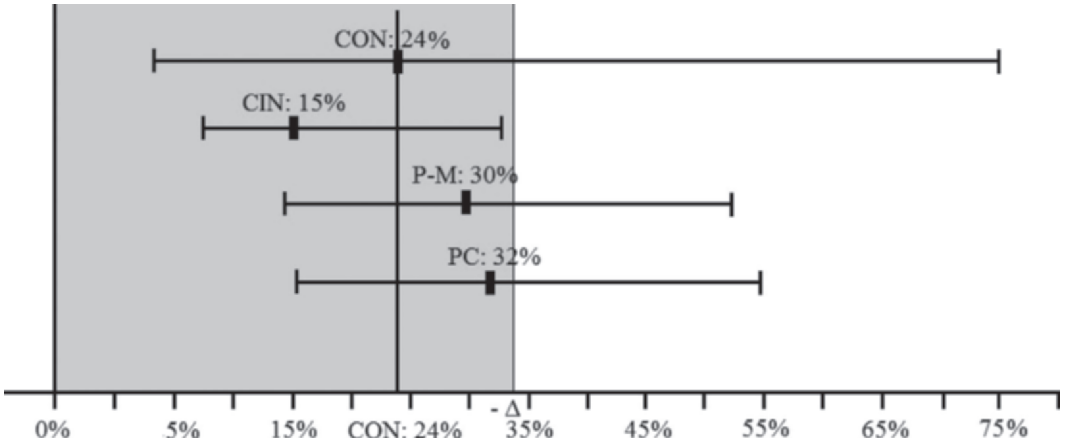

Figure 2. Noninferiority analysis of risk of new infection for quarters from cows treated with Cinnatube (CIN; New AgriTech Enterprises, Locke, NY; LSM = 0.15; 95% CI: 0.06–0.33), Phyto-Mast (P-M; Bovinity Health LLC, Narvon, PA; LSM = 0.30; 95% CI: 0.14–0.53), or Phyto-Mast and Cinnatube (PC; LSM = 0.32; 95% CI: 0.15–0.55) compared with cows treated conventionally (CON) with Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Florham Park, NJ) and Orbeseal (65% bismuth subnitrate; Zoetis) (LSM = 0.24; 95% CI: 0.03–0.75). The error bars indicate the bounds of the 95% CI and the shaded area indicates the region of noninferiority, with Δ as the predetermined margin of noninferiority (10%).

knowledge, this is the first controlled study to date examining the effect of herbal IMM products on milk production and milk quality. The inclusion of multiple breeds and ages of dairy cattle provides a better picture of how the products may affect a variety of cattle. This also provides more direct application to the organic dairy industry in the United States, as herds often include crossbreds and multiple breeds (Sato et al., 2005; Rotz et al., 2007; Mullen et al., 2013; Stiglbauer et al., 2013) and have older cattle than conventional herds (Stiglbauer et al., 2013). Although these herbal products were used as dry cow therapies in the current study, they are not labeled specifically for treatment of disease. Neither has undergone the FDA approval process for treatment of mastitis. Most of their ingredients are on the FDA Generally Recognized as Safe list for human consumption (US Food and Drug Administration, 2013b). Although the National Organic Standards Board can approve the use of herbal products in organic cattle, it cannot approve the use of an intramammary product labeled for mastitis treatment, as that is under FDA jurisdiction. The products tested in this trial have not undergone the FDA review process and thus cannot be marketed as treatments for mastitis.

In the development of intramammary treatments, it is important to consider that the mammary gland is very susceptible to irritation and that any intramammary infusions should be nonirritating (Sanderson, 1966). Several essential oils have documented cytotoxic activity (Bakkali et al., 2008), which raises concerns about the welfare of cows receiving herbal products. The current study indicates that Cinnatube, Phyto-Mast, or a combination of the 2 did not have an irritating effect on the udder, at least as measured by milk production at the cow level and by SCS at the cow and quarter levels compared with the positive (CON) and negative (NT) controls. Milk production was not adversely affected by the herbal products. Studies examining the effects of the herbal ingredients in the products tested in this trial have shown some of the essential oils to have anti-inflammatory activity (Table 1). The ingredients of the treatments in this trial did not appear to have that effect in the current study compared with no treatment. In trial 2, although 3 of the treatments (CON, P-M, and PC) had higher freshening SCS than NT, only CON had an SCSDiff much greater than zero, with a standard error smaller than its mean. We expected the SCS of CON to be reduced at freshening compared with NT, because a previous comparison of dry cow therapies reported lower SCS postcalving in quarters treated with cloxacillin and internal teat sealant compared with quarters treated with cloxacillin benzathine alone (Godden et al., 2003; Runciman et al., 2010).

The significance of year in the prediction of the difference in milk yield is likely due to the drought that occurred in 2009. The research herd used for trial 1 is a pasture-based research herd. The drought's negative effects on pasture quality and productivity likely negatively affected milk production.

The presence of infection at dry off was low (19.6%) compared with the 30 to 40% seen in some dry-off studies using whole-herd sampling (Godden et al., 2003; Bradley et al., 2010). Furthermore, the a priori sample size calculation for the noninferiority test accounted for

a 19% infection rate in each treatment, which was not achieved in the CON treatment. The sample size estimate was based on using a conservative initial infection rate relative to the 31% (Godden et al., 2003) and 50 to 60% (Bradley et al., 2010) seen in other trials evaluating antibiotics with internal teat sealants. Conventional therapy cured 75% of infections, slightly lower than previous studies examining the combination of intramammary antibiotics and teat sealants (Woolford et al., 1998; Godden et al., 2003), but with a very large confidence interval. We hypothesized that the herbal products would be significantly less effective than conventional therapy at curing infections during the dry period. Trial 2 showed that the herbal products had similar efficacy to conventional therapy despite numerical differences, but this reported similarity in efficacy was likely due to the low infection rate at dry off and resulting large confidence intervals for all treatments. Any antimicrobial activity of the herbal products was likely conferred by the previously reported antibacterial activity of several ingredients in each product tested (Table 1). Conversely, the herbal products did not cure significantly more infections than were spontaneously cured (NT treatment). Because Cinnatube was infused without pinching the teat, it is likely that Cinnatube entered the udder cistern and interacted with the Phyto-Mast during the dry period.

The authors accept that estimating bacteriological cure rate over the dry period based on a single milk sampling after calving could result in overestimation of the efficacy of a dry cow therapy; this is especially likely for bacteria such as *Staph. aureus*, which are shed intermittently from infected udder quarters. In the current study, only 4 of 30 initial cases of *Staph. aureus* infection were cured during the dry period, and those cured quarters were treated with CON (1 quarter), CIN (1 quarter), and PC (1 quarter). One of 30 initial cases cured was from an untreated cow.

The risk of acquiring a new infection during the dry period is highest during the beginning and end of the dry period (Cousins et al., 1980; Oliver and Mitchell, 1983; Smith et al., 1985) and is higher for cows with longer dry periods (Berry and Hillerton, 2007). Cows with longer dry periods that received any treatment except CON had a higher risk of new infection in the present experiment. Ideally, treatments administered during the dry period would remain in the mammary gland for the duration of the dry period to protect against infection. Thymol residues of Phyto-Mast were detected up to 4 h posttreatment in blood serum and up to 24 h in milk of goats (McPhee et al., 2011). Thymol is a component of *Thymus vulgaris* essential oil and has strong antibacterial activity against mastitis pathogens in vitro (Baskaran et al., 2009). Assuming this persistence translates to dairy cattle, Phyto-Mast does not appear to have the ability to remain in the cow's system long enough to be an effective dry cow therapy. This may explain the numerically higher new infection rate in P-M and PC cows. It does not, however, explain the similar rate of new infections among CON and all other treatments or the noninferiority of the herbal products compared with CON. It was expected that CON would have had a significant reduction in the rate of new infections compared with no treatment, as shown previously with antibiotics or teat sealants (Smith et al., 1967a,b; Huxley et al., 2002; Berry and Hillerton, 2002). Most studies evaluating teat sealants as dry cow therapy require cows to be uninfected or have a low SCC to receive teat sealant treatment. This qualification for treatment was not used in the present study and may explain some of the difference seen in new infection rates. The 95% CI for proportion of newly infected quarters was very large for CON and prevented CON from being significantly different from any other treatment. We failed to reject our hypothesis that the herbal products were the same as no treatment at preventing new infections, except for CIN, which had significantly fewer new infections than NT. Further research is recommended to determine the persistence of CIN in the udder and its potential for prevention of infection during the dry period.

CONCLUSIONS

Treatment with Cinnatube, Phyto-Mast, or a combination of Phyto-Mast and Cinnatube had no apparent negative effects on milk production or SCS in mature cows. These herbal products also had similar new infection rates to conventional antibiotic therapy. Although cure rates appeared to be similar among the herbal products and conventional therapy, further assessment with larger sample sizes and a higher initial infection rate is necessary to draw conclusions. This study was not able to detect a significant difference between no treatment, conventional treatment, and the herbal products.

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