

ABSTRACT

MULLEN, KEENA ANN ELIZABETH. Evaluation of Herbal Oils in Various Preparations for Treating Mastitis in Dairy Cattle. (Under the direction of Steven P. Washburn and Kevin L. Anderson.)

The organic dairy industry is growing rapidly in the United States and with its growth is an increasing need for organic treatments for mastitis. Mastitis, or udder inflammation, is often caused by bacterial infection and is conventionally treated with antibiotics. Antibiotics are also used at the end of lactation, known as dry cow therapy, to eliminate existing intramammary infections and prevent new infections from occurring before the next lactation. Organic dairies in the United States are prohibited from using antibiotics in their cattle and thus use alternatives for mitigating mastitis. Mastitis can be measured through culturing milk of cows or measuring the somatic cell count of the milk, which is an indicator of the level of inflammation present and is often transformed into a linear score (SCS). No research has been performed comparing organic and conventional dairies in the southeast where heat and humidity make quality milk production challenging. The goals of the research contained in this dissertation were to compare milk quality on organic and conventional dairies in the southeastern United States and to evaluate two herbal alternatives to antibiotics for use as dry cow therapy.

In the first experiment, organic and conventional dairies in North Carolina were compared during the warm months. Seven organically and 7 conventionally managed dairy herds in North Carolina were surveyed in 2010 to record differences in milking procedures,

mastitis detection and treatment, and to determine the prevalence of mastitis-causing organisms and milk quality for each management type. Overall infection rate, SCS, and cow-level prevalence of several mastitis-causing pathogens were not different between organic and conventional dairies surveyed. Because of the similar prevalence of mastitis-causing organisms in organic and conventional dairies in North Carolina, further studies were planned to evaluate alternatives to antibiotics for mastitis mitigation.

The second experiment evaluated two commercially available alternatives to antibiotics as dry cow therapy on organic and conventional dairies. Phyto-Mast® and Cinnatube™ are two intramammary products composed primarily of plant-based oils. In a study comparing Phyto-Mast, Cinnatube, Phyto-Mast and Cinnatube, no treatment, and conventional antibiotic and teat sealant dry cow therapy, the herbal products had no negative effects on milk production or SCS and similar cure and new infection rates to the cows receiving conventional dry cow therapy and the untreated cows. However, the cure rate was difficult to assess and compare because of a low initial infection rate.

The potential of Phyto-Mast to cure infections was investigated in the third experiment by testing the antibacterial activity of each of its plant-derived oil ingredients, alone and in combination, in vitro against 3 mastitis-causing pathogens in milk. Only essential oil of *Thymus vulgaris* (thyme) had a consistently significant antibacterial effect.

The fourth experiment examined Phyto-Mast again as a dry cow therapy, this time compared with an internal teat sealant, the combination of Phyto-Mast and internal teat sealant, and no dry cow therapy to determine if an internal teat sealant had any effect on the

efficacy of Phyto-Mast. Though the SCS and cure rates of cows receiving either Phyto-Mast, teat sealant, a combination of the two, or no treatment were not different among treatments, the combination of Phyto-Mast and teat sealant had significantly fewer new infections than no treatment.

Taken altogether, there is a need for alternatives to antibiotics for organic dairy producers throughout the United States. The herbal treatments tested here did not negatively affect milk production or SCS, and were not inferior to conventional dry cow therapy for preventing new infections from occurring during the dry period. Thyme oil, an ingredient of one of the herbal treatments, has significant antibacterial activity when cultured in milk.

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Evaluation of Herbal Oils in Various Preparations for Treating Mastitis in Dairy Cattle

by
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DEDICATION

To my parents, Jim and Carolyn, for their love and support.

BIOGRAPHY

Keena A. E. Mullen was born on August 23, 1988. She was raised in Northwest Washington State in the evergreen mountains, where she rode horses and learned to love the great outdoors. Her dairy experience started during her undergraduate career at Washington State University. She received a Bachelor of Science degree in Animal Science from Washington State University in 2009, completing the Honors Program with a thesis entitled “Ionic Liquids as Solvents for Lignocellulosic Biomass”. This research took place during the summer of 2008, when she participated in a National Science Foundation Research Experience for Undergraduates program in Chemical and Biomolecular Engineering at North Carolina State University. This experience fueled her interest in research. Though she was accepted into the College of Veterinary Medicine at Washington State University, she chose instead to pursue a Ph. D. at North Carolina State University in Animal Science.

During her tenure as a Ph. D. student, Keena expanded her knowledge of the dairy industry and was exposed to many different dairy management systems, including organic production and pasture-based dairies. She received multidisciplinary training including a minor in statistics and coursework at the College of Veterinary Medicine and in the Crop and Soil Science departments.

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LIST OF ABBREVIATIONS

CNS: coagulase-negative *Staphylococcus* species

d: day(s)

DHIA: Dairy Herd Improvement Association

DMI: dry matter intake

h: hour(s)

IMI: intramammary infection

IMM: intramammary

min: minute(s)

mL: milliliter(s)

SCC: somatic cell count

SCS: somatic cell score, a transformation of the somatic cell count using the following

formula: $\log_2 \left(\frac{\text{SCC}}{100,000} \right) + 3$

SE: standard error

yr: year(s)

*other abbreviations are defined at first use in each manuscript.

CHAPTER 1: LITERATURE REVIEW

MASTITIS

Mastitis is a pervasive and costly disease that afflicts mammary glands worldwide. Mastitis is inflammation of the mammary gland (Greek *mastos*, breast; *-itis*, inflammation). In the dairy industry, a clinical case of bovine mastitis can cost greater than \$100 U. S. and up to \$403 U. S. in high-yielding cows due to milk yield losses, increased mortality, and treatment costs (Bar et al., 2008; Cha et al., 2011). Mastitis is detrimental to the health of the cow, and its negative effects can impact cow reproduction, milk yield, and shelf life of dairy products derived from the cow's milk (Politis and Ng-Kwai-Hang, 1988; Ma et al., 2000; Schrick et al., 2001).

Etiology and Measurement

Mastitis is typically caused by intramammary bacterial infection but can also be caused by other microorganisms, such as yeasts, algae, or fungi. As many as 137 different microbial species and subspecies have been described as causative agents of mastitis (Watts, 1988).

Bacteria that cause mastitis have typically been separated into two sets of mutually exclusive pairings, one describing the source of the bacteria and the other describing the relative pathogenicity of the bacteria: contagious versus environmental and major versus minor. Contagious pathogens are usually transmitted from cow to cow, most often through inadequate hygiene in the milking parlor. Contagious pathogens are adapted to survive in the

bovine mammary gland (Bradley, 2002). These pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, and *Mycoplasma* spp. (National Mastitis Council, 1996, 2009). Environmental pathogens are often acquired through contact with soiled bedding or mud. Environmental mastitis is caused by coliform bacteria, *Enterococcus* spp., and *Streptococcus* spp. other than *agalactiae* (the “environmental streptococci”) (National Mastitis Council, 1997). Intramammary infections vary in severity depending upon the pathogen present. Clinical mastitis, where the cow shows signs of being sick (e. g., feverish or not eating), is considered a severe manifestation of intramammary infection. Subclinical mastitis is less severe, and the cow may not show outward signs of infection. Certain bacterial species are known to cause increases in somatic cell count (SCC) but are considered “minor” mastitis pathogens because they do not typically cause acute clinical mastitis; these are coagulase-negative *Staph.* spp. (CNS) (National Mastitis Council, 1996) and *Corynebacterium bovis* (LeVan et al., 1985; National Mastitis Council, 1996). “Major” bacterial mastitis pathogens are known to cause clinical mastitis and increase SCC; these include contagious pathogens (*Staph. aureus*, *Strep. agalactiae*, and *Mycoplasma* spp.) and environmental pathogens (*Escherichia coli*, *Klebsiella* spp., *Enterococcus* spp., and environmental streptococci) (National Mastitis Council, 1996). Both major and minor pathogens can cause chronic subclinical mastitis, including *Staph. aureus*, *Strep. agalactiae*, coagulase-negative *Staphylococcus* spp., and *C. bovis*. *Corynebacterium bovis* is considered a contagious pathogen, but does not generally cause as severe of mastitis as *Staph. aureus*, *Strep. agalactiae*, or *Mycoplasma* spp. (LeVan et al., 1985).

The cow has natural defense systems in place to prevent invasion of bacteria. These include the teat sphincter muscles, which are responsible for keeping the teat closed between milkings, the keratin lining of the inner surface of the teat canal, which has an antibacterial effect through both chemical and physical means, and cellular defenses (Sordillo et al., 1997). The main cellular defense system is comprised of mammary epithelial cells, macrophages, and neutrophils. Mammary epithelial cells are among the first cells to respond to invasion of pathogens into the mammary gland. Those epithelial cells, when triggered by elements of the bacterial cell wall or bacterial metabolites, produce pro-inflammatory cytokines such as interleukin (**IL**)-1 β and tumor necrosis factor α (**TNF α**) to trigger an immune response and recruit neutrophils to the site of infection (Oviedo-Boyso et al., 2007). Some bacterial cell wall molecules that can trigger this response include lipopolysaccharide (**LPS**) of gram-negative bacteria and lipoteichoic acid of some gram-positive bacteria, such as *Staph. aureus*. Pro-inflammatory cytokines include TNF α , IL-1 β , IL-6, IL-8, and interferon- γ . They function to modify endothelial permeability, regulate inflammatory tissue cell death, and bring blood cells to the inflammation site (Dinarello, 2000; Takeuchi and Akira, 2010). Anti-inflammatory cytokines, including IL-4, IL-10, IL-13, and transforming growth factor β suppress pro-inflammatory cytokine production (Dinarello, 2000). Macrophages predominate in milk from uninfected cows, and when they encounter bacteria in the mammary gland, they also release chemoattractant molecules to recruit neutrophils (Paape et al., 2002; Rainard and Riollet, 2006). Neutrophils predominate in milk during intramammary infection (Oviedo-Boyso et al., 2007). Once recruited to the infection site,

neutrophils eliminate bacteria through phagocytosis and respiratory bursts of hydroxyl and oxygen radicals (Rainard and Riollet, 2006). Reactive oxygen species can cause damage to mammary cells as well as to invading bacteria (Paape et al., 2002). Presence of cells in milk, whether they be damaged epithelial cells, macrophages, leukocytes, or other bacteria- or host-derived cells, is important when assessing a cow for the presence of mastitis. These cell types have historically been combined together for easy measurement. This measurement, SCC, is used by dairy farmers around the world to assess the prevalence of mastitis.

Mastitis can be measured directly by culturing milk or indirectly by measuring SCC. The SCC can be a good predictor of intramammary infection using a threshold of 200,000 cells/mL (Dohoo and Leslie, 1991). This threshold is also used as a cut-off value for predicting loss of milk quality (Schukken et al., 2003). In addition, SCC has an inverse relationship with milk production: as SCC increases, milk production decreases (Jones et al., 1984; Barkema et al., 1998), even when the concentration is adjusted for the dilution factor of higher milk production (Green et al., 2006). Somatic cell count is important as an indicator of intramammary infections and also carries economic significance, as dairy producers must comply with federal SCC regulations to be able to market their milk. Currently, SCC must not exceed 750,000 cells/mL in the United States (U. S. Food and Drug Administration, 2011). Furthermore, milk used for export to the European Union must be below 400,000 cells/mL on every farm based on a three-month geometric average (European Union, 2010). Dairy producers may also receive milk quality premiums for achieving SCC below certain thresholds.

TRADITIONAL MASTITIS MITIGATION

The National Mastitis Council, an organization composed of researchers, academicians, veterinarians, industry representatives, extension personnel, and regulatory agents, recommends a mastitis control plan based on current scientific knowledge about mastitis. The current ten-point program includes establishing herd udder health goals, maintaining a hygienic environment for cattle, using proper milking procedures, maintaining milking equipment regularly, keeping good records, managing clinical mastitis appropriately during lactation, managing dry cattle effectively, reducing the prevalence of contagious mastitis on-farm, regularly monitoring udder health status, and occasionally reviewing the mastitis control plan (National Mastitis Council, 2007).

Preventive Therapy

Mastitis prevention includes maintenance of a clean environment, adequate hygiene during milking, proper maintenance of milking machines, and use of prophylactic therapy at the end of lactation.

Maintenance of a clean environment for dairy cattle is essential because of the risk of environmental mastitis, especially post-milking. Cattle that leak milk prior to milking are not at as high of risk for mastitis because pre-milking teat sanitization and the process of milking eliminate potential pathogens and remove invading pathogens from the gland, respectively. However, the risk of environmental mastitis post-milking is of concern despite post-milking teat sanitization: it takes about 30 minutes after milking for the teat sphincter to close (Williams and Mein, 1987) and at least three hours for the teats to recover their size and

shape post-milking (Neijenhuis et al., 2001). Environmental cleanliness is important for cattle housed in confinement as well as for cattle with access to pasture. Bacterial species and counts in bedding are positively correlated with bacterial species and counts present on the teat skin, and these organisms present on the teat skin can cause mastitis (Rendos et al., 1975; Hogan et al., 1989; Hogan and Smith, 1997; Zdanowicz et al., 2004). In addition, SCC increases with increasing amounts of manure and mud present on the legs, flanks, abdomens, and udders of pasture-based dairy cows (Sant'Anna and Paranhos da Costa, 2011). Keeping the cow's environment clean is a straightforward practice for dairy farmers to employ in order to prevent mastitis from occurring.

Additionally, the milking parlor is an important place to practice good hygiene, as cows visit multiple times per day and most intramammary infections have been reported to occur during milking (Bushnell, 1984). It is also important to maintain the functionality of milking machines, as pulsation rates outside of acceptable limits and other machine-related failures (teat liner slips, etc.) can cause damage to the teat end and increase mastitis susceptibility (Mein et al., 2004). The three specific opportunities for mastitis mitigation in the milking parlor are before-milking teat sanitization, sanitizing the milking unit between cows, and post-milking teat sanitization. Pre-milking udder and teat sanitization reduces the risk of contaminating the milk with bacteria (Galton et al., 1982) and the use of a germicidal pre-milking teat dip reduces coliform counts in milk (Galton et al., 1986) and can help reduce the rate of new intramammary infections (Oliver et al., 1994). As of 2007, 79.0% of dairy herds in the United States used some type of germicidal pre-milking teat dip (USDA, 2008).

Cleaning the milking unit between cows reduces the possibility of infection being transmitted through the milk in the milking unit. Post-milking teat disinfection kills any bacteria on the teat that were obtained during milking (Olde Riekerink et al., 2012) and reduces the risk of infection with contagious mastitis pathogens (Pankey et al., 1984). Consistent use of post-milking teat dip is also associated with lower SCC (Erskine and Eberhart, 1991; Wenz et al., 2007; Dufour et al., 2011). Udder hygiene in the milking parlor is essential for mastitis prevention.

Dairy cattle also go through a “dry period” at the end of lactation before calving. This non-lactating period is to allow mammary gland involution before the next lactation. The typical recommended dry period length is 51-60 days, though shortening the dry period may have economic benefits (Bachman and Schairer, 2003). The dry period is an opportune time to use long-acting antibiotic therapeutics for mastitis because milk is not collected during this time, and therefore, residues from treatments in milk are not a concern. The purpose of dry cow therapy is to eliminate existing infections and prevent infections from occurring prior to the next lactation. The National Mastitis Council recommends treating all quarters of all cows with a long-acting intramammary antibiotic or an internal teat sealant or both (National Mastitis Council, 2007). This so-called “blanket” dry cow therapy has been associated with lower SCC in herds than not using dry cow therapy (Eberhart and Buckalew, 1972; Godden et al., 2003; Dufour et al., 2011). As of 2007, 72.3% of dairy herds in the United States used blanket dry cow therapies (USDA, 2008). Intramammary antibiotic therapy is used to control gram-positive pathogens, and use of an internal teat sealant provides a physical barrier to

prevent bacterial entry (especially environmental, gram-negative bacteria) during the dry period. This barrier effectively prevents infection with environmental bacteria (Huxley et al., 2002), and when combined with intramammary antibiotic therapy and used in high-SCC (>200,000 cells/mL) cows, can reduce the risk of treated cows being infected post-calving (Bradley et al., 2010).

Antibiotics

Antibiotics are used on dairy farms for treatment of calf enteritis, pneumonia, metritis, and foot rot as well as for mastitis, among other infections (Zwald et al., 2004; Sawant et al., 2005). Antibiotics are used to treat intramammary infections during lactation with varying success depending upon the pathogen present and the duration of infection (Wilson et al., 1999; Barkema et al., 2006). This review will focus on the use of antibiotics in dry cow therapy.

As of October 17, 2013, 34 different products in five different antibiotic classes were approved by the United States Food and Drug Administration (FDA) for treatment of mastitis via intramammary infusion, but not all are necessarily commercially available. Fourteen of those products are labeled specifically for use in lactating dairy cattle, 14 are labeled for use in dry cows only, and 6 are labeled for use in both lactating and dry cows. Antibiotics approved for dry cow therapy include β -lactams (ceftiofur, cephapirin, cloxacillin, penicillin G), macrolides (erythromycin), aminoglycosides (streptomycin), and aminocoumarins (novobiocin). The β -lactam class of antibiotics inhibits cell wall synthesis in targeted bacteria; the macrolides inhibit protein synthesis by binding to the 50s ribosomal subunit; the

aminoglycosides inhibit protein synthesis by binding to the 30s ribosomal subunit; the aminocoumarins inhibit DNA gyrase in the targeted bacteria, inhibiting their replication. Only one of the dry cow products, Orbenin DC (cloxacillin benzathine), is approved for prophylaxis for mastitis. The rest have specific indications for use in the presence of mastitis-causing organisms (U. S. Food and Drug Administration, 2013). In practice, many farmers use the other products for prophylaxis as well, as indicated by the overall percentage of American dairies using blanket dry cow therapy. Many farmers use multiple antibiotics on-farm; of 20 conventional dairies in Wisconsin that used blanket dry cow therapy, 18 used penicillin-dihydrostreptomycin, 15 used cephalosporin, and 3 used novobiocin as intramammary dry cow therapy (Pol and Ruegg, 2007a).

Residue Risk. One of the milk quality measurements dictated by the Pasteurized Milk Ordinance in the United States is the presence of antibiotic residues. In order to market their milk as Grade A, dairy producers must meet the following standards: raw milk must not exceed 100,000 colony forming units of bacteria per mL, must not contain drug residues, and the SCC must not exceed 750,000 cells per mL (U. S. Food and Drug Administration, 2011). Antibiotic residues are of concern because they are potentially allergenic to humans, their presence negatively affects production of cheese and other cultured dairy products, and because their presence above a certain established tolerance level indicates that the milk has been adulterated. Every FDA-approved antibiotic for use in dairy cattle has a recommended withdrawal time on its label to avoid residues in milk or meat from the treated animal. Dairy farmers in the United States are most likely to use antibiotics according to their past

experiences, but do consult the product label when determining withdrawal time (Zwald et al., 2004). Care must be taken when using intramammary antibiotics to avoid positive residue tests of milk. Of all antibiotic residues in milk from dairy farms in the United Kingdom, 25% came from intramammary dry cow treatments and over 50% came from lactating cow treatments (Booth and Harding, 1986). A high correlation between high SCC and risk of antibiotic residues in Wisconsin herds was reported by Ruegg and Tabone (2000), underlying the need to prevent mastitis with good management to avoid the need for antibiotics. Currently, antibiotic residue screenings mandated by the FDA only detect antibiotics in the β -lactam class (U. S. Food and Drug Administration, 2011), though testing for other classes is also performed. Testing of bulk tank milk in fiscal year 2012 resulted in only 0.017% samples positive for drug residues; only 0.02% of β -lactam tests run (3,720,542) were positive (National Milk Drug Residue Data Base, 2013).

Resistance Development. Antibiotic resistance in bacteria is a serious concern for both animal and human health. The cost of antibiotic resistance comes from multiple sources: increased mortality due to failed treatment, loss of efficacy of older antibiotics and thus the need for new antibiotics to be developed or higher doses required for efficacy, and the costs associated with isolation and treatment of humans (or animals) with multi-drug-resistant infections (Hawkey, 2008). When used, antibiotics should be delivered at a therapeutic dose. Sub-therapeutic doses of antibiotics contribute to the development of antibiotic resistance by accelerating the transfer of antibiotic resistance genes and increasing the prevalence of resistant bacteria. The FDA is concerned about the effect of antibiotic usage in livestock on

antibiotic resistance and the possible negative repercussions on human health (U. S. Department of Health and Human Services and U. S. Food and Drug Administration, 2013). The FDA encourages judicious use of antibiotics, and planned meetings in 2013 for public comment on requiring veterinary oversight for use of certain medically important antibiotics (U. S. Department of Health and Human Services and U.S. Food and Drug Administration, 2013). The issue of antibiotic resistance and livestock's contributions are increasingly global, and resistance to certain classes of antibiotics in livestock can transfer to bacterial infections in humans (Witte, 1998), though evidence of this is limited. Resistant bacteria from livestock can also be directly ingested by humans through contaminated food products (Catry et al., 2003). It has been recommended that antibiotics with the highest efficacy in their class be used in order to slow the development of resistance (Amyes et al., 2007).

Using an antibiotic on all cows at the start of the dry period is useful in mitigating mastitis, but may result in overuse of antibiotics because not all cows may be infected at the time of dry treatment (Berry and Hillerton, 2002). This potential overuse of antibiotics could contribute to the growing problem of resistance, though the benefits of preventing mastitis infections could well outweigh the risk of resistance development. Antibiotic therapy via the intramammary route is not always effective because of several challenges: the antibiotic not reaching the site of infection, antibiotic resistance in the mastitis pathogen, antibiotic interference with phagocytosis of pathogens, and inadequate intramammary antibiotic concentrations to eliminate infection (Sandholm et al., 1990).

Antibiotic resistance in mastitis pathogens has been reported around the world. A

review of penicillin resistance in *Staph. aureus* isolates from 43 different countries found that resistance exists in every country surveyed, and in many cases, prevalence of resistant isolates is increasing (Aarestrup and Jensen, 1998). A survey of *Staph. aureus* resistance to various antibiotics in the United States and other countries reported that a low level of resistance to β -lactams exists (de Oliveira et al., 2000). In Belgium, the percentage of β -lactam-resistant *Staph. aureus* increased from 36% in 1971 to almost 80% in 1974, plateauing for a decade before decreasing to around 50% in the mid-nineties (Devriese et al., 1997). However, two surveys from the United States found that resistance of mastitis pathogens to antibiotic mastitis therapies was static over time (Erskine et al., 2004) or even decreasing for some antibiotics, such as β -lactams (Makovec and Ruegg, 2003). Resistance can be overcome in some cases by using multiple antibiotics at dry off, such as penicillin and novobiocin (de Oliveira et al., 2000).

Studies examining antibiotic resistance in dairy cattle often use certified organic dairy herds as controls, because organic dairy producers in the United States are prohibited from using antibiotics in their cattle (Electronic Code of Federal Regulations, 2013). The risk of antibiotic resistance in zoonotic pathogens such as *E. coli* and *Staph. aureus* is higher in conventional dairies, likely due to exposure to synthetic antibiotics. A survey of *Campylobacter* isolates from cattle feces on organic and conventional farms in Wisconsin, using organic herds that had not used antibiotics for at least 3 years, reported that prevalence of *Campylobacter* and its resistance to ciprofloxacin, gentamicin, erythromycin, and tetracycline was no different between organic and conventional systems (Sato et al., 2004).

However, a survey on the same farms reported that fecal *E. coli* isolates from conventional farms had significantly higher rates of resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline (Sato et al., 2005a). Isolates of *Staph. aureus* from bovine milk in New York and Vermont herds were more likely to be resistant to antibiotics (ampicillin, penicillin, tetracycline) if they came from conventional herds (Tikofsky et al., 2003). The increasing concern over antibiotic usage in livestock has promoted the growth of the organic dairy industry, especially in the United States.

ORGANIC DAIRY PRODUCTION

Organic agriculture is a method of farming that “sustains the health of soils, ecosystems and people” (International Federation of Organic Agriculture Movements, 2012). Englishman Sir Albert Howard pioneered the modern concept of organic agriculture in the early 1900s, focusing on maintaining soil fertility in order to maintain the health of the crops, animals, and people on the farm. Walter Northbourne coined the term “organic”, referring to a system “having a complex but necessary interrelationship of parts, similar to that of living things” (Heckman, 2006). The terms “organic farming” and “sustainable agriculture” are often thought of as synonymous, as both incorporate land stewardship with food production. Indeed, an annotated bibliographic reference from the USDA covers both sustainable agriculture and organic history, as the two are intertwined (Gold and Gates, 2008). Organic agriculture was popularized in the United States by Jerome Rodale, who expanded upon Howard’s ideas but also helped to create the rift between organic and non-organic agriculture

still present today (Heckman, 2006). The rift developed during the “Green Revolution” from the 1940s through the 1970s. The Green Revolution was characterized by the breeding and introduction of high-yielding varieties of staple food crops (including wheat, maize, and rice) that also demanded higher input levels (Evenson and Gollin, 2003). Lauded as a movement to help feed the world, the Green Revolution prompted agricultural producers and researchers to focus their energies on high-input, high-output agriculture. Concerns have been raised about the sustainability of the intensive systems promoted during the Green Revolution (Evenson and Gollin, 2003). For example, repeated cropping of high-yielding varieties on the same land requires more synthetic fertilizer inputs to replace harvested crops. Using synthetic fertilizers rather than animal manures decreases the soil organic matter content faster, which consequently reduces water-holding capacity and increases the risk of topsoil erosion (Reeves, 1997). In addition, insufficient diversity in crop rotations can increase disease and pest susceptibility (Tilman et al., 2002). Intensive monoculture agriculture is contrary to the principles of organic agriculture, and thus proponents of the Green Revolution did not see eye to eye with the early adopters of organic agriculture. Even today, many conventional researchers and producers regard organic production as “farming the way our grandparents did”, a method antiquated and failing to take advantage of the technological advances that have increased productivity greatly since the Green Revolution. Despite criticism, the organic industry in the United States continued to grow during the Green Revolution, and continues to grow up to 20% per year (U. S. Department of Agriculture Economic Research Service, 2013). In 2010, organic food sales reached \$28.6 billion U.S., representing 4.0% of

the total food market. Organic dairy products are the second-largest category of organic foods and sales in 2010 were \$3.9 billion, representing almost 6% of the total dairy products market in the United States (Organic Trade Association, 2011).

Organic livestock farming requires farmers to allow livestock to express more of their natural behaviors, such as grazing, as compared to conventional high-production agriculture. One of the guiding principles of organic farming, in addition to environmental sustainability, is increasing the quality of life of livestock (Lund, 2006). Organic dairies tend to be smaller than conventional dairies and usually produce less milk per cow (Zwald et al., 2004; Sato et al., 2005b; Pol and Ruegg, 2007a). This can be advantageous, as lower-producing cows lose less milk production during mastitis (Hand et al., 2012). Lower production also places less stress on the cow which translates to a lower risk of disease.

An increasing number of consumers are concerned with animal welfare and the environmental impact of conventional animal production methods, fueling the growth of the organic dairy industry (Sundrum, 2001). Many consumers are also willing to pay more for dairy produced without the use of antibiotics (Olynk et al., 2010) and for milk with the USDA organic seal (Dhar and Foltz, 2005; Kiesel and Villas-Boas, 2007) as compared with conventional milk.

United States Organic Regulations

Federal standards for organic production were first created in 1990 with the Federal Organic Foods Production Act; official USDA labeling for organic products did not occur until 2002. Standards for organic farming in the United States are constantly evaluated,

updated, and changed through the National Organic Program's National Organic Standards Board.

In order to transition to organic production, a conventional dairy must manage its land organically for 3 years and its cattle organically for 1 year before organic certification can be obtained. Transition for the cattle can be concurrent with the third year of land transition. Land management, according to the organic standards, must maintain or improve soil health, must utilize biological control methods for pests, weeds, and disease management, and must not use synthetic products or municipal waste. Organic management of dairy cattle includes feeding 100% organic feed, allowing the cows to obtain at least 30% of their dry matter intake from pasture during the grazing period (at least 120 days out of the year), providing access to clean drinking water, shelter, room to exercise, and following a strict healthcare practice: organic animals may not receive antibiotics, hormones, or prophylactic medicines (except vaccinations) unless organic treatments do not work, in which case the farmer must not withhold conventional treatment to preserve the cow's organic status. If a substance that is not allowed in organic production is used, the animal and products from it permanently lose organic classification (Electronic Code of Federal Regulations, 2013).

International Organic Regulations

Organic regulations in different countries are similar in their requirement for animal well-being, including access to feed, water, shelter, and pasture, sufficient space to exercise their natural behaviors, and timely and adequate treatment of disease when prevention strategies fail (IFOAM, 2012). The main difference between organic regulations for dairy

cattle in the United States versus other countries is the ability to use antibiotics. Antibiotics are allowed as a last resort in many other countries: the European Union organic regulations allow the use of antibiotics when biotherapy (use of biological materials as therapeutics) and other treatments are deemed inappropriate (The Council of the European Union, 2007).

Danish organic dairy farmers whose goal was to eliminate antibiotic usage in their herds still wanted to be able to use antibiotics if necessary for animal welfare (Vaarst et al., 2006).

Sweden follows the European Union Organic Regulations, with a mandatory doubled withdrawal period for milk from cows treated with registered pharmaceuticals (KRAV, 2009). Canada also allows the use of antibiotics when biotherapies fail; written direction of the veterinarian is required, and withdrawal time must be twice the label indications or 14 days, whichever is longer. Cattle requiring more than 2 courses of antibiotic treatment must be re-transitioned to organic production over a year (Canadian General Standards Board, 2011). Many comparisons between organic and conventional dairies have been completed outside of the United States, so it is important to understand the difference in antibiotic regulations between countries when looking at milk quality.

Comparisons with Conventional Milk Quality

Studies comparing organic and conventional dairy production in the United States have been conducted in northern and midwestern states, with a focus on elucidating differences in management practices and milk quality between dairy systems. Organic dairies can follow nearly all of the National Mastitis Council recommendations for mastitis control, except for those involving the use of antibiotics for treatment. For example, most of the 30

organic herds surveyed in Wisconsin used postmilking teat dip (Sato et al., 2005b), as well as 90% of 192 organic herds surveyed in New York, Oregon, and Wisconsin (Stiglbauer et al., 2013). Up until 2002, organic farms in the United States could use antibiotics. Even without the National Organic Program standards, only 6.3% of organic herds in Michigan, Minnesota, New York, and Wisconsin used intramammary dry cow therapy in 2000-2001, compared to 98% of conventional herds surveyed (Zwald et al., 2004). In that same study the authors were concerned that the inability of organic dairy farmers to use proven antibiotics could lead to increases in udder health problems and ultimately decreased milk quality (Zwald et al., 2004). The data of Zwald et al. (2004) included documentation that organic dairy farms did have higher SCC than the conventional farms but such observations are not consistent with the current body of literature comparing milk quality on organic and conventional dairies.

Even with differences in regulations, there are no clear patterns of differences in SCC between organic and conventional dairies in the United States or other countries. Most comparisons of organic and conventional dairies in the United States have reported no significant differences in bulk tank SCC (Sato et al., 2005b; Pol and Ruegg, 2007a; Stiglbauer et al., 2013). Some European studies have also reported similar bulk tank SCC between management types, such as in the United Kingdom (Ellis et al., 2007; Haskell et al., 2009), Norway (Valle et al., 2007) and Sweden (Hamilton et al., 2006; Fall et al., 2008). One study of dairies in Michigan, Minnesota, New York, and Wisconsin reported higher SCC in organic dairies compared to conventional dairies (Zwald et al., 2004). Similarly,

higher bulk tank SCC were also seen in organic herds in Norway (Hardeng and Edge, 2001), the United Kingdom (Hovi and Roderick, 2000), and Switzerland (Roesch et al., 2007). There is evidence from Finland that organic milk has significantly higher SCC than conventional milk in the summertime (Luukkonen et al., 2005). In contrast, bulk tank SCC were lower in organic herds (273,000 cells/mL) than in conventional herds (559,300 cells/mL) in spring and summer in New York and Vermont (Tikofsky et al., 2003). In Denmark, herds that had been organic for at least 10 years had significantly lower bulk tank SCC than conventional herds, but newer organic herds had similar SCC to conventional herds in that study (Bennedsgaard et al., 2003). Herds that had been transitioned to organic production for just two years in Denmark had slightly higher SCC than conventional herds (Vaarst et al., 2003). The same trend was observed in a study in the Netherlands, where SCS (a logarithmic transformation of SCC) was higher in first parity Holsteins in organic herds than conventional herds for up to 6 years after transitioning (Nauta et al., 2006). Risk factors for high SCC have been evaluated in United States organic herds in New York, Oregon, and Wisconsin; factors that significantly increased SCC included larger herd size, more years in the dairy industry, presence of *Staph. aureus* in bulk tank milk cultures, not using segregation or a bucket milker for infected or high SCC cows, feeding less grain per day, and not using of anionic salts in transition cow diets (Cicconi-Hogan et al., 2013). There are no consistent indications that organic dairies have significantly higher or lower SCC than conventional dairies.

Though the literature comparing mastitis-related microorganisms in organic and

conventional dairies is sparse, it seems that organic producers face the same challenges as conventional producers. Prevalence of mastitis-causing pathogens was significantly different in Wisconsin organic and conventional herds with bulk tank SCC over 250,000 cells/mL. Conventional farms had more CNS (38% vs. 30%), *Strep. spp.* (18% vs. 15%), and coliforms (6% vs. <1%), whereas organic farms had a higher prevalence of *Strep. agalactiae* (4% vs. 2%) and there was no difference in *Staph. aureus* presence (Pol and Ruegg, 2007b). In a survey of California mastitis test-positive (score 2+) quarters from Switzerland, only the prevalence of *Streptococcus spp.* was significantly different between production types (70% in organic, 48.3% in conventional). Prevalence of *Staph. aureus*, *C. bovis*, CNS, and *E. coli* was no different between organic and conventional herds (Roesch et al., 2007). More primary research is needed to examine mastitis sources and ramifications of mastitis on organic farms (Wilhelm et al., 2009). Although the same mastitis-causing bacteria are present on organic farms and conventional farms, because of the inability of organic producers to use antibiotics, alternatives to antibiotics are needed for mastitis control.

ALTERNATIVE MASTITIS THERAPIES

Alternative treatments for mastitis and other ailments of cattle have been used for millennia before the development of modern, “Western” medicine. These alternative treatments come from cultural or ethnic traditions and have been labeled as “complementary and alternative medicine” (CAM) today. Treatments associated with CAM are not necessarily focused on relieving a specific symptom; instead, they consider the well-being of

the whole animal. Some modalities of CAM include acupuncture, botanical medicine, chiropractic and physical therapy, homeopathy, massage, and nutraceutical medicine. The complexity of some CAM treatments makes them difficult for Western-trained veterinarians to learn in addition to the rigorous training required in their current curricula. For example, traditional Chinese medicine includes physical therapy (acupuncture primarily) and herbal medicine. Chinese herbs have different energy levels (hot through cold) and different tastes; both of these attributes must be considered in relation to the symptoms of the patient (Xie, 2011). The intense level of personalization of CAM, such as Chinese herbal medicine, makes it both difficult to learn and difficult to evaluate scientifically. However, thousands of years of anecdotal evidence of efficacy may warrant investigation of CAM by veterinarians. Lin et al. (2003) proposed a sustainable veterinary medicine, in which CAM is used for disease prevention and modern medicine is reserved for clinical illness. This approach to veterinary medicine is based on the theory of maintaining treatment availability by preserving the functional integrity of treatments, using antibiotics only when necessary (Lin et al., 2003). In the same focus as sustainable veterinary medicine, alternatives to antibiotics have potential value for augmenting the toolbox of dairy veterinarians and producers.

Organic Dairy Production and the Need for Alternatives to Antibiotics

Mastitis management is potentially more difficult on organic farms in the United States, because certified organic dairy farms in the United States are prohibited from using antibiotics in their cattle (Electronic Code of Federal Regulations, 2013). Currently, there are no approved intramammary mastitis treatments that do not contain antibiotics (U. S. Food

and Drug Administration, 2013). Another challenge for organic producers is their relationship to farm advisors, such as veterinarians or nutritionists. Many veterinarians are skeptical of organic livestock husbandry and working with organic regulations, including some in Denmark (Vaarst et al., 2003). Organic farmers in the United States are less likely than conventional farmers to have veterinarians regularly visit the farm (Stiglbauer et al., 2013), either because of having a healthier herd (Lund, 2006) or because the farmers cannot use many of the treatment suggestions from veterinarians due to differences in underlying values (Lund, 2006). Indeed, a majority of veterinarians in the United States are highly skeptical of treatments with several years to thousands of years of anecdotal efficacy but relatively few double-blind clinical studies (Wynn and Wolpe, 2005). Regardless, mastitis remains an issue in both organic and conventional dairy herds. Because organic dairy farmers around the world must abide by either restrictions on or prevention of antibiotic usage, they use a variety of alternatives to antibiotics for treating mastitis.

Alternative Mastitis Therapies Currently in Use

Organic dairies in the United States use alternatives to antibiotics for treating and preventing mastitis. For example, 20 organic dairy farmers in Wisconsin have been reported to use whey-based products (9 farms; oral, intravenous, intramuscular, or subcutaneous), garlic tinctures (7 farms; oral or in vulva), *Aloe vera* (6 farms; oral, intramuscular, intramammary, or in vulva), vitamin C (5 farms; intramuscular, intramammary, or intravenous), aspirin (4 farms; oral), homeopathy (4 farms; oral or in vulva), a multivitamin supplement (4 farms; oral), vegetable oils (4 farms; topical), corticosteroids (2 farms;

intramuscular or intramammary), electrolytes (1 farm; oral), probiotics (1 farm; intramammary), or vitamin B (1 farm; intramuscular) for clinical mastitis treatment (Pol and Ruegg, 2007a). Dry cow treatments on those Wisconsin farms included ultra-filtered bovine whey products (5 farms; oral, intramuscular, or subcutaneous), vitamin supplements (3 farms; oral or intramuscular), microbial supplements (2 farms; intramammary), vitamin C (2 farms; intramammary), *Aloe vera* (2 farms; oral), homeopathy (1 farm), and olive oil (1 farm; intramammary) (Pol and Ruegg, 2007a). Prophylactic treatment of disease is not permitted by the USDA organic standards, so if certified organic farmers were to use any treatment, they would first have to verify presence of an infection at dry off. A significant number of mastitis cases occur during the dry period in organic cows, as seen in a United Kingdom study where 20% of all clinical mastitis cases were during the dry period (Weller and Bowling, 2000). Organic farmers in a Wisconsin survey used anti-inflammatory drugs and frequent stripping out of quarters to manage clinical mastitis, as well as whey products, herbs, mineral oil, vinegar, vitamin C, and selenium for general mastitis treatments (Sato et al., 2005b). Danish organic farmers used peppermint ointment on the udder for mild mastitis, and had experimented with homeopathy. Cows with high SCC or clinical mastitis on those farms were often used for suckling calves, and chronic mastitis cases had mastitic quarters dried off (Vaarst et al., 2006). German organic farmers treated disease using conventional medicine (53%), homeopathy (27%), or herbal medicine and home remedies (20%) (Krutzinna et al., 1996).

The steady growth of the organic dairy industry has also increased the amount of

resources available to organic farmers. These resources range from extension programs to books and many have mastitis treatment recommendations for farmers. Books are available on organic dairy farm management (Padgham, 2006), veterinary care of organic dairy cattle (Coleby, 2001; Karreman, 2007), and even as specific a topic as using homeopathy in cattle (Sheaffer, 2003; Dupree, 2010). Regarding veterinary treatments for organic dairy cattle, many recommendations have been made. For example, Dr. Paul Dettloff lists the nine veterinary tools used in organic herds: tinctures, homeopathy, essential oils, *Aloe* products, whey products, botanicals, vitamins, trace and macro elements, and probiotics in the book *Organic Dairy Farming: A Resource for Farmers* (Padgham, 2006). Dr. Dettloff goes on to define each of the tools: tinctures are extracts of plants or minerals that are alcohol or glycerin-based. These are administered orally or in the vulva. The basic principle of homeopathy is that of “likes treating likes”; for example, in order to treat inflammation, a homeopathic remedy based on an inflammatory substance would be used. Essential oils are highly concentrated and thus should only be used topically. *Aloe* products are thought to be immunostimulatory. They are often used orally or topically. Whey products “contain antigens and stimulate the immune system to produce antibodies” (Padgham, 2006). Botanical treatments are plant products used whole either for oral or topical use. Trace and macro elements are vital for reproductive function and should be balanced in the diet. Probiotics are another potential tool, but are thought to be less important as the whole farm becomes healthier (soils, forages, animals) (Padgham, 2006). Another veterinarian with knowledge of alternative practices who has written books on veterinary management of dairy

cattle is Dr. Hubert Karreman. Dr. Karreman recommends a two-pronged approach in mastitis therapy for farmers not using antibiotics: the immune system must be stimulated and the infected quarters should be infused with an effective herbal product. He recommends that, if intramammary plant-based treatments are used, milk should not be sold from treated cows and that antibiotic residue tests should be used to ensure the quality of the milk after treatment (Wynn and Fougère, 2007). Though these treatments are recommended by knowledgeable veterinarians, peer-reviewed studies examining the efficacy of alternative treatments are limited or lacking (Ruegg, 2009).

Efficacy of Alternative Mastitis Therapies

The scientific literature is lacking in evaluations of some of the alternative treatments mentioned for mastitis. Inferences can be made from literature examining the activity of mastitis treatments in vitro, in other species, or from the limited clinical trials available. There is a large body of literature on ethnobotany, traditional Chinese medicine, and other uses of alternative medicines, the scope of which is beyond this review. Here the focus will strictly be on peer-reviewed scientific studies.

Whey-based Products. Whey-based products have some evidence of stimulating the immune system of cattle. Whey-based products are composed of whey isolated from cows either naturally resistant to mastitis or hyperimmunized and challenged with killed mastitis-causing bacteria intramammarily. Extensive studies have demonstrated the activity of whey-based products for human consumption (Madureira et al., 2007); some of these functions may also occur during administration to cattle, including the antibacterial properties (mainly

through the actions of lactoferrin) and immunostimulatory properties. For example, subcutaneous administration of an ultrafiltered whey product to post-parturient cows resulted in increased bactericidal capacity of immune cells (Roth et al., 2001). Neutrophil function was increased in mid-lactation cows injected subcutaneously with a whey product (Kehrli et al., 1989).

Homeopathy. Homeopathy has some efficacy for treatment of mild to moderate clinical mastitis caused by environmental organisms, as seen in a randomized clinical trial (Werner et al., 2010). Another randomized clinical trial compared homeopathy, antibiotic treatment, and a placebo for treatment of clinical mastitis. The antibiotic treatment reduced acute signs (body temperature, acute inflammation, California mastitis test results, and bacteriology) the most in the first 7 days following onset and was significantly better for cows than the placebo, but it was not significantly more effective than homeopathy (Hektoen et al., 2004).

Vitamin Supplements. In addition to providing dietary requirements, vitamins can be used as supportive therapy during an episode of mastitis. Vitamin research relating to bovine mastitis has mainly focused on vitamins E, A, and β -carotene. A meta-analysis revealed that vitamin E supplementation, on average, reduced the risk of intramammary infection 14% and reduced SCC by 70% (Moyo et al., 2005). Vitamin A, as well as its precursor β -carotene, is essential for epithelial cell integrity and sustaining the udder's natural defense system against mastitis. The relation of supplementation of vitamin A and β -carotene to mastitis severity and incidence is unclear according to the literature (Heinrichs et al., 2009).

Supplementation with other vitamins has been linked to changes in milk production and potentially has effects on mastitis prevention or recovery. Vitamin B₁₂ and folic acid have been used to increase milk production and metabolic efficiency in periparturient cattle (Graulet et al., 2007). Vitamin C concentration in plasma is decreased during mastitis (Matsui, 2012), so supplemental vitamin C during a case of mastitis would help return the cow to homeostasis. Infusion of pro-vitamin D [25-hydroxyvitamin D(3)] directly into an udder infected with *Streptococcus uberis* reduced the bacterial counts and decreased the amount of clinical signs of mastitis (USDA-ARS, 2012). Vitamin supplementation can help cows overcome mastitis challenges, but mainly just by helping the cow meet her daily requirements.

Herbal Treatments. No studies currently available validate the use of garlic or *Aloe vera* in vivo as therapy for bovine mastitis. Antibacterial effects of garlic are well-reported (Cowan, 1999; Wilson and Demmig-Adams, 2007). Garlic also has anti-inflammatory effects, antioxidant effects, and the ability to cause contact dermatitis if applied in a high concentration (Wilson and Demmig-Adams, 2007). The use of undiluted garlic in cattle could have negative implications for animal well-being. In addition, from personal experience, oral administration of raw garlic to humans causes severe throat irritation that persists for an hour or more. *Aloe vera* has been used as an immune stimulant in both humans and animals with no adverse reactions (Zago, 2009; Padgham, 2006). A review of controlled human clinical trials through 1999 reported that *Aloe vera* gel applied topically to a wound site speeds the healing process and when taken orally, can lower blood glucose in diabetic

people (Vogler and Ernst, 1999). Garlic and *Aloe vera* are frequently used by organic farmers for treatment of cows with mastitis, despite the lack of scientific literature examining these treatments in cattle.

Many herbal essential oils have a long history of use for treatment of bacterial infections and inflammation and represent possible alternatives to synthetic antibiotics. Herbal extracts also possess multiple different antibacterial molecules (Cowan, 1999; Mahady, 2005), theoretically making it more difficult for bacteria to develop resistance. Essential oils are obtained from plants by distillation through boiling water or steam (Kalemba and Kunicka, 2003), and can also be extracted using another oil (olive oil, canola, etc.) (Padgham, 2006). Yield of essential oil using distillation is relatively low. For example, *T. vulgaris* yielded only 2.83 g of essential oil per kg of dried herb (Tsai et al., 2011). Many studies of essential oils examine single molecules present in the oils in an attempt to isolate the activity of the “active ingredient” molecule(s). However, it is important to not only examine the singular components of essential oils, but also to study the essential oils as a whole because of the amount of component molecules and their potential synergistic activity (Raffa and Pergolizzi, 2011). It likely will also be easier for dairy farmers to access whole plant essential oils rather than their derivatives for treatment of mastitis.

Investigating the antibacterial properties of essential oils requires the use of specific testing techniques. Conventional antibiotic resistance testing uses antibiotic-impregnated disks placed on an agar plate inoculated with the bacteria of interest. After incubation, the zone of inhibition around the disks is measured and compared to the reference of the Clinical

and Laboratory Standards Institute. This method is an initial screening for antibiotic sensitivity, useful for evaluating the sensitivity of a pathogen to antibiotics for therapeutic treatment. This evaluation method does not work well for essential oils because it is difficult to determine the amount of essential oil in the disk, the active components of the essential oil may volatilize during incubation, and the oil may spread across the surface of the agar plate, creating an anaerobic environment and thus artificially increasing its apparent antibacterial activity against aerobic pathogens. A test commonly used to evaluate antibacterial activity of antibiotics is the Minimum Inhibitory Concentration (**MIC**) method. The MIC method involves adding a standardized concentration of bacteria to a series of test tubes containing growth media (broth), and adding the antibacterial agent in dilutions to the series of tubes. Tubes are incubated for a pre-set period of time, and then the concentration of bacteria remaining is assessed. The tube in the series at the lowest concentration of antibacterial agent tested with no bacterial growth contains the MIC of the antibacterial agent. The MIC method is more adequate for essential oil testing. An emulsification agent, such as Tween 80, may be added to get the essential oil into solution (Hood et al., 2003). The results of the essential oil treatments are presented as percentage of bacterial growth relative to the control bacterial culture without added essential oil, or the minimal concentration that inhibits bacterial growth (Kalemba and Kunicka, 2003). The broth dilution method is also the most consistent across replications for multiple different essential oils (Hood et al., 2003). Only studies using the broth dilution method for examining the antibacterial efficacy of essential oils are presented here.

Evaluation of individual molecules from essential oils can be easier than investigating the whole oil, as some molecules are water-soluble like many synthetic antibiotics and therefore can be tested using other methods than the broth dilution method. Components of essential oils rank as follows for antibacterial efficacy: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons (Kalemba and Kunicka, 2003). Many components are also chiral compounds with enantiomers of possibly differing biological activity (Kalemba and Kunicka, 2003). Some common classes and mechanisms of action of several essential oil compounds are presented in Table 1.1.

Two herbal preparations currently available in the United States are promising possible mastitis treatments. One product, Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), is composed of canola oil extractions of four different herbs: *Angelica dahuricae* (Bai zhi), *Angelica sinensis* (Dong quai), *Gaultheria procumbens* (wintergreen), *Glycyrrhiza uralensis* (Chinese licorice), and also contains essential oil of *Thymus vulgaris* (garden thyme). These herbal oils are combined with other ingredients into a 12 mL tube for intramammary administration. The other product, Cinnatube (New AgriTech Enterprises, Locke, NY), contains essential oils of *Calendula officinalis* (marigold), *Cinnamomum spp.* (cinnamon), *Eucalyptus globulus* (eucalyptus), and *Melaleuca alterniflora* (tea tree) as well as beeswax in an oil base for intramammary administration. Few studies exist that have examined the effects of those essential oils on mastitis in cows; most studies have examined their effects in other species and in vitro. The ingredients of Phyto-Mast have anti-inflammatory, analgesic, or antibacterial properties (McPhee et al., 2011). Many of the ingredients of Phyto-Mast have

anti-inflammatory activity. For example, *A. dahuricae* reduced the inflammatory response of LPS-treated murine macrophages (Kang et al., 2008). Polysaccharides from *A. sinensis* decreased TNF α and increased IL-10 production in rats with experimentally induced colitis, effectively lowering the inflammatory response (Liu et al., 2003). Glycyrrhizin, a component of *G. uralensis*, promoted IL-10 production in mouse liver dendritic cells with hepatitis, and had a strong anti-inflammatory effect when administered prior to induction of experimental hepatitis (Abe et al., 2003). When administered 30 minutes before and 1 and 6 hours after spinal cord injury in mice, glycyrrhizin decreased inflammation, tissue injury, and reactive oxygen species production (Genovese et al., 2009). Glycyrrhizin also had anti-inflammatory activity without adverse reactions when infused (400 mg in a 20mg/mL formula) into the mammary glands of lactating cattle with CNS infections (Kai et al., 2003). *T. vulgaris* has anti-inflammatory activity similar to dexamethasone on murine macrophages stimulated by LPS (Vigo et al., 2003) and had anti-inflammatory activity on human acute monocytic leukemia cells treated with LPS (Tsai et al., 2011). *G. procumbens* essential oil contains a molecule that hydrolyzes to methyl salicylate (Poppenga, 2002) and thus has an analgesic effect. *T. vulgaris* is also a strong antibacterial agent. Thymol, a component of *T. vulgaris* essential oil, can degrade the LPS of gram-negative bacteria at a 1% concentration (Helander et al., 1998). Thymol also had bactericidal activity in milk against the mastitis-causing pathogens *Staph. aureus*, *Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis*, and *E. coli* at 1.2%, 0.9%, 0.9%, 0.9%, 1.4%, and 1.5%, respectively (Baskaran et al., 2009). That study also examined eugenol, carvacrol, and *trans*-cinnamaldehyde; results are presented in Table

1.2.

The ingredients of Cinnatube have anti-inflammatory and antibacterial properties. *Calendula officinalis* has historically been used to treat mastitis in dairy cows (Jost, 1984). Dairy farmers in British Columbia used *C. officinalis* to treat wounds and diarrhea (Lans et al., 2007). Essential oil extracts from the flowers of *C. officinalis* have anti-inflammatory activity in mice and expedite wound healing on thermal burns in rats (Muley et al., 2009). Essential oils of *Cinnamomum* spp. and *Eucalyptus globulus* also have antibacterial activity in vitro (Cowan, 1999). One component of *Cinnamomum* spp., *trans*-cinnamaldehyde, has strong antibacterial activity versus several common mastitis pathogens at less than 0.5% concentration (Baskaran et al., 2009; Table 1.2). Oil of *M. alterniflora* (tea tree oil) has antibacterial activity in vitro, and is caustic when applied topically undiluted (Rotblatt and Ziment, 2002). However, at a 10% concentration, the oil effectively reduced clinical signs of chronic dermatitis in dogs when applied topically twice daily for 4 weeks (Fitzi et al., 2002). *M. alterniflora* is one of the more antibacterial essential oils, likely due to its high phenol content. It effectively kills *E. coli* and *Staph. aureus* in vitro at concentrations of 0.25% and 0.50% vol/vol, respectively (Carson et al., 1995).

Though these studies examined each essential oil or oil component alone, essential oils can also have synergistic effects. For example, the antibacterial activity of *T. vulgaris* essential oil was greater than the sum of its individual compounds, carvacrol and thymol, against *Staph. epidermidis*, *Staph. aureus*, and *E. coli* in vitro (Iten et al., 2009). This has important implications for the adoption of essential oils or their components as antibacterial

treatments. A blessing and curse of essential oils is that the concentration of bioactive compounds in each plant will differ depending upon the nutrition status or disease state of the plant, the growing conditions, and other highly variable environmental factors. This reality is a blessing by altering the concentrations of antibacterial molecules, theoretically making it more difficult for bacteria to develop resistance because the quantity and type of antibacterial compounds present will change. The curse is that developing an essential oil into a consistent, marketable product is difficult depending upon which components are used to standardize the formula.

Research interest in using essential oils in dairy animals is increasing. Prior to 2005, a search for “essential oils” in the *Journal of Dairy Science* yielded only 20 manuscripts. From 2005 until October 2013, that same search yielded 45 manuscripts, many of which described feeding essential oils to dairy cattle. While in the rumen, essential oils can improve the fermentation profile through decreased methanogenesis and inhibition of deamination (Calsamiglia et al., 2007). Feeding essential oils raises the concern that residues of the oils could enter the milk, causing off-flavors or possibly triggering antibiotic detection tests. A study from 2013 did not detect any residues in milk from feeding double the recommended dose of thymol, carvacrol, cinnamaldehyde, or diallyl disulfide (Hallier et al., 2013). Use of essential oils directly in the mammary gland has a greater potential for milk residues. A 2011 study reports on Phyto-Mast given to goats intramammarily and examined the fate of thymol in milk and blood plasma up to 10 days following treatment; thymol residues were detectable in plasma only 15 minutes to 4 hours post-treatment. Residues in milk were detectable until

24 hours post-treatment (McPhee et al., 2011). These residues times are generally shorter than those of synthetic antibiotics, making them even more appealing for replacing antibiotics. Preliminary work has been completed to examine the use of essential oils for treatment of mastitis in dairy cattle, but more must be accomplished to understand the possible value of these natural treatments.

Herbal essential oils are promising alternatives to antibiotics because of their availability, biodegradability, and lower risk of side effects as compared with traditional medicine (Kalemba and Kunicka, 2003). The effects of herbal essential oils on bovine mammary tissue, however, must be assessed to ensure the well-being and productive ability of the cow being treated, especially because of documented cytotoxic effects of several essential oils (Bakkali et al., 2008). Nevertheless, more scientific studies are needed to examine the possible role of essential oils in treating mastitis in dairy cattle. This research has direct implications for both the organic and conventional dairy sectors and could provide a more antibiotic-resistance-resistant alternative for mastitis treatment.

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Table 1.1: Common classes and mechanisms of action of essential oil compounds¹

	Class of component; antibacterial mechanism of action	Component name	Essential oils
↑ Increasing antibacterial activity	Phenol; disturbance of cytoplasmic membrane, enzymatic inhibition	Thymol, carvacrol, eugenol, catechol, pyrogallol, cinnamaldehyde	Thyme, savory, oregano, clove, cinnamon
	Ketones; complex with cell wall	Thujone, camphor, menthone, carvone, flavones, flavonoids	Sage, peppermint, licorice
	Alcohols; compromise cytoplasmic membrane	Terpinin-4-ol, α -terpineol, geraniol, citronellol, menthol, linalool	Tea tree, geranium, peppermint, lavender
	Ethers; respiratory inhibition	Anethole, 1,8-cineole	Fennel, eucalyptus, rosemary

¹Table adapted from data from Cowan, 1999, Carson et al., 2002, Kalembe and Kunicka, 2003, Kubo et al., 2008, and Storia et al., 2011.

Table 1.2: Minimum bactericidal concentration of several essential oil components on mastitis pathogens in milk (expressed as percentage vol/vol) adapted from Baskaran et al., 2009

Pathogen	<i>trans</i> - cinnamaldehyde	eugenol	carvacrol	thymol
<i>Staph. aureus</i>	0.45	1.4	1.2	1.2
<i>Strep. agalactiae</i>	0.4	0.8	0.8	0.9
<i>Strep. dysgalactiae</i>	0.4	0.4	0.8	0.9
<i>Strep. uberis</i>	0.45	0.4	1.2	1.4
<i>E. coli</i>	0.45	1.4	1.2	1.5

**CHAPTER 2: COMPARISONS OF MILK QUALITY ON NORTH CAROLINA
ORGANIC AND CONVENTIONAL DAIRIES**

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ABSTRACT

The organic dairy industry is growing rapidly across the United States and has recently expanded into the southeastern states. To date, no published comparisons of milk quality exist between organic and conventional dairies in the southeastern United States. Maintaining high milk quality is challenging in this region due to the longer periods of high heat and humidity. The objective of this observational study was to compare milk quality on organic and conventional dairies in North Carolina during the warm summer months of the year. Data were compared from 7 organically and 7 conventionally managed herds in North Carolina. To assess milk quality, milk samples were aseptically collected from each functional quarter of each cow in the milking herds at the time of sampling and linear somatic cell scores (SCS) were obtained for individual cows. A total of 4,793 quarter milk samples (2,526 conventional, 2,267 organic) were collected from 1,247 cows (652 conventional, 595 organic). Milk samples were cultured and bacterial growth was identified using protocols consistent with those of the National Mastitis Council (Verona, WI). Subclinical mastitis was defined as the presence of $SCS \geq 4$ and also a microbiological infection in at least 1 quarter. The proportion of cows with subclinical mastitis did not differ between conventional (20.8%) and organic (23.3%) herds. No significant difference was observed between herd management types in the proportion of cows without microbiological growth in milk samples. Also, no significant differences were observed between organic and conventional herds for cow-level prevalence of *Staphylococcus aureus*, coagulase-negative *Staphylococcus* spp., *Streptococcus* spp., or *Corynebacterium* spp. Two of the organic herds

had a notably higher prevalence of *Corynebacterium* spp. and higher SCS. Coliforms were found in 5 of 7 conventional herds and in only 1 of 7 organic herds. Mean SCS did not differ between conventional (3.3 ± 0.2) and organic (3.5 ± 0.2) herds. Despite differences in herd management, milk quality was remarkably similar between the organic and conventional dairies compared for this study.

INTRODUCTION

The organic industry of the United States is growing steadily due to increasing consumer demand. In 2010, organic food sales reached \$28.6 billion, representing 4.0% of the total United States food market. Organic dairy products are the second-largest category of organic foods and sales in 2010 were \$3.9 billion, representing almost 6% of all marketed dairy products in the United States (Organic Trade Association, 2011). The growth of the United States organic dairy industry is fueled by the willingness of some consumers in the United States to pay more for dairy products produced without the use of antibiotics and with documented pasture access for dairy cattle (Olynk et al., 2010).

Mastitis, or mammary inflammation, represents a significant economic challenge to the dairy industry. Mastitis management is potentially more difficult on organic farms in the United States because certified organic dairy farms in the United States are prohibited from using antibiotics in their cattle. However, treatment cannot be withheld from a sick animal, and if an animal is treated with antibiotics, it cannot return to the organic herd and products from it cannot be sold as organic (USDA National Organic Program, 2013). Organic

regulations in the United States emphasize prevention rather than treatment of disease. Organic cattle must also have year-round access to the outdoors, at least a 120-d grazing season on organic pastures, and obtain at least 30% of their DMI from those pastures during the grazing season (USDA National Organic Program, 2013).

Few studies have compared milk quality in organic and conventional production systems in the United States. Most of those studies have been performed in Wisconsin (Sato et al., 2005; Pol and Ruegg, 2007b) or other northern states (Zwald et al., 2004; Stiglbauer et al., 2013). No studies comparing organic and conventional dairy production in the southeastern states have been published. The heat and humidity of the southeast provide favorable conditions for the growth of environmental bacteria and increase the risk of mastitis. Heat stress compromises the udder's defense mechanisms (Giesecke, 1985) and increases SCS in Holstein and Jersey cattle (Smith et al., 2013). The southeast United States also faces significant challenges with regards to meeting lower SCC limits (Norman et al., 2000).

Mastitis is a challenge for many southeast dairy producers, particularly in summer, and the limited availability of effective treatments for mastitis in organic dairy cattle raises questions about the ability to maintain high quality milk in organic herds in the region. Currently, 6 certified organic dairy herds produce milk in North Carolina. One pasture-based research herd manages half of its cattle using organic health care standards, whereas the other half of the herd is managed conventionally. The objective of the current study was to compare milk quality in organic versus conventional dairies in North Carolina during the

warm months.

MATERIALS AND METHODS

Regulatory Compliance

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

Farm Selection and Surveys

In 2010, owners of all certified organic dairy farms in North Carolina ($n = 6$) were contacted and all agreed to participate in the study. Six conventionally managed dairy farms of similar size and geographical locations were recruited by personal contact of the authors. Also, a pasture-based research farm was included in this study. That research herd in North Carolina has managed half of its herd using organic health standards since 2009 and those organic cattle graze in transitioned pastures that can be certified organic. The other half of the herd is managed with conventional disease treatments, including antibiotic therapy at dry off and antibiotic treatment of mastitis. All cows in the research herd meet the 30% of DMI organic pasture requirement but they did receive supplemental concentrates that were not organically produced. Both management groups are milked in the same parlor, but otherwise do not have contact with each other. The research herd was considered as 2 separate herds in the analysis.

A written survey was developed by one of the authors (L. G. Sparks) to evaluate herd

health management practices related to milk quality. Questions included basic farm demographics, milking protocols, fly control, and detection and treatments of subclinical and clinical mastitis. Farm demographics included breed(s) of cattle, number of lactating cattle, number of people milking cows, number of years certified organic, and the most recent bulk tank SCC. Surveys were given to farmers to fill out during farm visits while samples were being collected. Farmers were not compensated for participating in the study, but they were provided all milk culture results from cows in their respective herds.

Sample Collection

Milk samples were aseptically collected once from each functional quarter of all lactating cows in each herd between May and October of 2010. The average temperature during those months was 23°C (74°F) with an average of 70% relative humidity, with temperatures ranging from 17.9 to 27.4°C (64.3 to 81.3°F) and relative humidity ranging from 40% to 101% (State Climate Office of North Carolina, 2013). Only those cows that contributed milk to the bulk tank were sampled as cows with clinical mastitis were not part of the protocol for the current study. Briefly, teats were pre-dipped with the teat germicide used on the farm and wiped dry using the normal milking preparation procedure of the farm being sampled; 2 to 4 streams of foremilk were expressed and then teat ends were cleaned using cotton balls soaked in 70% isopropanol. Samples were collected into 12-mL vials and cooled, then frozen overnight prior to milk culture. Linear SCS were obtained for individual cows within the sampled herds from the most recent monthly DHIA test if they participated in monthly testing. In those herds not on a monthly testing schedule with DHIA (n = 4), milk

samples were collected from each cow, taking an equal amount of milk from each functional teat into a tube containing a bronopol tablet preservative. The samples were shipped to the United DHIA Laboratory (Blacksburg, VA) for SCC analysis. Monthly test data were given as SCS and thus results here are presented as SCS. The SCS was calculated using the formula

$$\log_2 \left(\frac{SCC}{100,000} \right) + 3$$
 to obtain the base 2 logarithmic transformation as recommended by

Shook (1982).

Milk Culture

Microbiological analysis was performed in the Milk Quality and Mastitis Laboratory at the College of Veterinary Medicine at North Carolina State University. Microbiological identification was performed using methods consistent with those of the National Mastitis Council (NMC, 1999). Briefly, 0.01mL of milk from each sample was plated on Trypticase soy agar with 5% sheep blood (BD, Sparks, MD), incubated at 37°C, and examined for growth after 24 h and again after 48 h. Samples were considered contaminated if 3 or more dissimilar colony types were present. Otherwise, all colonies were identified using standard microbiological procedures (NMC, 1999). Streptococci were distinguished from enterococci using the Christie, Atkins, and Munch-Petersen (CAMP) test, esculin hydrolysis, and growth on bile esculin agar with azide (Hardy Diagnostics, Santa Maria, CA). If required, species were determined using the API20 Strep identification system (bioMérieux Inc., Durham, NC). 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), oxidase testing, and the API 20E identification system (bioMérieux Inc.), if required.

Culture results were recorded and are reported on a per-quarter basis. However, quarter milk culture data were pooled within cow to facilitate comparison with SCS, which was available on a cow basis. Cows with only a single bacterial species in 1 or more quarters were classified as being infected with that organism on a cow basis; these species included *Staph. aureus*, CNS, *Streptococcus* spp. other than *Streptococcus agalactiae*, and *Corynebacterium* spp. Cows with more than 1 bacterial species were classified as having mixed infections. To facilitate statistical analysis, only cows with infections attributed to one of the organisms listed above were considered. Cows with either *Corynebacterium* spp. or CNS or both were classified as cows with minor species infections. Subclinical mastitis was defined as the presence of a SCS ≥ 4 and presence of any microbiological infection in 1 or more quarters.

Statistical Analyses

The MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) was used to analyze SCS data, with a general linear mixed model incorporating management type as a fixed effect and farm within management type as a random effect. Management type was included in the model to allow for comparisons between organic and conventional dairies. Farm within management type was also included in the model to account for variation in farm management and farm size within the organic and conventional groupings. Farms were not grouped by size due to the small sample size and consequent low statistical power to

detect differences between size groups. The model for this analysis was $Y = X\beta + Z\gamma + \varepsilon$, where Y is the continuous variable SCS and the expectation of Y , $E(Y) = X'\beta$, $X\beta$ represents the effect of management type, $Z\gamma$ represents the effect of farm within type, and ε is the error term. Results are given as least squares means.

Logistic regression was used to model the cow-level prevalence of different bacterial infections. This was performed using the GLIMMIX procedure of SAS (version 9.2, SAS Institute Inc.). The model incorporated management type as a fixed-effect factor, SCS as a quantitative fixed-effect explanatory variable, and farm within management type as a random-effect factor. Results were recorded as presence or absence of infection of each pathogen in each cow. The result of interest, $\pi = P(\text{infection} = 1)$; that is, π is the probability

of an infection being present, was modeled using the logit function $Y = \text{logit}\left(\frac{\pi}{1-\pi}\right)$. The

logistic regression model was $Y = X\beta + Z\gamma + \varepsilon$, where β represents a vector of fixed effects including management type and SCS, X is the incidence matrix of fixed effects, $Z\gamma$ accounts for the random effect of farm within management type, and ε is the random effect of the residuals due to modeling with R side effects. Least squares means were transformed

using the equation $\hat{\pi} = \frac{1}{1 + \exp(X\hat{\beta})}$ to express least squares means on a probability scale.

Results are reported as least squares means except in Table 2.3, where raw data are presented. Differences were considered significant at $P < 0.05$.

RESULTS

Farm Survey

Farm Demographics. Dairy herds surveyed contained Holstein, Jersey, Guernsey, Brown Swiss, and crossbred cattle and ranged in size from 18 to 157 lactating cattle, as shown in Table 2.1. Only 5 herds had a single breed of cattle, in all cases Holsteins (Table 2.1). The 6 certified organic herds had transitioned to organic production in 2007 or 2008 and had been organic for at least 2 yr at the time of this survey. The research herd had been managing half of its cattle following US Department of Agriculture (**USDA**) organic health care practices since 2009. Annual milk production ranged from 5,942 to 11,612 kg in conventional herds, with 3.5 to 4.7% fat and 2.9 to 3.1% protein. Organic herds produced between 4,536 and 7,031 kg milk annually, with 3.3 to 4.6% fat and 2.9 to 3.7% protein. The most recent bulk tank SCC reported ranged from 180,000 to 390,000 cells/mL in conventional herds and from 83,000 to 500,000 cells/mL in organic herds (Table 2.1). All herds except 1 organic herd used fly control. Conventional farms used topical control (5 farms), bait (4 farms), ear tags (2 farms), traps (1 farm), or a cow vacuum (1 farm) for fly control. Organic farms used topical control (3 farms), traps (3 farms), biological controls (4 farms), repellents (3 farms), or a cow vacuum (1 farm) for fly control.

Milking Procedures. Eight of the 14 herds had 2 people in the milking parlor during milking time, as shown in Table 2.1. Nine of the 14 farms (5 conventional and 4 organic) reportedly checked foremilk by hand-milking before attaching the milking unit (i.e. pre-strip; Table 2.1). At the time of the survey, all herds used premilking teat dip. Nine farms used an

iodine-based premilking teat dip, 4 used hydrogen peroxide-based teat dips, and 1 used a dilute bleach solution (Table 2.1). All herds used an iodine-based postmilking teat dip.

Mastitis Detection, Treatment, and Prevention. All herds used either the California mastitis test, SCC records, or both, to detect subclinical mastitis (data not shown). All herds except 1 conventional herd treated subclinical mastitis; the organic herds used a range of treatments from essential oils to topical garlic application at the tailhead (Table 2.2). Clinical mastitis was most often detected by checking foremilk (9 farms) or by visual assessment of behavior and the mammary gland (7), as shown in Table 2.2. All conventional herds used an intramammary antibiotic to treat clinical mastitis (Table 2.2). Treatments for clinical mastitis in organic dairy cattle mirrored organic treatments used for subclinical mastitis (Table 2.2).

All conventional herds used an antibiotic as part of the dry off protocol and 4 herds also used an internal bismuth subnitrate teat sealant (Orbeseal, Zoetis Inc., Florham Park, NJ). Only 1 organic herd used an intramammary treatment (Phyto-Mast; Penn Dutch Cow Care, Narvon, PA) at dry off. The rest of the organic herds did not use any treatments at dry off. Three organic herds gradually reduced milking frequency at the end of lactation just before dry off (Table 2.2).

Milk Quality

A total of 4,793 quarter milk samples (2,526 conventional, 2,267 organic) were collected from 1,247 cows (652 conventional, 595 organic). Eighty-three quarter samples (3.3%) of 2,526 collected from conventional cows and 50 quarter samples (2.2%) of 2,267 collected from organic cows were unusable due to contamination and are not included in the

microbiological analyses.

Somatic cell score did not differ significantly between conventional (3.3 ± 0.2) and organic (3.5 ± 0.2) herds ($P = 0.75$), although great variation existed among herds within both management types (Figure 2.1). The proportion of cows with subclinical mastitis did not differ significantly ($P = 0.72$) between conventional (20.8%) and organic (23.3%) herds.

The proportion of cows with negative microbiological results did not differ between conventional (49.8%) and organic (34.1%) dairies ($P = 0.27$). No significant differences were observed between management type for cow-level prevalence of *Staph. aureus* (4.1% conventional vs. 4.7% organic; $P = 0.75$), CNS (20.6% conventional vs. 14.1% organic; $P = 0.17$), *Streptococcus* spp. (2.2% conventional vs. 2.9% organic, $P = 0.66$), or *Corynebacterium* spp. (2.8% conventional vs. 6.6% organic; $P = 0.33$). The proportion of cows with minor species infections (CNS, *Corynebacterium* spp., or both CNS and *Corynebacterium* spp.) also did not differ between management types (26.5% conventional vs. 30.4% organic; $P = 0.64$).

The frequency of intramammary infections in individual quarter milks is presented in Table 2.3. In all conventional herds and the majority of organic herds, CNS was the organism most frequently isolated from infected quarters. Presence of *Staph. aureus* alone in quarter milk ranged from 0.5 to 4.8% in conventional herds and from 2.7 to 6.8% in organic herds (Table 2.3). *Corynebacterium* spp. (presumed *bovis*) were frequently found in organic herds, but percentages of infected glands were quite variable, ranging from 0 to 63.5%. High prevalence of *Corynebacterium* spp. was related to high average herd SCS in 2 organic herds

(Table 2.3; Figure 2.1; Figure 2.2b). The prevalence of environmental pathogens varied greatly by farm, with the highest frequency of coliforms found in herd 7 (conventional) and the highest proportion of *Streptococcus* spp. (not *agalactiae*) found in herd 13 (organic). Coliform organisms were found in 1 of the 7 organic herds and were detected in 5 of the 7 conventional herds. Table 2.3 also shows that the most common result was a single organism per quarter. For example, the large majority of *Staph. aureus* quarter infections were single and not mixed infections (e.g. *Staph. aureus* and CNS).

DISCUSSION

The objective of this study was to assess milk quality and mastitis management practices in similarly sized organic and conventional dairies in North Carolina. This study provides a snapshot of similarities and differences between regionally matched dairies during the warmest months of the year in a region of the United States with a history of challenges regarding milk quality. Interpretation of the data should be done cautiously, as this single sampling of each farm may not adequately represent milk quality over multiple years.

Milk quality on organic farms is of great interest to both consumers and dairy scientists. The potential for variation in milk quality on organic dairies as compared to conventional dairies is certainly of concern with the steady growth of the organic dairy industry. We expected that SCS might be greater in organic dairies than conventional dairies because the weather over the duration of the study was highly conducive to heat stress and organic producers would not have access to antibiotics for use in managing udder health. The

average temperature-humidity index during the study was above 72, the standard index value used to indicate heat stress in dairy cattle (Armstrong, 1994). Despite these potential challenges, our results indicate that no difference existed in SCS between the organic and conventional dairy herds sampled. Comparisons are difficult to make between United States and international organic dairies due to differences in regulations on use of antibiotics (Ruegg, 2009). For example, European Union organic regulations allow the use of antibiotics to treat disease when the use of non-synthetic products is inappropriate, but with an extended withdrawal time (The Council of the European Union, 2007). Even with differences in regulations, no clear patterns of differences exist in SCC between organic and conventional dairies in the United States or other countries. Most previous comparisons of organic and conventional dairies in the United States have reported no significant differences in bulk tank SCC (Sato et al., 2005; Pol and Ruegg, 2007b; Stiglbauer et al., 2013, Cicconi-Hogan et al., 2013). Some studies in Norway (Valle et al., 2007) and Sweden (Hamilton et al., 2006; Fall et al., 2008) have also reported similar bulk tank SCC between management types. One study of dairies in Michigan, Minnesota, New York, and Wisconsin reported higher SCC in organic dairies compared to conventional dairies (Zwald et al., 2004). Higher bulk tank SCC were also seen in organic herds in Norway (Hardeng and Edge, 2001), the United Kingdom (Hovi and Roderick, 2000), and Switzerland (Roesch et al., 2007). Evidence from Finland suggests that organic milk has significantly higher SCC than conventional milk in the summertime (Luukkonen et al., 2005). In contrast, bulk tank SCC was lower in organic herds (273,000 cells/mL) than in conventional herds (559,300 cells/mL) in spring and summer in New York

and Vermont (Tikofsky et al., 2003). No clear relationships exist between SCC and organic or conventional dairy management.

Successful mastitis prevention strategies include application of a germicidal teat dip postmilking and treating all cows with intramammary antibiotics at dry off; both of these management practices are associated with lower SCC than not using teat dip or treating all cows at dry off (Erskine and Eberhart, 1991; Wenz et al., 2007; Dufour et al., 2011). All farms that we surveyed used an iodine-based postmilking teat dip. Most organic farms previously surveyed in the United States use postmilking teat dip (Sato et al., 2005; Stiglbauer et al., 2013). Many of the conventional farms used US Food and Drug Administration-approved intramammary antibiotics on all cows at dry off. A considerable majority of conventional herds in the United States use antibiotic dry cow therapy on all cows (USDA, 2007). The use of blanket dry cow therapy is not possible in United States organic dairies because the USDA prohibits administration of any drug in the absence of illness in organic dairy cattle, except for vaccines (USDA National Organic Program, 2013). Many organic farms use gradual cessation of milking to dry off their cows, as seen in our survey (Table 2.2) as well as in most organic farms surveyed in Wisconsin (Ruegg, 2009). Gradual cessation of milking at dry off has been associated with less milk leakage post-dry off compared to abruptly drying off (Zobel et al., 2013). Less milk leakage could ostensibly allow for more rapid formation of the keratin teat plug, providing a natural source of resistance to mastitis during the dry period.

Treatment and prevention of mastitis in organic dairy cattle in the United States is

challenging because most products used by organic producers for mastitis treatment have limited scientific evidence of efficacy. Organic farmers in this survey used Phyto-Mast, garlic, essential oils, or *Aloe vera* for treating subclinical and clinical mastitis. Organic dairy farmers in Wisconsin have been reported to use whey-based products, garlic tinctures, *Aloe vera*, vitamin C, aspirin, homeopathy, and vegetable oils to treat clinical mastitis (Pol and Ruegg, 2007b). No studies currently available validate the use of garlic, essential oils, or *Aloe vera* as therapy for bovine mastitis. One study has shown that Phyto-Mast has similar efficacy to antibiotic therapy for curing infections when used as a dry-off treatment (Mullen et al., 2012), but Phyto-Mast has not been evaluated for treatment of mastitis during lactation. Intravaginal or intramammary treatments used by organic farmers in the United States have not been approved by the FDA for treatment of mastitis. Further evaluation of alternatives to antibiotics is essential to determine effective treatments for organic dairy producers to use as mastitis therapy when infections occur.

A wide range of SCS was observed among farms within management types, which could be due, in part, to prevalence of certain bacteria such as *Corynebacterium* spp. and *Staph. aureus*. Infection with *Corynebacterium bovis* has been shown to increase SCC 2 to 3 times relative to uninfected cows (NMC, 1996). The 2 organic herds with the highest SCS also had very high rates of infection with *Corynebacterium* spp. (Table 2.3; Figure 2.1; Figure 2.2b) which is problematic because of the known relationship between *C. bovis* infection and elevated SCC. We believe that isolated *Corynebacterium* spp. were *C. bovis*, though the authors recognize that a small chance (~3%) exists that this is an incorrect

assumption based on the lack of further testing for speciation (Huxley et al., 2004). The infection rate in those organic herds is of concern because of the lack of treatments for mastitis that are approved by the FDA and the USDA National Organic Program. The increase in SCC for some organic herds in this study is also of concern for milk quality.

Organic herds had a numerically higher cow-level prevalence of *Staph. aureus* in this study (4.7% of cows infected vs. 4.1% of conventional cows infected). Other studies on organic herds in the United States have found the prevalence of *Staph. aureus* to be numerically different, either lower (Tikofsky et al., 2003) or higher (Sato et al., 2004; Pol and Ruegg, 2007b; Cicconi-Hogan et al., 2013). The prevalence of *Staph. aureus* on Wisconsin dairies with 6-mo average bulk tank SCC $\geq 250,000$ cells/mL was significantly greater in organic herds compared with conventional herds (Pol and Ruegg, 2007a), and presence of *Staph. aureus* is a risk factor for high SCC on organic dairies in the United States (Cicconi-Hogan et al., 2013). The probability of curing a *Staph. aureus* infection decreases as cow age and duration of infection increase (Barkema et al., 2006). Organic dairies tend to have older cows than conventional dairies (Stiglbauer et al., 2013), and the lack of approved treatments for bovine mastitis in organic cattle could also contribute to the higher prevalence of *Staph. aureus* in organic dairies. The relatively higher prevalence of *Staph. aureus* in organic dairies could also be related to lack of use of practices such as dry-cow treatment and challenges in controlling fly populations: synthetic insecticides are not permitted for use on organic dairies (USDA National Organic Program, 2013). Horn flies (*Haematobia irritans*) are blood-feeding flies active during the summer months that have been shown to be possible vectors

for *Staph. aureus* on dairies in North Carolina (Anderson et al., 2012). In the case of those organic herds with high prevalence of *Corynebacterium* spp. and *Staph. aureus*, we recommend selectively culling cows with high SCC and presence of IMI. Aggressively culling for *Staph. aureus* mastitis, effective fly control, and maintaining a clean environment are essential to reducing incidence of *Staph. aureus* mastitis on both organic and conventional herds.

CONCLUSIONS

Despite differences in mastitis treatment availability, organic and conventional dairies surveyed in North Carolina had similar cow-level SCS and prevalence of several mastitis-causing bacteria including *Staphylococcus aureus*, *Streptococcus* spp., *Corynebacterium* spp., and coagulase-negative *Staphylococcus* spp. Our data indicate that conventional and organic dairy farmers in the southeastern United States face similar challenges in mastitis management and milk quality during the warm months of the year.

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Table 2.1. Demographics of 7 organic and 7 conventional dairy herds in North Carolina as reported by farmer surveys¹

Date Sampled	Farm	Type ²	No. of milking cows	Breeds ³	Milkers ⁴ (no.)	DHIA testing	Prestrip ⁵	Predip	Postdip	Most recent bulk tank SCC/mL
June 3, 2010	1	C	111	H	2	Yes	Yes	Iodine	Iodine	252,000
June 10, 2010	2	C	48	H, J, X	2	Yes	Yes	Iodine	Iodine	306,000
September 14, 2010	3	C	136	H, J	1.5	Yes	No	Iodine	Iodine	380,000
August 31, 2010	4	C	150	H, J, X	2	Yes	Yes	Hydrogen peroxide	Iodine	
October 11, 2010	5	C	80	H, J, G, B	2	No	No	Dilute bleach (1:1)	Iodine	350,000
May 26, 2010	6	C	18	H	1.5	Yes	Yes	Hydrogen peroxide	Iodine	180,000
October 5, 2010	7	C	109	H, J	1	Yes	Yes	Hydrogen peroxide	Iodine	390,000
June 14, 2010	8	O	47	H, J, X	2	Yes	Yes	Iodine	Iodine	306,000
August 3, 2010	9	O	97	H	2.5	No	Yes	Iodine	Iodine	500,000
June 7, 2010	10	O	85	H, X	2	No	No	Iodine	Iodine	260,000
July 21, 2010	11	O	157	H, J, X	2	Yes	Yes	Iodine	Iodine	167,000
July 29, 2010	12	O	123	H, J, X	1	Yes	No	Iodine	Iodine	286,000
July 29, 2010	13	O	19	H	1	No	No	Iodine	Iodine	83,000
July 8, 2010	14	O	67	H	2	Yes	Yes	Hydrogen peroxide	Iodine	450,000

¹This study was conducted between May and October of 2010 on all certified organic dairies (n = 6) in North Carolina, 6 conventional dairies of similar size and geographic location, and a research herd managed half conventionally (farm 2) and half organically (farm 8). Organic cows in that herd received health care according to US Department of Agriculture organic standards but consumed conventional concentrate feed supplements.

²Type: C = conventional; O = organic.

³Breeds: H = Holsteins; J = Jerseys; X = Holstein x Jersey crossbreds; G = Guernsey, B = Brown Swiss.

⁴Average number of people milking the cows at any one time. Some farms (2, 3, 6, 8) had rotations that involved multiple different milkers over time.

⁵Hand-milking a few strips before milking machine attachment.

Table 2.2. Detection and treatment of mastitis and dry-off treatments used by 7 conventional and 7 organic dairy farms in North Carolina¹

Farm	Type ²	Subclinical mastitis treatment ³	Clinical mastitis detection	Clinical mastitis treatment ³	Dry off protocol
1	C	Cephapirin sodium, pirlimycin hydrochloride, or ceftiofur hydrochloride	Check foremilk, behavior	Flunixin meglumine, cephalosporin sodium, pirlimycin hydrochloride, or ceftiofur hydrochloride	Penicillin-dihydrostreptomycin and bismuth subnitrate
2	C	Pirlimycin hydrochloride	Check foremilk, behavior, also assessed using CMT ⁴	Pirlimycin hydrochloride	Penicillin-dihydrostreptomycin and bismuth subnitrate
3	C	Pirlimycin hydrochloride or ceftiofur hydrochloride	Visual, confirmed using CMT	Hetacillin potassium or ceftiofur hydrochloride	Penicillin-dihydrostreptomycin
4	C	Cephapirin sodium or pirlimycin hydrochloride	Check foremilk	Cephapirin sodium or pirlimycin hydrochloride	Penicillin-dihydrostreptomycin and bismuth subnitrate
5	C	Cephapirin sodium, pirlimycin hydrochloride, or ceftiofur hydrochloride	Visual	Cephapirin sodium, pirlimycin hydrochloride, or ceftiofur hydrochloride	Benzathine cloxacillin or cephalosporin benzathine
6	C	Nothing	Check foremilk	Ceftiofur hydrochloride	Ceftiofur hydrochloride
7	C	Ceftiofur hydrochloride	Check foremilk, also assessed using CMT	Pirlimycin hydrochloride	Cephapirin benzathine and bismuth subnitrate
8	O	Phyto-Mast ⁵	Check foremilk, also assessed using CMT, visual, SCC	Phyto-Mast	Phyto-Mast
9	O	Phyto-Mast, garlic oral or in vulva	Visual	Phyto-Mast, garlic oral or in vulva	No treatment
10	O	Essential oils, garlic tinctures	Visual	Essential oils, garlic tinctures	3 mo dry
11	O	Phyto-Mast	Check foremilk	Garlic tinctures, Phyto-Mast	No treatment
12	O	Garlic, <i>Aloe vera</i> , Echinacea, topical rub	Check foremilk, SCC	Garlic, <i>Aloe vera</i> , topical rub	Milk 1 time/d for 4 to 5 d and dry off
13	O	Strip out, garlic at tailhead, oral <i>Aloe vera</i>	Behavior, also assessed using CMT	Strip out, garlic at tailhead, oral <i>Aloe vera</i>	First 3 d milk every other milking, every 2 d for 2 times, wait 1 wk, milk out if there is still milk
14	O	Garlic tincture in vulva	Check foremilk	Garlic tincture in vulva	Dry and 1 wk later milk out

¹This study was conducted between May and October of 2010 on all certified organic dairies (n = 6) in North Carolina, 6 conventional dairies of similar size and geographic location, and a research herd managed half conventionally (farm 2) and half organically (farm 8). Organic cows in that herd received health care and pasture access according to USDA organic standards but consumed conventional concentrate feed supplements.

²Type: C = conventional management; O = organic management.

³In some mastitis cases farmers may use more than one treatment.

⁴CMT: California mastitis test.

⁵Phyto-Mast is an intramammary herbal product (Penn Dutch Cow Care, Narvon, PA).

Table 2.3. Frequency of udder quarters with various intramammary infections on 7 conventional and 7 organic dairies in North Carolina¹

Farm	Conventional							Organic						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No growth	356	168	412	399	253	56	261	156	165	264	476	353	58	44
<i>Bacillus</i> spp.	0	0	0	0	1	0	1	0	0	0	0	0	0	0
CNS	37	6	56	87	20	7	42	9	32	28	45	53	3	8
CNS – <i>Corynebacterium</i> spp.	1	0	0	4	0	0	2	0	3	0	1	0	1	2
Coliforms	1	0	1	3	1	0	3	0	0	0	0	0	0	1
<i>Corynebacterium</i> spp.	3	0	17	14	11	1	27	2	120	2	11	6	0	162
<i>Enterococcus</i> spp.	0	1	4	0	2	4	1	0	0	0	1	1	0	3
<i>Enterococcus</i> spp., CNS	0	0	1	1	0	0	0	0	0	0	1	0	0	0
<i>Enterococcus</i> spp., <i>Corynebacterium</i> spp.	0	0	0	0	0	0	0	0	2	0	1	0	0	1
<i>Nocardia</i> spp.	0	2	0	0	0	0	1	0	0	0	0	0	0	0
Other ²	0	0	0	0	1	0	5	0	1	0	1	0	1	3
<i>Pseudomonas</i> spp.	0	0	0	0	0	0	10	0	0	0	0	0	0	0
<i>Staphylococcus aureus</i>	8	1	25	21	12	3	6	8	17	22	26	22	2	12
<i>Staph. aureus</i> , CNS	0	0	0	1	0	0	0	0	0	2	0	0	0	0
<i>Staph. aureus</i> , <i>Corynebacterium</i> spp.	0	0	0	1	0	0	0	0	4	0	0	0	0	4
<i>Staph. aureus</i> , <i>Strep.</i> spp. ³	0	0	4	3	0	0	0	0	1	0	2	2	0	1
<i>Strep.</i> spp.	10	1	1	16	4	0	15	1	14	5	4	12	7	13
<i>Strep.</i> spp., CNS	4	2	4	4	1	0	1	0	4	1	0	5	0	1
<i>Strep.</i> spp., <i>Corynebacterium</i> spp.	0	0	0	5	0	0	1	0	1	0	0	0	1	0
<i>Strep. agalactiae</i>	1	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Trueperella pyogenes</i>	0	0	0	0	2	0	1	0	0	1	1	0	0	0
Total mammary glands (quarters)	421	181	525	559	308	71	378	176	364	325	570	454	73	255

¹This study was conducted between May and October of 2010 on all certified organic dairies (n = 6) in North Carolina, 6 conventional dairies of similar size and geographic location, and a research herd managed half conventionally (farm 2) and half organically (farm 8). Organic cows in that herd received health care and pasture access according to USDA organic standards but consumed conventional concentrate feed supplements.

²One quarter infected with yeast and *Streptococcus* spp. (Farm 5); 2 *Pseudomonas* spp. and CNS, 2 *Pseudomonas* spp. and *Pasteurella*, and 1 *Streptococcus* spp. and *E. coli* (Farm 7); 1 yeast (Farm 9); 1 *Trueperella pyogenes* and *Streptococcus* spp. (Farm 11); 1 *Serratia marcescens* (Farm 13); 1 *Corynebacterium* spp. and *E. coli*, 1 gram-negative, and 1 *Streptococcus agalactiae* and *Corynebacterium* spp. (Farm 14).

⁴*Strep.* spp.: *Streptococcus* spp. other than *agalactiae*.

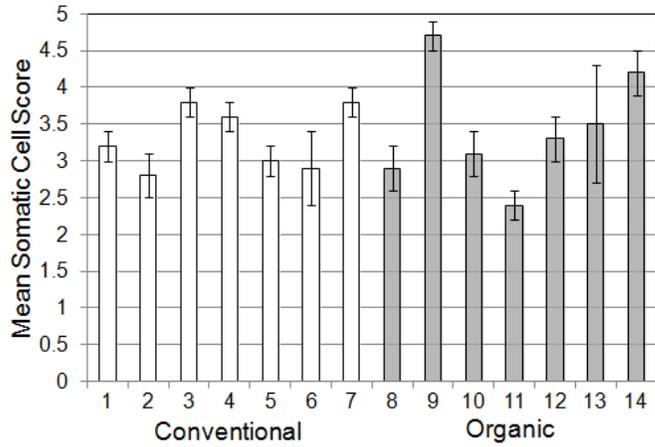
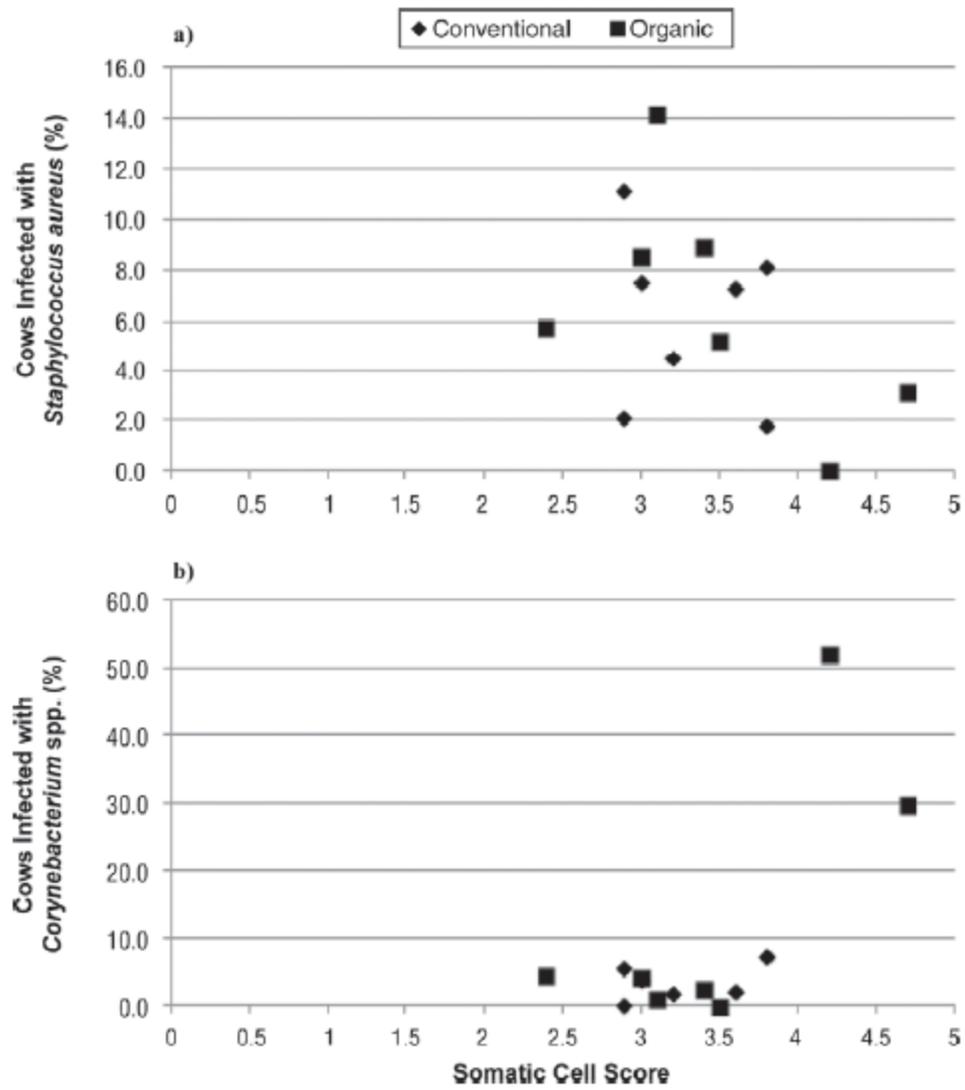


Figure 2.1. Mean SCS of 7 conventional and 7 organic dairies in North Carolina. This study was conducted between May and October of 2010 on all certified organic dairies (n = 6) in North Carolina, 6 conventional dairies of similar size and geographic location, and a research herd managed half conventionally (farm 2) and half organically (farm 8). Organic cows in that herd received health care according to US Department of Agriculture organic standards but consumed conventional concentrate feed supplements.

Figure 2.2. Relationship between herd mean SCS and percentage of cows infected with 2 contagious mastitis-causing organisms [*Staphylococcus aureus* (a) and *Corynebacterium* spp., (b)]. This study was conducted between May and October of 2010 on all certified organic dairies (n = 6) in North Carolina, 6 conventional dairies of similar size and geographic location, and a research herd managed half conventionally and half organically. Organic cows in that herd received health care and pasture access according to US Department of Agriculture organic standards but consumed conventional concentrate feed supplements.



**CHAPTER 3: EFFECT OF TWO HERBAL INTRAMAMMARY TREATMENTS ON
MILK QUALITY AND QUANTITY 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 U. S. are restricted from using antibiotics and must consider an alternative or no dry cow therapy. The current study compared 2 herbal treatments 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 one research farm and Trial 2 included 4 commercial farms plus the research herd over 2 yr. Treatments included 1) a conventional IMM antibiotic and teat sealant (penicillin-dihydrostreptomycin and bismuth subnitrate; CON); 2) an herbal IMM product purported to act as a teat sealant (Cinnatube™; CIN); 3) an herbal IMM product (Phyto-Mast®; P-M); 4) Phyto-Mast and Cinnatube (PC); or 5) no dry cow therapy (NONE). 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 post-treatment 305-d mature equivalent milk production (Trial 1), somatic cell score change from dry off to freshening at the cow and quarter level (Trials 1 and 2), and milk microbiology change over the dry period (Trial 2). There were 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 were also not significantly different among treatments in either trial. Cure rates were not

significantly different among treatments, but only 19.6% of all quarters were infected at dry off. The proportion of quarters with new infections at 3 to 5 days post-calving was not significantly different among treatments, except for between CIN and NONE. Percentages 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 NONE. The efficacy of the herbal treatments was similar to conventional therapy and the herbal products had no apparent adverse effects.

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 \$100 U. S. (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 67 to 82% (Smith et al., 1967a; Hillerton and Berry, 2005). Mastitis treatment and dry cow therapy are normally accomplished using IMM antibiotics labeled for treatment of gram-positive bacterial infections (U. S. Food and Drug Administration, 2013). 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 U. S. Total sales of organic fluid milk products doubled between 2006 and 2011, representing 4% of the total fluid milk market in 2011 (AMS-USDA, 2012). Certified organic dairy farms in the U. S. 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 (Electronic Code of Federal Regulations, 2013a). Organic standards focus on disease prevention through allowing cattle to exercise natural behaviors by mandating pasture access and allowing the use of vaccinations (Electronic Code of Federal Regulations, 2013a). Administration of medication in the absence of illness is prohibited in organic dairy production in the U. S., except for vaccinations. However, provisions may be made by organic certifiers for herbal products that are allowed by the National Organic Standards Board.

Mastitis is a challenge for organic dairy farmers for two reasons. One is prevalence of mastitis; organic dairy farms in the U. S. 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 there are no alternatives to antibiotics currently approved by the Food and Drug Administration for IMM treatment of mastitis and accepted by the U. S. National Organic Program, making it more difficult for organic dairy farmers to manage this costly and prevalent disease. Scientists have expressed concerns that the inability of organic dairy

farmers to use approved antibiotics for mastitis therapy could lead to increases in udder health problems, leading to decreased milk quality (Zwald et al., 2004).

In the evaluation of alternatives to antibiotics, it is important to consider that the mammary gland is very susceptible to irritation and that any intramammary infusions should be non-irritating (Sanderson, 1966). The mammary gland is more susceptible to infection and subsequent inflammation during the dry period when the mammary gland is in a state of transition from lactation to involution 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 non-irritating. Though organic dairy farmers in the U. S. have been reported to use a wide variety of mastitis treatments (Ruegg, 2009), no scientific studies evaluating the effect of those treatments on the mammary gland *in vivo* have been published to date.

The objective of this study was to determine the effects of administration of two 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 two herbal products when used as dry cow therapy. The 5 treatments, assessed in both trials, were a synthetic IMM antibiotic (penicillin-dihydrostreptomycin; Quartermaster; Zoetis, Kalamazoo, MI) plus internal teat sealant (bismuth subnitrate; Orbeseal; Zoetis, Kalamazoo, MI), an herbal internal teat sealant (Cinnatube; New AgriTech Enterprises,

Locke, NY), an herbal IMM product (Phyto-Mast; Penn Dutch Cow Care, 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, Ohio), and its ingredients comply with the USDA National Organic Standards Board regulations. The components of Phyto-Mast and Cinnatube are listed in Table 3.1.

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 in 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 conventional antibiotic and teat sealant (**CON**), 40 Phyto-Mast (**P-M**), and 40 no treatment (**NONE**). In calving season 2010-2011, 116 cows were used and 2 additional treatments were included: 22 CON, 24 Cinnatube (**CIN**), 24 P-M, 24 Phyto-Mast and Cinnatube (**PC**), and 23 NONE. 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 post-calving milk production, and 305-d mature equivalent milk production for the lactation following treatment (**PostLact305MEM**). Somatic cell score (**SCS**) 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, sterile milk samples were collected, then teat ends were cleaned with 70% 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, 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, 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 post-dipped 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 days 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, one research farm, and two 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 only assessed on the research herd because managers of all 4 of the 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 on-farm (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 and considering first breed, then lactation number, 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 pre-milking preparation. Normal pre-milking preparation included pre-dip, stripping each quarter 3 to 5 times, and wiping off pre-dip after 30 or more seconds' contact time. Collected milk samples were immediately stored on ice and kept cold until 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 a 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 days post-calving. 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 at North Carolina State University. 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 either 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. Presence of infection. If a bacterial species was present at ≥ 100 colony forming units/mL, it was recorded as an IMI in the quarter cultured except for CNS, in which case at least 200 colony forming units/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 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 they were 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. All 4 infection status categories (cure, new IMI, no change in IMI status, no change, still not infected) were mutually exclusive.

Statistical Analyses

Statistical analyses for Trial 1 were performed using mixed linear models (PROC MIXED) in SAS version 9.2 (Cary, NC). The hypothesis for Trial 1 was that the herbal treatments (CIN, P-M, and PC) had the same effect on milk quality and quantity as NONE 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. Since proving equality of treatments is statistically impossible, a pre-experiment margin of noninferiority (Δ) must be defined (Piaggio et al., 2006). This Δ value is established based on the range of efficacy of treatments acceptable in a clinical setting or the results of previous research. The null hypothesis (H_0) tested in this study's noninferiority trial is that herbal treatments are inferior to CON, and the alternative hypothesis (H_A) is 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, also written as

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

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 probability 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).

All statistical analyses for Trial 2 were performed at the quarter level using SAS (version 9.2; Cary, NC). 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 treatments 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 non-inferiority of one herbal treatment 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 rates of 87% and 33% for CON and an herbal treatment, respectively. The NONE treatment was also included in trial 2 to test the hypothesis that the herbal treatments (CIN, P-M, and PC) were no different in cure rate or new infection rate than NONE.

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 = 1st lactation pre-dry off, group 2 = 2nd lactation pre-dry off, group 3 = 3rd and greater lactation pre-dry off), dry period length (short = less than 45 d, normal = 45 to 60 d, long = greater than 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 somatic cell score (SCS) to more closely approximate the Normal distribution; this transformation was performed using the formula $\log_2\left(\frac{SCC}{100,000}\right)+3$ to obtain the base 2 logarithmic transformation as recommended by Shook (1993). Model selection for both trials was performed similar 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 less than 0.20. Once univariate selection was complete, all main-effect interactions with treatment were included in the model. The final model selection step involved backwards 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 errors. Differences among 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 NONE), 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 NONE (Table 3.2). 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 post-treatment sampling date. There were no clinical mastitis cases or signs of visible irritation to the udder during the dry period or during the first 5 days of lactation.

Difference between PostLact305MEM and PrevLact305MEM. Raw mean milk yield difference (PostLact305MEM minus PrevLact305MEM) was 523 ± 1895 kg for CON cows, 82 ± 1758 kg for CIN cows, -108 ± 1639 kg for P-M cows, 40 ± 1424 kg for PC cows, and 84 ± 1298 kg for NONE cows. The final model used to predict milk yield difference included treatment, breed, lactation number, first test date post-partum milk production, year, last calving date, TD01_SCS, PrevLactLastSCS, and the interaction of treatment and breed. Least squares means are given in Table 3.3. With the large standard errors within treatment

groups for changes in production from one lactation to the next, there were no significant differences among treatments for milk yield difference. Similarly, there were 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 post-partum 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 test date post-partum milk production increased, so 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 2802 kg) significantly greater than 0 in treatments CON, NONE, and PC, respectively. In contrast, milk yield differences for other breed and treatment combinations were not different from 0. Cows that dried off in 2009 had a lower milk difference (-605 ± 1017 kg) than cows that dried off in 2010 (-29 ± 288 kg) or 2011 (1948 ± 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. There were 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 3.3. 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 0. 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 0 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 PostLact305MEM and treatment was significant because cows producing between 4,536 and 9,072 kg (10,000 to 20,000 lb) of milk and receiving PM 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 3.3.

Previous lactation last SCS and TD01_SCS least squares means are presented in Table 3.3. Though TD01_SCS was significantly lower in P-M cows than in PC cows, the difference in SCS from dry off to freshening was not significantly different.

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 of 2010 and March of 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 NONE). Contamination rates ranged from 5.1% in the research herd to 13.3% in one 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 NONE (Table 3.5). 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 for of SCSDiff revealed no significant differences among treatments. The results of the mixed model regression are shown in Table 3.4. Dry periods less than 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 longer than 60 d had an average SCSDiff of 0.15. Of all possible results of treatment, only new IMI had an SCSDiff significantly different from 0. 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 NONE Jerseys and CON Jerseys were significantly different from each other ($P = 0.04$) with the difference in favor of no treatment. Only three treatment and breed combinations had SCSDiff significantly different from 0: CON Jerseys and PC crossbreds had SCSDiff > 0 and NONE Jerseys had SCSDiff < 0 .

IMI at Dry Off. A 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 *Staph. aureus* (14.6% or 30 quarters) and *Strep. spp.* other than *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 3.6.

IMI at 3 to 5 Days Post-Calving. Most samples (804 or 77.0%) had no infection present 3 to 5 days post-calving. The most prevalent organism in the post-calving sample was CNS, present in 82 of the 240 quarters with IMI (34.2%). *Strep. spp.* other than *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 post-calving for each treatment are given in Table 3.6.

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 3.1. While none of the herbal treatments reached the zone of noninferiority, the 95% confidence interval for CON overlaps the intervals for all of the herbal treatments. Results of the regression are

shown in Table 3.7. Increased SCC at dry off was associated with less chance of cure. Somatic cell score difference less than 0 was associated with a higher chance of cure.

Coagulase-negative staphylococci were 50.5% of all of the 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 3.6). However, analysis comparing the other 4 treatments showed no differences in cure rate for CNS or *Corynebacterium* spp. Number of quarters cured by pathogen are listed in Table 3.8. 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 (NONE; 9 of 19). There were 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 Post-Calving. Mixed model logistic regression results of the proportion of quarters with new infections postpartum are shown in Table 3.7. Quarters treated with CIN were significantly less likely to experience a new infection than quarters treated with NONE ($P = 0.03$). There were no significant

differences in the cure rates among the other treatments. Higher post-calving SCC was associated with higher probability of new infection. Jerseys were the most likely to have a new infection post-calving ($35 \pm 15\%$), followed by Holsteins ($29 \pm 10\%$) and crossbreds ($17 \pm 8\%$). Jerseys were more likely ($P = 0.08$) than crossbreds to have a new infection present post-calving. The interaction of the 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 longer than 60 days. Length of the dry period was also included in the model though it was not significant.

New infection rates for specific organisms could not be calculated due to the low number of new infections, especially in CON cows. When IMI were grouped into gram-positive, gram-negative, other, and mixed infections, there were no significant differences among 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 two herbal IMM products on milk production and milk quality when administered as a dry cow therapy. To the authors' 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 provide

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 U. S., as herds often include crossbreeds and multiple breeds (Sato et al., 2005; Rotz et al., 2007; Stiglbauer et al., 2013; Mullen et al., 2013) and have older cattle than conventional herds (Stiglbauer et al., 2013). Though 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 Food and Drug Administration (**FDA**) approval process for treatment of mastitis. Most of their ingredients are on the FDA Generally Recognized as Safe list for human consumption. Though 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. Technically speaking, the treatments tested in this trial are not illegal in the sense of FDA labeling but have not undergone the FDA review process and thus cannot be marketed as treatments for mastitis.

Now, as well as early 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 non-irritating (Sanderson, 1966). Several essential oils have documented cytotoxic activity (Bakkali et al., 2008), which raises concerns about the welfare of cows receiving herbal treatments. The current study indicates that Cinnatube, Phyto-Mast, and a combination of the two did not have an irritating effect on the udder, at least as measured by milk production at the cow level and SCS at the cow and quarter level compared to the positive (CON) and negative (NONE) controls. Milk production was not

adversely affected by the herbal treatments. 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 3.1). The ingredients of the treatments in this trial did not appear to have that effect in the current study as compared with no treatment. In trial 2, although three of the treatments (CON, P-M, and PC) had higher freshening SCS than NONE, 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 no treatment, because a previous comparisons of dry cow therapies reported lower SCS post-calving 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 impacts on pasture quality and productivity likely negatively impacted milk production.

The presence of infection at dry off was low (19.6%) compared to the 30-40% 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 had 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 31% (Godden et al., 2003) and 50-60% (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). We hypothesized that the herbal treatments would be significantly less effective than conventional therapy at curing infections during the dry period. Trial 2 shows that the herbal treatments 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 treatments was likely conferred by the previously reported antibacterial activity of several ingredients in each product tested (Table 3.1). Conversely, the herbal treatments did not cure significantly more infections than were spontaneously cured (NONE 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 of consideration for bacteria such as *Staphylococcus aureus*, which are shed intermittently from infected udder quarters. In the current study, only 4 of 30 initial cases of *S. 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., 1979; Oliver et al., 1983; Smith et al.,

1985), and 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 hours post-treatment in blood serum and up to 24 hours 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. It was expected that CON would have had a significant reduction in the rate of new infections compared to no treatment, as shown previously with antibiotics or teat sealants (Smith et al., 1967b; Smith et al., 1967c; 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% confidence interval 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 treatments were the same as no treatment at preventing new infections, except for CIN which had significantly fewer new infections than

NONE. 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 treatments also had similar new infection rates to conventional antibiotic therapy. Though 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 treatments.

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Table 3.1. Phyto-Mast¹ and Cinnatube² herbal oil ingredients, major chemical components, and bioactivity

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

¹Intramammary treatment for improving milk quality; Penn Dutch Cow Care, Narvon, PA.

²Intramammary treatment for use as an internal teat sealant, New AgriTech Enterprises, Locke, NY.

Table 3.2. 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)

Treatment ¹	Breed of Cow						Total
	Holstein		Jersey		Crossbred		
	#	Lactation	#	Lactation	#	Lactation	
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
NONE	13	5.2 ± 2.5	11	4.6 ± 1.6	56	3.5 ± 1.3	80
Total	44		45		240		329

¹Treatments: CON = conventional intramammary dry cow therapy including 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); CIN = herbal internal teat sealant (Cinnatube; New AgriTech Enterprises, Locke, NY); P-M = herbal intramammary treatment for improving milk quality (Phyto-Mast; Penn Dutch Cow Care, Narvon, PA); PC = treatment with Phyto-Mast and Cinnatube; NONE = no treatment. The CIN and PC treatments were only assessed for two of the three years.

Table 3.3. 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

	CON (n = 79)	CIN (n = 44)	P-M (n = 83)	PC (n = 43)	NONE (n = 80)
PrevLact305MEM ²	7584 ± 278	6249 ± 516	7426 ± 290	6638 ± 516	6726 ± 277
PostLact305MEM ³	7339 ± 269	7012 ± 499	7600 ± 281	7957 ± 499	7293 ± 269
Milk Difference [*]	-245 ± 348	763 ± 646	175 ± 363	1322 ± 645	570 ± 347
PrevLactLastSCS ⁴	3.29 ± 0.26	2.96 ± 0.37	3.34 ± 0.28	3.86 ± 0.39	3.43 ± 0.26
TD01_SCS ⁵	3.07 ± 0.32 ^{ab}	3.70 ± 0.56 ^{ab}	2.91 ± 0.31 ^a	3.29 ± 0.54 ^b	3.20 ± 0.34 ^{ab}
SCS Difference [*]	-0.71 ± 0.42	0.54 ± 0.67	-1.20 ± 0.45	-0.37 ± 0.66	-0.40 ± 0.44

¹Treatments: CON = conventional intramammary dry cow therapy including 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); CIN = herbal internal teat sealant (Cinnatube; New AgriTech Enterprises, Locke, NY); P-M = herbal intramammary treatment for improving milk quality (Phyto-Mast; Penn Dutch Cow Care, Narvon, PA); PC = treatment with Phyto-Mast and Cinnatube; NONE = no treatment. The CIN and PC treatments were only assessed for two of the three years.

²PrevLact305MEM: Previous lactation 305-day mature equivalent milk production LS means ± SE, in kg.

³PostLact305MEM: Post-treatment lactation 305-day mature equivalent milk production LS means ± SE, in kg.

⁴PrevLactLastSCS: Last recorded somatic cell score of the lactation before treatment.

⁵TD01_SCS: Somatic cell score of the first test date post-partum.

* Although numerically variable, there were no significant differences among treatments for the difference in ME 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.

^{ab}Treatments with different superscripts within a row are significantly different ($P < 0.05$).

Table 3.4: Mixed model regression results for somatic cell score 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 *1 st 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

¹Conventional = Quatermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Kalamazoo, MI) and Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); Cinnatube (New AgriTech Enterprises, Locke, NY); Phyto-Mast (Penn Dutch Cow Care, Narvon, PA).

Table 3.5: Quarter-level somatic cell scores 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

¹Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Kalamazoo, MI) and Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); Cinnatube (New AgriTech Enterprises, Locke, NY); Phyto-Mast (Penn Dutch Cow Care, Narvon, PA). Estimates given are LS means ± SE.

^{abc}Estimates with different letters within a column are significantly different ($P < 0.05$).

Table 3.6. Frequency of IMI 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)¹

¹CON = Quatermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Kalamazoo, MI) and Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); CIN = Cinnatube (New AgriTech Enterprises, Locke, NY), an herbal internal teat sealant; P-M = Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), an herbal intramammary product for improving milk quality; PC = Phyto-Mast and Cinnatube; NONE = No treatment. Percentages given are percentage of all observed quarters receiving that specific treatment that were infected with the specific organism.

²Other gram-positives includes *Bacillus* spp., *Nocardia* spp., and *Trueperella pyogenes*.

³Gram-negatives include *Enterobacter aerogenes*, *Escherichia coli*, and *Klebsiella* spp.

Table 3.6 Continued

	IMI present at dry off					IMI present 3 to 5 DIM				
	CON	CIN	P-M	PC	NONE	CON	CIN	P-M	PC	NONE
Gram-positive										
CNS	0 (0%)	19 (8.26%)	27 (10.59%)	29 (13.55%)	18 (7.47%)	2 (1.92%)	12 (5.22%)	20 (7.84%)	24 (11.22%)	24 (9.96%)
<i>Corynebacterium</i>	0 (0%)	9 (3.91%)	15 (5.89%)	8 (3.74%)	11 (4.56%)	0 (0%)	12 (5.22%)	10 (3.92%)	12 (5.61%)	6 (2.49%)
spp.										
<i>Enterococcus</i> spp.	1 (0.96%)	0 (0%)	0 (0%)	1 (0.47%)	2 (0.83%)	1 (0.96%)	3 (1.30%)	1 (0.39%)	2 (0.93%)	2 (0.83%)
<i>Staph. aureus</i>	2 (1.92%)	7 (3.04%)	7 (2.75%)	9 (4.21%)	5 (2.07%)	0 (0%)	5 (2.17%)	10 (3.92%)	8 (3.74%)	5 (2.07%)
<i>Strep. spp. (not</i>	1 (0.96%)	2 (0.87%)	1 (0.39%)	3 (1.40%)	6 (2.49%)	1 (0.96%)	11 (4.78%)	17 (6.67%)	8 (3.74%)	15 (6.22%)
<i>agalactiae)</i>										
Other gram-	0 (0%)	1 (0.43%)	1 (0.39%)	0 (0%)	0 (0%)	0 (0%)	3 (1.30%)	0 (0%)	2 (0.93%)	0 (0%)
positives ²										
Total gram-	4 (3.85%)	38 (16.5%)	51 (20.00%)	50 (23.36%)	42 (17.43%)	4 (3.84%)	46 (20.00%)	58 (22.75%)	56 (26.17%)	52 (21.58%)
positives										
Gram-negative										
Total gram	0 (0%)	0 (0%)	1 (0.39%)	0 (0%)	1 (0.41%)	0 (0%)	1 (0.43%)	4 (1.57%)	0 (0%)	1 (0.41%)
negatives ³										
Other										
Yeast	0 (0%)	0 (0%)	1 (0.39%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.47%)	0 (0%)
Mixed infections	0 (0%)	5 (2.17%)	8 (3.14%)	3 (1.40%)	1 (0.41%)	0 (0%)	1 (0.43%)	6 (2.35%)	3 (1.40%)	7 (2.90%)
Total infected	4 (3.85%)	43 (18.70%)	61 (23.92%)	53 (24.77%)	44 (18.26%)	4 (3.85%)	48 (20.87%)	68 (26.67%)	60 (28.04%)	60 (24.90%)
quarters										
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

Table 3.7: Mixed model logistic regression results for cure rate and new infection rate models for 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
Cure Rate					
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	
New Infection Rate					
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

¹Conventional = Quatermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Kalamazoo, MI) and Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); Cinnatube (New AgriTech Enterprises, Locke, NY); Phyto-Mast (Penn Dutch Cow Care, Narvon, PA).

²SCS 3 to 5 days postpartum-SCS at dry off.

Table 3.8: Proportion of IMI cured and proportion of new IMI by conventional, herbal, or no dry cow therapy during the dry period in 5 dairies in North Carolina¹

Organism	Proportion of IMI cured					New IMI during the dry period				
	CON ^{2,3}	CIN	P-M	PC	NONE	CON	CIN	P-M	PC	NONE
Gram-positive										
<i>Bacillus</i> 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
<i>Corynebacterium</i> spp.	0/0	3/9	10/15	2/8	5/11	0	4	4	7	3
<i>Enterococcus</i> spp.	1/1	0/0	0/0	1/1	0/2	1	3	1	2	1
<i>Nocardia</i> spp.	0/0	0/1	1/1	0/0	0/0	0	1	0	0	0
<i>Staphylococcus aureus</i>	1/2	1/7	0/7	1/9	1/5	0	0	4	1	2
<i>Streptococcus</i> spp. (not agalactiae)	1/1	1/2	0/1	3/3	1/6	1	11	12	8	14
<i>Trueperella pyogenes</i>	0/0	0/0	0/0	0/0	0/0	0	0	0	1	0
Gram-negatives 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	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%)

¹Infections are recorded as number of quarters cured/number of quarters infected at dry off.

²CON = Quatermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Zoetis, Kalamazoo, MI) and Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); CIN = Cinnatube (New AgriTech Enterprises, Locke, NY); P-M = Phyto-Mast (Penn Dutch Cow Care, Narvon, PA); PC = Phyto-Mast and Cinnatube; NONE = No treatment.

³The conventional treatment was only assessed on the research herd due to antibiotic use limitations on the other dairies.

⁴Gram-negative infections present included *Enterobacter aerogenes*, *Escherichia coli*, and *Klebsiella* spp.

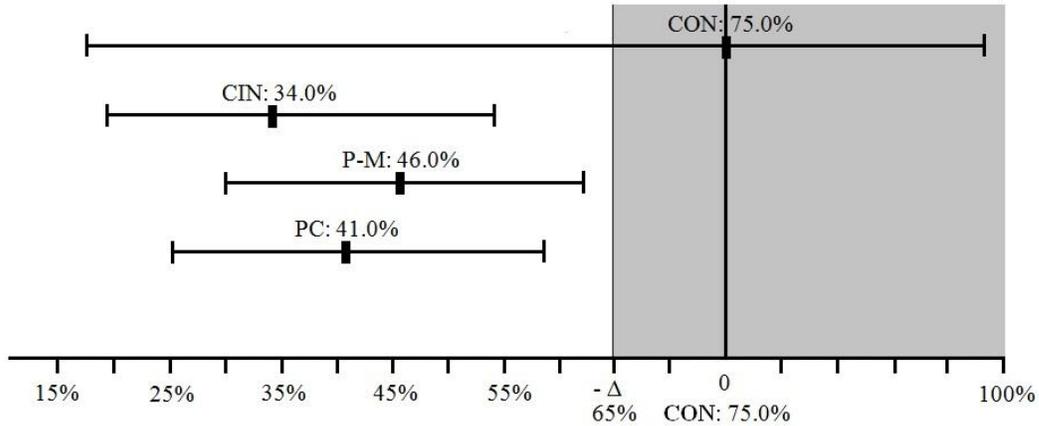


Figure 3.1. Noninferiority analysis of probability of cure for quarters from cows treated with Cinnatube (CIN; New AgriTech Enterprises, Locke, NY; LSM = 0.34; 95% CI: 0.19 to 0.53), Phyto-Mast (P-M; Penn Dutch Cow Care, Narvon, PA; LSM = 0.46; 95% CI: 0.30 to 0.62), or Phyto-Mast and Cinnatube (PC; LSM = 0.41; 95% CI: 0.26 to 0.60) compared with cows treated with 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) (CON; LSM = 0.75; 95% CI: 0.18 to 0.98). The error bars indicate the bounds of the 95% confidence intervals and the shaded area indicates the region of noninferiority, with Δ as the predetermined margin of noninferiority (10%).

**CHAPTER 4: AN IN VITRO ASSESSMENT OF THE ANTIBACTERIAL ACTIVITY
OF PLANT-DERIVED OILS**

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ABSTRACT

There is need for non-antibiotic treatments for mastitis in the growing organic dairy industry. There is anecdotal evidence that plant-derived oils have efficacy for treatment of mastitis in dairy cattle. The potential mechanisms of action of plant-derived oils in mastitis therapy have not been well studied. The objective of the current study was to evaluate the antibacterial activity of the plant-derived oil components of Phyto-Mast®, an herbal intramammary mastitis treatment, against three mastitis-causing pathogens: *Staphylococcus aureus*, *Streptococcus uberis*, and *Staphylococcus chromogenes*. The plant-derived oils evaluated were *Thymus vulgaris* (thyme), *Gaultheria procumbens* (wintergreen), *Glycyrrhiza uralensis* (Chinese licorice), *Angelica sinensis*, and *Angelica dahuricae*. Broth dilution testing according to CLSI standard protocol was performed using ultrapasteurized whole milk instead of broth. Controls included milk only (negative control), milk + bacteria, and milk + penicillin-streptomycin (positive control, at 1% and 5% dilutions). 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 three 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. Of all the individual plant oils tested, only thyme oil had consistent antibacterial activity against all three pathogens tested. Thyme oil at concentrations $\geq 2\%$ completely inhibited bacterial growth in all replications. Some combinations of the other plant-derived oils were consistently antibacterial across all 3 replications, although various combinations of

the other plant-derived oils did not show typical dose-response effects. We conclude that only thyme essential oil had consistent antibacterial activity against the 3 mastitis-causing organisms tested in vitro. Further evaluation of the physiological effects of plant-derived oils on mammary tissue is recommended to determine the suitability of these treatments for mastitis therapy.

INTRODUCTION

Consumers are concerned about the use of antibiotics in livestock production systems and the potential contribution to the development of antibiotic-resistant bacteria. One particularly important application of antibiotics in dairy cows is dry cow therapy, which is used to eliminate existing intramammary infections and prevent new intramammary infections from occurring during the non-lactating period. As of 2007, 72.3% of dairy herds in the United States used intramammary antibiotic dry cow therapy on all cows (USDA, 2008). Using an antibiotic on all cows at the start of the dry period has been associated with higher milk quality in the lactation following treatment (Eberhart and Buckalew, 1972; Godden et al., 2003; Dufour et al., 2011), but may result in overuse of antibiotics because all cows may not be infected at the time of dry off (Berry and Hillerton, 2002). In a recent study of organic and conventional dairies, 50% of conventional cows and 34% of organic cows did not have intramammary infections (Mullen et al., 2013) and use of antibiotics on such cows would be contraindicated. Antibiotic resistance has been documented in mastitis pathogens worldwide (Aarestrup and Jensen, 1998) but there is no evidence that antibiotic-resistant

mastitis pathogens have increased in the United States as of nearly a decade ago (Makovec and Ruegg, 2003; Erskine et al., 2004). Regardless, some consumers in the United States indicate a preference for milk produced from cattle raised without the use of antibiotics and with pasture access (Olynk et al., 2010). Organic dairy farms that are certified by the United States Department of Agriculture (**USDA**) meet those specifications. Milk that specifically has the USDA organic seal on its label does result in a willingness of some consumers to pay more for such milk (Dhar and Foltz, 2005; Kiesel and Villas-Boas, 2007).

Organic dairies in the United States are prohibited from using synthetic antibiotics in all cattle, except when antibiotics are required to save the animal's life. If antibiotic therapy is administered, the animal forfeits its organic status and no milk or meat from it can be sold as organic (Electronic Code of Federal Regulations, 2013). Organic certification by the USDA also requires mandatory pasture access for at least 120 d of the year so that cows consume at least 30% of their DMI from pasture during the grazing season. Further, use of synthetic hormones is prohibited in organic systems but use of vaccines is allowed.

The growth of the U. S. organic dairy industry coupled with the presence of mastitis-causing pathogens underlies the need for effective alternatives to antibiotics. The U. S. organic dairy industry is growing rapidly, from just 38,196 cows in 2000 to 249,766 cows in 2008, which represented 2.7% of all dairy cows in the U. S. in 2008 (USDA ERS, 2010). In 2010, organic dairy products represented almost 6% of the entire U. S. dairy market (Organic Trade Association, 2011). According to a 2009 USDA report, the U. S. organic market is growing more rapidly than domestic production can supply (Greene et al., 2009). This rapid

growth is coupled with a growing need for alternatives to antibiotics, as U. S. organic dairies have similar (Mullen et al., 2013) or higher (Pol and Ruegg, 2007a) overall intramammary infection rates compared with conventional dairies. Furthermore, organic dairies face the same common mastitis-causing bacteria, including *Staph. aureus*, *Streptococcus* spp., and CNS. A survey of North Carolina organic farms reported similar prevalence of *Staph. aureus*, *Streptococcus* spp., CNS, and *Corynebacterium* spp. in organic and conventional herds (Mullen et al., 2013). A study conducted on dairies with a 6-mo average bulk tank SCC over 250,000 cells/mL recorded significantly higher prevalence of *Staph. aureus*, *Streptococcus* spp., CNS, and *Strep. agalactiae* in organic herds compared with conventional herds (Pol and Ruegg, 2007a). Because organic dairies have similar or higher prevalence of mastitis-causing pathogens, effective alternatives to antibiotics must be available for treating mastitis in organic production systems.

Organic dairies have been reported to use a wide variety of treatments for mastitis, from *Aloe vera* to vitamin supplements (Pol and Ruegg, 2007b). Peer-reviewed clinical efficacy studies evaluating those alternatives are scarce (Ruegg, 2009). However, there are anecdotal reports on the efficacy of plant essential oils for treatment of mastitis in dairy cattle (Karreman, 2007) and components of several plant oils have shown inhibitory effects against mastitis-causing pathogens in vitro (Baskaran et al., 2009).

Plant oils are the main ingredients in one intramammary product, Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), labeled for improvement of milk quality and approved for use in organic production by the Ohio Ecological Food and Farm Association (Columbus,

OH). Phyto-Mast has some effectiveness in curing infections during the dry period when used as dry cow therapy (Mullen et al., in review). This antibacterial action is likely due to the essential oil of *Thymus vulgaris* (thyme) included in the formula. Essential oil of thyme has strong antibacterial activity (Cowan, 1999; Kalemba and Kunicka, 2003) and contains a phenolic molecule called thymol that has strong activity versus gram-negative bacteria (Helander et al., 1998) and common mastitis-causing pathogens (Baskaran et al., 2009). The plant oil ingredients of Phyto-Mast and their proposed biological activity are given in Table 4.1.

Because Phyto-Mast has been evaluated as a dry cow therapy, we investigated the antimicrobial activity of each herbal component of Phyto-Mast in milk for potential future application as a dry cow therapy or for treatment of mastitis during lactation. Antimicrobial activity was assessed versus *Staphylococcus aureus*, *Staphylococcus chromogenes*, and *Streptococcus uberis*.

MATERIALS AND METHODS

Source and Preparation of Bacterial Cultures

One isolate each of *Staph. aureus*, *Staph. chromogenes*, and *Strep. uberis* were obtained from mastitis cases on North Carolina dairy farms diagnosed by the Milk Quality and Mastitis Laboratory at the College of Veterinary Medicine at North Carolina State University. Isolates were plated on Trypticase soy agar with 5% sheep blood (**TSA**) (Becton, Dickinson and Company, Franklin Lakes, NJ). The purity of each culture was confirmed by

gram-stain, morphology on mannitol salt agar (*Staph. aureus* and *Staph. chromogenes*), and morphology on blood agar (*Strep. uberis*). Bacteria were grown on TSA plates for 18 h in preparation for testing, then kept in the refrigerator for the duration of the experiment. For each replication of each treatment tested, 1 colony was removed from the refrigerated plate and placed into 3 mL of autoclaved Mueller-Hinton broth. Bacteria were grown to the midpoint of log phase (6 h for *Staph. aureus* and *Staph. chromogenes* (Fujikawa and Morozumi, 2006), 3 h for *Strep. uberis* (Almeida and Oliver, 1993) before beginning the experiment.

Herbal Oils

Canola oil extractions of 4 different herbs and essential oil of thyme were obtained from Herbal Vitality, Inc. (Sedona, AZ). The 4 herbs included *Angelica dahuricae*, *Angelica sinensis*, *Gaultheria procumbens* (wintergreen), and *Glycyrrhiza uralensis* (Chinese licorice). The plant oils were kept refrigerated in brown glass bottles to prevent light degradation or volatilization of the oils.

Experimental Design

The herbal oils were tested at concentrations of 0.5%, 1%, 2%, and 4% (vol/vol). These concentrations would theoretically be achievable in the pre-dry off udder quarter provided that milk production was between 0.30 and 2.45 kg/quarter/d (0.67 to 5.4 lb/quarter/d). Because of the known antibacterial activity of thyme essential oil, it was tested by itself and at concentrations of 1%, 2%, and 3% (vol/vol). The other 4 herbal oils were tested alone and in all possible combinations for a total of 15 different treatments at each of

the total concentrations tested (0.5%, 1%, 2%, and 4%). The combination treatments were prepared by mixing equal amounts of each of the oils to a total volume of 5 mL in a 10 mL vial. For the 6 combinations of just two oils, 2.5 mL of the two oils were added to a 10 mL vial resulting in a mixture that contained 50% (vol/vol) of each of the oils. For the 4 three-oil combinations, 1.67 mL of each of the 3 oils was combined in a 10 mL vial resulting in a mixture that contained 33% (vol/vol) of each of the oils. For the four-oil combination, 1.25 mL of each of the oils was added to a 10 mL vial, resulting in a mixture containing 25% (vol/vol) of each of the oils. To ensure all treatments had the same conditions pre-testing, 5 mL of each of the oils (including thyme essential oil) were pipetted into their own 10 mL vial. Oil-containing vials were kept sealed and refrigerated when not in use. All oil-containing vials were vortexed for 15 s before beginning each trial.

Several controls were included in every testing of each treatment. The two negative controls were milk alone, to ensure that pasteurization was successful, and milk + bacteria + penicillin-streptomycin, to make sure that the tested bacteria were sensitive to antibiotics and to have the same antibiotic control as was used in the dry cow therapy study (Mullen et al., 2012). The milk + bacteria + penicillin-streptomycin treatment was tested at 1% and 5% concentrations. Phyto-Mast was also tested at 1, 2, 3, and 4% concentrations. Canola oil was tested at 1% and 70% concentrations to determine if it had an antibacterial effect without the herbal extract. The positive control was milk + bacteria, to ensure that the bacteria could grow in the milk. Three replicates were run of every concentration of every treatment and control for each bacteria tested. Replicates were randomized by date, bacteria, and treatment

to minimize experimental bias.

Antibacterial Activity Testing

The herbal oils were tested using a modified Clinical and Laboratory Standards Institute protocol for broth dilution testing (Clinical and Laboratory Standards Institute, 2008). Whole ultra high-temperature pasteurized and homogenized organic milk was purchased from a grocery store and used instead of Mueller-Hinton broth as the growth medium.

Vials were prepared for testing each control and treatment by first adding a calculated volume of milk, then adding the volume of treatment solution required to establish the percent by volume to be tested (0.5%, 1%, 2%, or 4%). Following addition of treatments to milk, vials were vortexed for 1 min 30 s. Ten microliters of the inoculated Mueller-Hinton broth were added, then the test vials were vortexed for another 15 s and placed in an incubator at 37° C for 24 h. Test vials contained a total of 1 mL of liquid.

Following incubation, vials were vortexed for 15 s. Serial dilution was used to determine bacterial counts, using a 0.1 mL aliquot from the vial and sterile 0.85% saline solution to create 8 10-fold dilutions. The dilutions were plated on eighths of a TSA plate and incubated for 24 h at 37° C. Colony forming units of all dilutions were recorded. Colony counts from the 1:1 x 10⁸ dilution were used in the analysis, as colonies were clearly separated and countable (<100 colonies) for all iterations of the experiment at this dilution.

Results are reported as growth of bacteria in the treatment sample relative to the growth of the milk + bacteria control. Treatment and concentration combinations were only

considered successful at reducing bacterial growth if at least 2 replications resulted in reduction of bacterial growth compared to the control, and the third either resulted in reduction of bacterial growth or had a similar amount of colony forming units as the control. Treatment and concentration combinations that did not reduce bacterial growth compared with the control were defined as those that had similar or more bacterial growth present than contemporary controls in all 3 replications of the experiment.

RESULTS

Both concentrations of penicillin-streptomycin were bactericidal in all replications of all bacteria tested, resulting in no detectable bacterial growth after the final incubation. Every test run of milk only also resulted in no bacterial growth for all replications of all bacteria. The positive control, milk + bacteria, had bacterial growth for all replications of all bacteria. All replications of canola oil at both 1% and 70% had no effect on bacterial growth.

Efficacy against Staphylococcus aureus

Thyme oil killed all bacteria present at concentrations of 2 and 3% (Figure 4.2). Other treatments that reduced growth of *Staph. aureus* compared to control included *Angelica dahuricae* + *Angelica sinensis* + *Glycyrrhiza uralensis* at 2%, *Angelica dahuricae* at 1%, *Angelica sinensis* + *Gaultheria procumbens* + *Glycyrrhiza uralensis* at 1%, and *Gaultheria procumbens* + *Glycyrrhiza uralensis* at 2% (Figure 4.1).

Treatments that did not significantly reduce bacterial growth relative to the control sample of *Staph. aureus* included *Gaultheria procumbens* at 2%, *Angelica dahuricae* +

Angelica sinensis at 0.5 and 2%, *Angelica dahuricae* + *Angelica sinensis* + *Gaultheria procumbens* + *Glycyrrhiza uralensis* at 0.5, 1, and 4%, and Phyto-Mast at 1 and 4%.

Efficacy against Staphylococcus chromogenes

Thyme oil at 2 and 3% was bactericidal, preventing *Staphylococcus chromogenes* from multiplying and killing all bacteria that were inoculated into the thyme-containing vials (Figure 4.2). Treatments that successfully reduced growth of *Staph. chromogenes* included *Angelica dahuricae* + *Glycyrrhiza uralensis* at 1%, Phyto-Mast at 2%, *Gaultheria procumbens* + *Glycyrrhiza uralensis* at 1%, *Gaultheria procumbens* at 0.5%, *Angelica dahuricae* + *Angelica sinensis* + *Glycyrrhiza uralensis* at 4%, and *Angelica dahuricae* + *Gaultheria procumbens* + *Glycyrrhiza uralensis* at 1%.

Treatments that did not significantly reduce bacterial growth relative to the control sample of *Staph. chromogenes* included *Angelica sinensis* at 0.5%, *Glycyrrhiza uralensis* at 2%, *Angelica dahuricae* + *Glycyrrhiza uralensis* at 0.5%, and Phyto-Mast at 1%.

Efficacy against Streptococcus uberis

Thyme oil at 2 and 3% was bactericidal versus *Streptococcus uberis* and the 1% concentration also reduced growth of *Strep. uberis* significantly (Figure 4.2, Table 4.2). The other treatment that successfully reduced growth of *Strep. uberis* was *Angelica sinensis* + *Gaultheria procumbens* at 1% (Table 4.2).

Treatments that did not reduce bacterial growth relative to the control sample of *Streptococcus uberis* included *Angelica dahuricae* at 4%, *Angelica dahuricae* + *Angelica sinensis* at 2%, *Angelica dahuricae* + *Gaultheria procumbens* at 2 and 4%, *Angelica*

dahuricae + *Glycyrrhiza uralensis* at 1 and 4%, *Angelica sinensis* + *Glycyrrhiza uralensis* at 1%, *Gaultheria procumbens* + *Glycyrrhiza uralensis* at 1%, *Angelica dahuricae* + *Angelica sinensis* + *Gaultheria procumbens* at 2%, and *Angelica dahuricae* + *Angelica sinensis* + *Glycyrrhiza uralensis* at 0.5 and 2%.

DISCUSSION

Consumer concerns over antibiotic usage in livestock and the growth of the organic dairy industry encourage the development of alternatives to antibiotics for use in dairy cows. Organic dairy producers use alternatives to antibiotics (Pol and Ruegg, 2007b) because they face challenges with mastitis similar to those of conventional dairy producers. Scientific evidence demonstrating the efficacy of these alternative treatments is lacking (Ruegg, 2009). The current study measured the antibacterial activity of plant-derived oil components of an herbal product against mastitis-causing pathogens.

The broth dilution method for assessing antibacterial efficacy is the most consistent method for assessment of different essential oils (Hood et al., 2003). However, using broth for testing compounds that ultimately may be used as intramammary dry cow therapy does not accurately simulate the environment where the compounds will function or work. Whole milk was used in this experiment to simulate the environment of the udder. It is suspected that the lipids found in whole milk have the hydrophobic properties that may bind or decrease the antimicrobial properties of essential oils against mastitis-causing pathogens (Burt, 2004). This potential association between the herbal oils and the milk fat, as well as

other lipophilic molecules in milk, is the reason that whole milk was chosen as the in vitro model for evaluating the antibacterial activity of the herbal oil ingredients of Phyto-Mast.

Herbal essential oils are promising alternatives to antibiotics because of their availability, biodegradability, and lower risk of side effects compared to traditional pharmaceutical preparations (Kalemba and Kunicka, 2003). However, the results of the current study indicate that not all plant oils are valuable as antimicrobial agents for mastitis treatments (from an antibacterial perspective). Only thyme oil had consistent antibacterial activity against all pathogens tested, and only at 2% or greater concentration. Although some of the other treatments demonstrated antibacterial activity at certain concentrations, this activity was not seen at the highest concentrations and thus does not follow a typical antibiotic dose-response curve, where an increasing dosage would have a greater antibacterial effect. In one case, different concentrations of the same treatment (*Angelica dahuricae* + *Glycyrrhiza uralensis*) both seemed to promote and to prevent bacterial growth. There was no expectation that any of the oils tested would actually promote bacterial growth. However, the testing method used resulted in large variations in the amount of control bacterial growth among replications of the experiment. As such, even those treatments reported to promote bacterial growth relative to their respective control did not have more growth than what was seen in at least some of the controls observed throughout the experiment. Comparisons between the plant-derived oils used in this experiment and those listed in the literature are difficult to make because typically extractions of the plants' oils use the more potent steam-distilled essential oils (Kalemba and Kunicka, 2003). All of the oils used in this experiment

(except for thyme essential oil) were extracted into canola oil. However, the plant-derived oils that had inconsistent antibacterial activity in the current study have been reported to have other biological effects as noted in Table 4.1.

The proprietary composition of Phyto-Mast was not made available to the authors for the purposes of this research, and so the concentrations of ingredients tested here may not be representative of the full formula of Phyto-Mast. Though this study determined that most of the plant-derived oil components of Phyto-Mast did not have consistent antibacterial activity, further research is needed to determine if thyme essential oil maintains its documented antibacterial activity when combined with the other plant-derived oils. Since this research has been completed, the formula of Phyto-Mast has been altered to include more thyme essential oil (H. Karreman, personal communication).

Thyme essential oil contains several different molecules with documented antibacterial activity (Cowan, 1999). Thymol has been examined in milk against mastitis-causing pathogens *in vitro* with strong antibacterial activity around 1% concentration (vol/vol) (Baskaran et al., 2009). Thymol and the caffeic acids contained in thyme essential oil are thought to inhibit enzyme function as one mode of action (Cowan, 1999). Because thyme oil possesses multiple different antibacterial molecules, it is important to look at the activity of the plant oils as a whole because of the potential synergistic activity of the component molecules (Raffa and Pergolizzi, 2011). Thyme oil shows promise as an alternative to synthetic antibiotics. The pharmacokinetics of thymol in reference to its food safety have been examined in dairy goats: after Phyto-Mast administration in the udder,

thymol residues were detectable up to 4 h post-administration in plasma and up to 24 h post-administration in milk (McPhee et al., 2011). Further research is needed to establish the effect of thyme oil on the mammary gland after infusion to ensure the safety of the cow receiving treatment, especially for multiple or repeated dosing as is typically used with intramammary infections. Based on our research, thyme essential oil concentrations in the range of 2 to 3% (vol/vol) in milk appear to have antibacterial activity against some mastitis-causing pathogens. Further research is necessary to determine if the addition of other plant-derived oils to thyme essential oil affects the antibacterial activity of thyme essential oil.

CONCLUSIONS

Essential oil of thyme at 2 and 3% exhibits consistent antibacterial activity against the three mastitis-causing pathogens tested (*Staph. aureus*, *Staph. chromogenes*, and *Strep. uberis*). Some concentrations of other tested plant-derived oils and oil combinations had antibacterial activity, but none demonstrated a typical dose response curve of increasing antibacterial efficacy at increasing plant-derived oil concentrations. Further investigation into the role of these and other plant oils as mastitis therapies is recommended.

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Table 4.1. Phyto-Mast¹ plant oil ingredients and bioactivity

Ingredient	Common name	Bioactivity	References
<i>Angelica dahuricae</i>	Bai zhi	Anti-inflammatory	Kang et al., 2008
<i>Angelica sinensis</i>	Chinese angelica	Immunomodulatory	Liu et al., 2003
<i>Gaultheria procumbens</i>	Wintergreen	Analgesic	Poppenga, 2002
<i>Glycyrrhiza uralensis</i>	Chinese licorice	Anti-inflammatory	Abe et al., 2003; Kai et al., 2003, Genovese et al., 2009
<i>Thymus vulgaris</i>	Thyme	Antibacterial	Helander et al., 1998; Kalemba and Kunicka, 2003; Tsai et al., 2011

¹Intramammary herbal preparation for improving milk quality; Penn Dutch Cow Care, Narvon, PA.

Table 4.2: Plant-derived oils and combinations of oils effective in reducing mastitis-causing bacterial growth in milk in vitro¹

¹Plant oils tested are essential oils (*T. vulgaris*) or canola oil extracts (*A. dahuricae*, *A. sinensis*, *G. procumbens*, *G. uralensis*) of plants. The table includes only those concentrations of treatments that reduced bacterial growth in all three replications as compared to a milk + bacteria control. Canola oil by itself had no antibacterial action when tested at 1% and 70% vol/vol.

²*A. dahuricae* = *Angelica dahuricae*; *A. sinensis* = *Angelica sinensis*; *G. procumbens* = *Gaultheria procumbens* (wintergreen); *G. uralensis* = *Glycyrrhiza uralensis* (Chinese licorice); *T. vulgaris* = *Thymus vulgaris* (thyme).

³As compared to a milk + bacteria control sample. Means reported are the average of three repetitions of each treatment and concentration combination \pm SE.

⁴Phyto-Mast contains all of the listed plant oils and is an intramammary herbal preparation used for improving milk quality (Penn Dutch Cow Care, Narvon, PA).

Table 4.2 Continued

Bacteria	Treatment ²	Concentration (% vol/vol)	Percentage of Control Bacterial Growth ³
<i>Staph. aureus</i>	<i>A. dahuricae</i> , <i>A. sinensis</i> , <i>G. uralensis</i>	2	71 ± 15
	<i>A. dahuricae</i>	1	62 ± 20
	<i>A. sinensis</i> , <i>G.</i> <i>procumbens</i> , <i>G. uralensis</i>	1	56 ± 29
	<i>G. procumbens</i> , <i>G.</i> <i>uralensis</i>	2	41 ± 5
	<i>T. vulgaris</i>	2	0 ± 0
		3	0 ± 0
	Penicillin-streptomycin	1	0 ± 0
		5	0 ± 0
<i>Staph. chromogenes</i>	<i>A. dahuricae</i> , <i>G. uralensis</i>	1	93 ± 7
	<i>G. procumbens</i> , <i>G.</i> <i>uralensis</i>	1	93 ± 7
	Phyto-Mast ⁴	2	60 ± 31
	<i>G. procumbens</i>	0.5	47 ± 29
	<i>A. dahuricae</i> , <i>A. sinensis</i> , <i>G. uralensis</i>	4	33 ± 33
	<i>A. dahuricae</i> , <i>G.</i> <i>procumbens</i> , <i>G. uralensis</i>	1	33 ± 33
	<i>T. vulgaris</i>	2	0 ± 0
		3	0 ± 0
	Penicillin-streptomycin	1	0 ± 0
		5	0 ± 0
<i>Strep. uberis</i>	<i>A. sinensis</i> , <i>G. procumbens</i>	1	65 ± 24
	<i>T. vulgaris</i>	1	8 ± 8
		2	0 ± 0
		3	0 ± 0
	Penicillin-streptomycin	1	0 ± 0
		5	0 ± 0

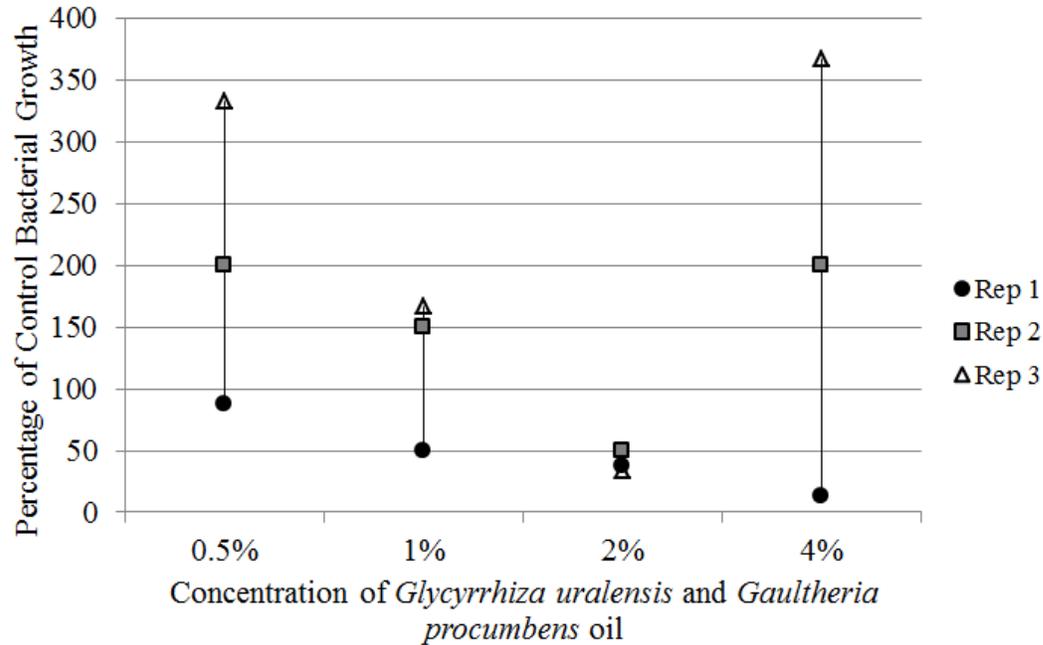


Figure 4.1: Growth of *Staphylococcus aureus* in organic milk treated with varying concentrations of *Glycyrrhiza uralensis* (Chinese licorice) and *Gaultheria procumbens* (wintergreen) oils. Plant oils were extracted into canola oil and then added to whole ultra high-temperature pasteurized and homogenized organic milk. The 2-combination oil extract (*Glycyrrhiza uralensis* and *Gaultheria procumbens*) was tested at equal concentrations of both oils at a total of 0.5%, 1%, 2%, and 4% in milk (vol/vol) to simulate potentially achievable levels in the pre-dry off udder quarter. Three replicates were performed at each concentration level.

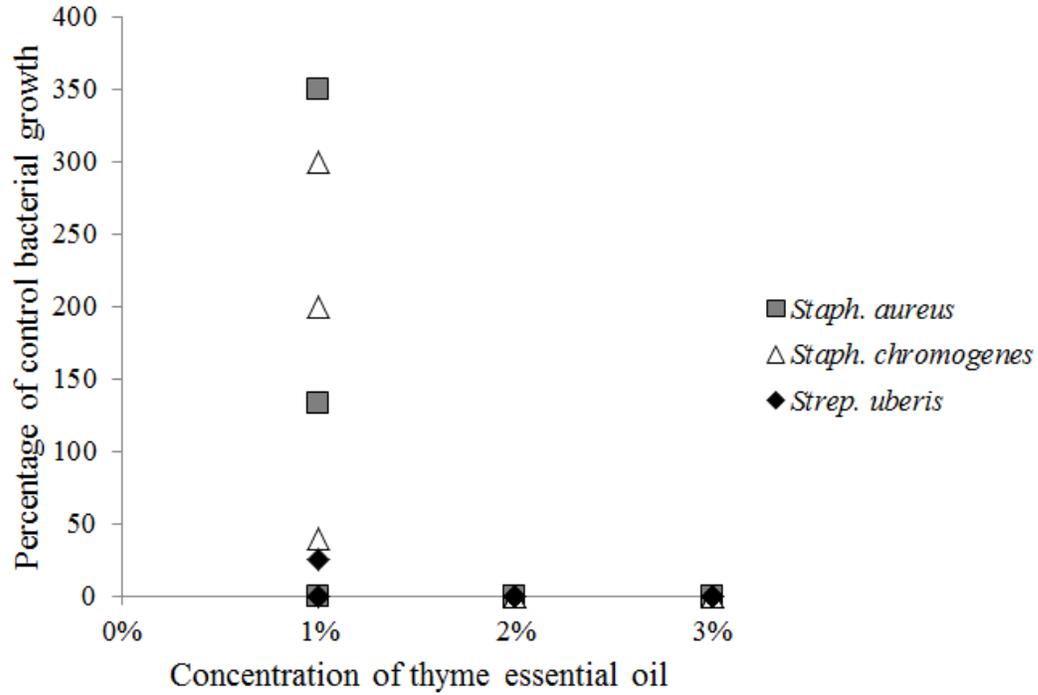


Figure 4.2. Growth of 3 mastitis-causing bacteria treated with varying concentrations of thyme (*Thymus vulgaris*) essential oil in organic milk (vol/vol). Thyme essential oil was tested at 1%, 2%, and 3% to simulate concentrations achievable in the pre-dry off udder quarter, with three replicates at each concentration level. Whole ultra high-temperature pasteurized and homogenized organic milk was used to grow bacteria. Values are a percentage of the control bacterial growth.

**CHAPTER 5: EFFICACY OF AN HERBAL INTRAMAMMARY PRODUCT AND A
TEAT SEALANT, BOTH ALONE AND IN COMBINATION, WHEN USED AS DRY
COW THERAPY**

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ABSTRACT

Dry cow therapy is used to eliminate existing intramammary infections (IMI) and to prevent new IMI from occurring during the dry period. In conventional herds, dry cow therapy includes intramammary administration of antibiotics, an internal teat sealant, or both. Certified organic dairies in the United States are prohibited from using antibiotics even though they face the same challenges as conventional dairies with regard to mastitis. One herbal intramammary product, Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), contains several plant-derived oils with antibacterial or anti-inflammatory activity, but does not remain in the udder for an extended period of time. The objective of this study was to examine the efficacy of Phyto-Mast as a dry off therapy alone or in combination with an internal teat sealant (Orbeseal; Zoetis, Kalamazoo, MI) compared with internal teat sealant alone or no dry cow therapy. Somatic cell score (SCS) differences (SCS at 5 to 7 d postpartum minus SCS at dry off) were -0.56 ± 0.70 for untreated quarters, -1.72 ± 0.74 for quarters receiving teat sealant, -0.22 ± 0.71 for quarters receiving Phyto-Mast, and -0.87 ± 0.69 for quarters receiving both Phyto-Mast and teat sealant. Teat sealant quarters had a significantly lower SCS difference than Phyto-Mast quarters. Somatic cell scores at 5 to 7 d postpartum were 4.50 ± 0.19 for untreated quarters, 3.66 ± 0.19 for quarters receiving teat sealant, 4.51 ± 0.18 for quarters receiving Phyto-Mast, and 4.55 ± 0.19 for quarters receiving Phyto-Mast and teat sealant. There were no differences among treatments for curing infections during the dry period. Determination of cure rates was made difficult by the low level (10.8%) of infections present at dry off. New infection rate obtained during the dry

period was $22.1 \pm 5.5\%$ in untreated quarters, $6.9 \pm 3.5\%$ in quarters receiving teat sealant, $10.2 \pm 3.8\%$ in quarters receiving Phyto-Mast, and $3.9 \pm 2.4\%$ in quarters receiving Phyto-Mast and teat sealant. Quarters receiving both Phyto-Mast and Orbeseal had significantly fewer new infections than untreated quarters.

INTRODUCTION

The need for non-antibiotic mastitis treatment and prevention is increasing with the growth of the organic dairy industry in the U.S. Between 2006 and 2011, organic fluid milk sales doubled, representing 4% of the total U. S. fluid milk market in 2011 (AMS-USDA, 2012) and 6% of the entire U. S. dairy market in 2010 (Organic Trade Association, 2011). Organic milk commands a premium over conventional milk, making organic production economically desirable to many farmers. Studies have noted that some consumers are willing to pay more for the organic label on milk (Dhar and Foltz, 2005; Kiesel and Villas-Boas, 2007). Producing organic milk is challenging in the U. S. because the use of antibiotics is prohibited on-farm (Electronic Code of Federal Regulations, 2013), despite the fact that organic dairies have a prevalence of mastitis similar to or greater than conventional dairies (Pol and Ruegg, 2007; Cicconi-Hogan et al., 2013, Mullen et al., 2013). In order to control mastitis on their farms, organic producers need proven alternatives to antibiotics for mastitis treatment.

One non-antibiotic dry cow therapy with proven efficacy is an internal teat sealant composed of 65% bismuth subnitrate (Orbeseal; Zoetis, Kalamazoo, MI). The 65% bismuth

subnitrate formula has been significantly more effective than no treatment at reducing the incidence of new infections in the dry period and at calving in cows with low (< 200,000 cells/mL) SCC (Woolford et al., 1998; Huxley et al., 2002; Berry and Hillerton, 2002b). This teat sealant has been available since 1978 and is often used in combination with an antibiotic for dry cow therapy (Woolford et al., 1998; Godden et al., 2003; Bradley et al., 2010). The action of the teat sealant is to form a plug in the teat canal, physically blocking bacteria from entering and it often persists for the duration of the dry period (Woolford et al., 1998).

The use of a teat sealant could also be of assistance on U. S. organic dairy farms, though it is not yet approved for use in organic dairy cattle. Organic dairy farmers use a variety of non-antibiotic strategies for mastitis control and dry cow therapy (Ruegg, 2009; Mullen et al., 2013). One commercially available herbal intramammary product, Phyto-Mast® (Penn Dutch Cow Care, Narvon, PA), has been examined for potential suitability as a dry cow therapy on several farms as compared with conventional antibiotic + teat sealant therapy and was found to have similar efficacy in preventing new infections from occurring during the dry period (Mullen et al. [b], in review). Phyto-Mast is approved for use in organic dairy herds by the Ohio Ecological Food and Farm Association (Columbus, Ohio) and its ingredients comply with the USDA National Organic Standards Board regulations. Phyto-Mast is composed of essential oil of *Thymus vulgaris* (thyme) and canola oil-derivatives of *Angelica dahuricae*, *Angelica sinensis*, *Gaultheria procumbens* (wintergreen), and *Glycyrrhiza uralensis* (Chinese licorice). Essential oil of thyme contains thymol, a molecule with reported antibacterial activity against mastitis pathogens in vitro (Baskaran et al., 2009;

Mullen et al. [a], in review). Thymol was measured in milk and blood of goats administered Phyto-Mast to determine the persistence of Phyto-Mast in the udder. Thymol was detected up to 4 hr post-treatment in blood serum and up to 24 hr in milk (McPhee et al., 2011).

Assuming this persistence translates to dairy cattle, Phyto-Mast does not remain in the udder long enough to be an effective dry cow therapy. However, the use of an internal teat sealant may help Phyto-Mast remain in the udder for a longer period of time.

The objective of the current study was to determine if Phyto-Mast is as effective as a conventional teat sealant alone (Orbeseal), or if the two work synergistically if used together as dry cow therapy.

MATERIALS AND METHODS

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.

Experimental Design

Data were collected from a 100+-cow seasonal calving, pasture-based dairy herd at the Center for Environmental Farming Systems in Goldsboro, NC. That herd contained Holstein, Jersey, and various percentage crosses of those breeds and the calving season was from October of 2012 through January of 2013. Half of the herd has been managed using organic health care standards since 2009, and those cattle graze in transitioned pastures that

can be certified as organic. The other half of the herd receives conventional disease treatments when necessary, including antibiotic treatment of mastitis and antibiotic therapy at dry off. All cows in the herd meet the 30% of DMI organic pasture requirement but they received supplemental concentrates and stored forages that were not organically produced.

A total of 87 cows were used for this study. Treatments were balanced by breed, lactation number, and calving due date. Twenty-two cows received no treatment (**NONE**) at dry off, 21 cows received Orbeseal (**ORB**; 65% bismuth subnitrate; Zoetis, Kalamazoo, MI), 22 cows received Phyto-Mast (**P-M**; Penn Dutch Cow Care, Narvon, PA), and 22 cows received Phyto-Mast and Orbeseal (**P-M+ORB**).

Data Collection and Treatment Application

Duplicate quarter milk samples were collected from all functional teats at the time of dry off. Before sample collection, teats were pre-dipped with 0.5% iodine and wiped with single-use paper towels after 30 s. Teat ends were scrubbed with cotton balls soaked in 70% isopropanol, changing cotton balls until they were visibly clean following teat contact. Duplicate milk samples were collected into pre-labeled 12 mL vials after 1 to 3 squirts of foremilk were discarded. Quarter milk samples were collected into 90 mL vials containing a bronopol tablet preservative for SCC analysis at the United DHIA laboratory (Blacksburg, VA). Following sample collection, teat ends were cleaned again with 70% isopropanol-soaked cotton balls. Treatments were administered by inserting the tip of the product tube into the teat canal and infusing the product, with the exception of ORB. During administration of ORB, the area of the teat just below the udder was pinched to ensure that

ORB did not enter the udder cistern. For cows receiving P-M+ORB, P-M was administered first, then the teat ends were cleaned with 70% isopropanol-soaked cotton balls, then ORB was infused as previously described. Duplicate quarter milk samples were collected at 5 to 7 d postpartum using the same method as for the dry off milk samples.

Milk Microbiology

Assessment of microbiological content of the milk samples was completed in the Milk Quality and Mastitis Laboratory at the College of Veterinary Medicine at North Carolina State University. 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. If one sample was contaminated, the duplicate was cultured. If the duplicate was also contaminated, the quarter result was recorded as contaminated and was not considered in the analysis. If the duplicate was not contaminated, then its result was used in the analysis for the quarter tested.

Definitions

Presence of infection. If a bacterial species was present at ≥ 100 colony forming units/mL, it was recorded as an IMI in the quarter cultured except for CNS, in which case at least 200 colony forming units/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 samples.

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 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 and not as a cure.

No change in IMI status. Quarters infected with the same microorganism postpartum as they were 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. All 4 infection status categories (cure, new IMI, no change in IMI status, no change, still not infected) were mutually exclusive.

Statistical Analyses

All statistical analyses were performed at the quarter level using SAS (version 9.2; Cary, NC). Before model selection, diagnostic tests were conducted 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 $\log_2\left(\frac{SCC}{100,000}\right)+3$ to obtain the base 2 logarithmic transformation as recommended by Shook (1993).

The mixed linear model (MIXED) procedure was used to analyze SCS data and the generalized linear mixed model (GLIMMIX) procedure was used to analyze the microbiological outcomes, because results were recorded as presence or absence of infection

of each pathogen in each quarter. The results of interest for the microbiological outcome models, $\pi_{cure} = P(cure = 1)$ and $\pi_{new} = P(new = 1)$; that is, π is the probability of an infection being cured or newly infected, were modeled using the logit function $Y = \text{logit}\left(\frac{\pi}{1-\pi}\right)$. In all cases, cow was incorporated as a random effect. Cow was incorporated into the model to account for the fact that treatments were assigned on a cow basis, not a quarter basis. Categorical covariates 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 = 1st lactation pre-dry off, group 2 = 2nd lactation pre-dry off, group 3 = 3rd and greater lactation pre-dry off), month of postpartum sampling, quarter, organism group present at dry off (gram-positive, gram-negative, no growth, mixed, or other), and organism group present postpartum. Continuous covariates offered to each model included dry off date, date of postpartum sampling, number of days dry, dry off SCS, postpartum SCS, and the difference between postpartum and dry off SCS (**SCSDiff**). Model selection was performed similar to Arruda et al. (2013), and began with univariate analysis of the aforementioned variables, using cured infection rate, new infection rate, and SCSDiff as dependent variables. Variables were retained in the model if the univariate analysis yielded a *P*-value less than 0.20. Once univariate selection was completed, all main-effect interactions with treatment were included in the model. The final model selection step involved backwards 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.

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

RESULTS

A total of 1,287 quarter samples (675 from dry off, 612 from postpartum) including duplicates were collected from the 87 cows enrolled in this study. Of those quarter samples, 12 were contaminated (1 from dry off, 11 from postpartum). Samples from the same cow taken at dry off and postpartum were paired, and any pairs missing one or both sampling points were removed from the analysis data set. A total of 279 paired quarter samples from 76 cows were included in the analysis. Those 76 cows were evenly distributed among the treatment groups, with 19 cows in each of the 4 treatment groups. Total numbers of quarters assessed from each treatment group were also similar (68 no treatment, 71 ORB, 72 P-M, and 68 P-M+ORB).

Treatments were reasonably balanced by breed in the final data set for analysis. Each of the 4 treatment groups had between 4 and 8 Holstein udder quarters, between 4 and 8 Jersey quarters, and between 55 and 60 crossbred quarters. Average lactation number for cows in this study was 2.6 ± 1.7 , with no significant differences among treatment groups.

Quarter-Level SCS

Mean SCS at dry off for all quarters in the analysis was 3.7 ± 1.5 , ranging from 0.3 to 8.1. Dry off SCS means were 3.70 ± 0.19 for NONE, 3.73 ± 0.19 for ORB, 3.29 ± 0.18 for

P-M, and 4.31 ± 0.19 for P-M+ORB. Quarters receiving P-M treatment had significantly lower SCS than P-M+ORB quarters ($P = 0.001$), but there were no significant differences among any other treatments for SCS at dry off. Quarters that were infected at dry off had an SCS of 5.35 ± 0.29 at dry off, whereas quarters with no infections present at dry off had SCS of 3.57 ± 0.10 . Cows with gram-positive infections or mixed infections at dry off had similar dry off SCS (5.36 ± 0.30 and 5.20 ± 1.45 , respectively). The SCS at dry off of quarters with infections was significantly higher ($P < 0.001$) than the SCS at dry off of uninfected quarters.

Somatic cell score postpartum for all quarters in the analysis was 4.3 ± 1.5 , ranging from -0.2 to 8.8. This range, in SCC, is 4,000 cells/mL to 33,000,000 cells/mL. Postpartum SCS means were 4.50 ± 0.19 for NONE, 3.66 ± 0.19 for ORB, 4.51 ± 0.18 for P-M, and 4.55 ± 0.19 for P-M+ORB. Quarters receiving no treatment had significantly higher SCS than ORB quarters ($P = 0.009$), and the postpartum SCS of ORB quarters was significantly lower than the SCS of P-M quarters ($P = 0.007$) and P-M+ORB quarters ($P = 0.005$). Quarters with gram-positive infections at dry off had a postpartum SCS of 5.29 ± 0.30 , quarters with mixed infections at dry off had a postpartum SCS of 3.66 ± 1.51 , and quarters with no IMI at dry off had SCS of 4.20 ± 0.10 . The postpartum SCS of quarters with gram-positive infections was significantly higher than the postpartum SCS of quarters uninfected at dry off.

SCS Difference. Mean SCSDiff was -0.56 ± 0.70 for NONE, -1.72 ± 0.74 for ORB, -0.22 ± 0.71 for P-M, and -0.87 ± 0.69 for P-M+ORB. Treatment ($P = 0.041$), month of postpartum sampling ($P = 0.005$), and organism group present at dry off ($P = 0.002$) were significant in predicting SCSDiff. The interaction of treatment and month of postpartum sampling was not

significant in the model ($P = 0.185$), but was examined to assess the significance of month of postpartum sampling (Table 5.1). ORB had a significantly lower SCSDiff than P-M ($P = 0.030$). There were no other significant differences among treatments. Only ORB had a SCSDiff significantly different from 0. Mean SCSDiff was -0.45 ± 0.63 for quarters of cows that calved in October, -0.45 ± 0.66 for quarters of cows that calved in November, -2.12 ± 0.69 for quarters of cows that calved in December, and -0.36 ± 1.02 for quarters of cows that calved in January. Quarters of cows calving in December had significantly lower SCS difference than cows calving in October or November. The differences in each treatment by month of postpartum sampling are given in Table 5.1. Quarters of cows calving in December and receiving the P-M+ORB treatment had a very low SCSDiff relative to the other combinations of treatment and freshening month. Those quarters of December-calving cows had a higher dry off SCS (4.6 ± 1.7) in comparison with quarters of cows calving in other months (dry off SCS range 3.3 ± 1.4 to 3.7 ± 1.4). Quarters with a gram-positive infection at dry off had a SCSDiff of -0.67 ± 0.47 , quarters with mixed infections at dry off had a SCSDiff of -2.51 ± 1.64 , and quarters with no evidence of IMI at dry off had a SCSDiff of 0.65 ± 0.26 . Quarters with gram-positive infections at dry off had a significantly lower SCSDiff than quarters uninfected at dry off.

Milk Microbiology

IMI at Dry Off. Of all 279 quarters in the analysis, only 30 (10.8%) were infected at dry off. This included 11 NONE quarters (16% of all NONE quarters), 5 ORB quarters (7% of all ORB quarters), 5 P-M quarters (7% of all P-M quarters), and 9 P-M+ORB quarters

(13% of all P-M+ORB quarters). The majority of infections present at dry off were caused by gram-positive organisms (97%), with the remainder of infections caused by multiple organisms (Table 5.2). No quarters were infected with gram-negative organisms at dry off.

IMI at 3 to 5 Days Postpartum. The total number of infected quarters was 47 (16.8% of all quarters in the analysis). Fourteen of those quarters (5% of all quarters) had the same infection present postpartum as they did at dry off. Most infected quarters were in NONE cows (24 quarters, or 35% of all NONE quarters), followed by P-M (11 quarters, or 15% of all P-M quarters), ORB (7 quarters, or 10% of all ORB quarters), and P-M+ORB (5 quarters, or 7% of all P-M+ORB quarters). The majority of postpartum infections were caused by gram-positive organisms (40 quarters, or 85%), followed by multiple organisms (6 quarters, or 13%) and gram-negative organisms (1 quarter, or 2%).

Effect of Treatment on Probability of Cure. The final model for predicting proportion of quarters cured only contained treatment as a predictor. There were no significant differences among treatments for apparent ability to cure infections. Least squares means \pm SE for cure rates were $9.1 \pm 9.3\%$ in untreated quarters, $40.0 \pm 23.5\%$ in ORB quarters, $40.0 \pm 23.5\%$ in P-M quarters, and $77.8 \pm 14.9\%$ in P-M+ORB quarters.

Effect of Treatment on Probability of New Infection Postpartum. Proportion of quarters with new infections were $22.1 \pm 5.5\%$ for NONE, $6.9 \pm 3.5\%$ for ORB, $10.2 \pm 3.8\%$ for P-M, and $3.9 \pm 2.4\%$ for P-M+ORB. The combination treatment, P-M+ORB, had a significantly ($P = 0.030$) lower rate of new infection than in untreated quarters. Treatment ($P = 0.01$) and somatic cell score at 5 to 7 d postpartum ($P = 0.008$) were used to predict the

proportion of newly infected quarters. Quarters with a higher SCS at 5 to 7 d postpartum had a higher probability of having a new infection.

DISCUSSION

Dry cow therapy is an important part of an effective mastitis management protocol. However, concern over antibiotic usage in livestock and a growing organic dairy industry prompt exploration of alternatives to antibiotics for dry cow therapy. The current study examined two alternatives to antibiotics as dry cow therapy, both alone and in combination. This study used all cows in a research herd in order to more closely mimic a commercial dairy farm than many dry cow studies previously performed that only used cows or herds with SCC < 250,000 cells/mL. Somatic cell counts on organic and conventional dairies in the U. S. are variable and not always below 250,000 cells/mL. In North Carolina, bulk tank SCC for organic and conventional dairies have been reported to range from 83,000 cells/mL to 500,000 cells/mL (Mullen et al., 2013). Organic and conventional dairies compared in New York, Oregon, and Wisconsin also had a large range of bulk tank SCC, from 41,000 cells/mL to 724,000 cells/mL (Cicconi-Hogan et al., 2013).

When evaluating new dry cow therapies, it is important to assess the impact on the mammary gland as it is very susceptible to irritation (Sanderson, 1966) and more so during the dry period (Oliver and Sordillo, 1988). In the present study, SCC was assessed at the quarter level at dry off and 5 to 7 d postpartum to determine if the tested dry cow therapies had an effect on the mammary gland. Somatic cell count is frequently elevated during the

immediate postpartum period, but reduces as quickly as 3 d postpartum (Barkema et al., 1999), with most of the reduction in SCC occurring during the first 2 wk postpartum (Dohoo, 1993). The desirable outcome of SCSDiff is a negative value, which indicates that SCS was reduced during the dry period likely due to the treatment administered. All treatment groups tested including no treatment had a negative SCSDiff from dry off to postpartum. Because none of the treatments had a SCSDiff significantly different from untreated cows, the authors conclude that the treatments had no significant positive or negative effects on the underlying level of udder inflammation. There was a clear advantage in SCS difference to calving in December, likely due to the higher SCS at dry off in December-calving cows compared with cows calving in other months. The higher SCS in December-calving cows at dry off could have made it easier to achieve a larger difference in SCS from dry off to the postpartum sampling. Using the same logic, the apparent advantage in SCSDiff of cows infected at dry off versus cows uninfected at dry off can be attributed to the relatively high SCS of cows infected at dry off.

The low rate of infection at dry off (11% of all samples) made it difficult to assess the ability of each treatment to cure infections. However, there was sufficient power to detect differences between NONE and P-M+ORB. Effective dry cow therapies typically eliminate more infections than the number spontaneously cured (Smith et al., 1967a; Smith et al., 1967b; Berry and Hillerton, 2002a; Bhutto et al., 2011), which was not seen in this study. Previously, the addition of an internal teat sealant to antibiotic therapy conferred no advantage in bacteriological cure rates compared to use of antibiotic therapy alone (Woolford

et al., 1998; Godden et al., 2003; Bradley et al., 2010). This could be related to the observation that quarters infused with an oil-based intramammary antibacterial product as well as an internal teat sealant had lower retention of the teat sealant at the end of the dry period than cows treated with the teat sealant alone (Bradley et al., 2010). Phyto-Mast has been evaluated as a dry cow therapy, but did not have significantly greater effectiveness at curing infections than no dry cow therapy with or without an herbal teat sealant (Mullen et al., [b] in review). The cure rate of P-M + ORB was numerically higher than that of P-M alone and ORB alone, and may be significant if examined again in a herd with a higher rate of infection at dry off. The cure rate of P-M + ORB was very close (78%) to the 80-90% cure rates reported for antibiotic and teat sealant dry cow therapy (Godden et al., 2003; Cook et al., 2005; Bradley et al., 2010).

Teat sealants have traditionally been used to serve as a physical plug to prevent infections from occurring during the dry period, to augment or replace the keratin plug that would naturally form at cessation of lactation. All treatments tested in this study had a numerically lower rate of new infections than no dry cow therapy. Use of a teat sealant alone as dry cow therapy typically significantly reduces new infection rate compared with no dry cow therapy (Woolford et al., 1998; Berry and Hillerton, 2002b; Cook et al., 2005) and the combination of teat sealant and antibiotic dry cow therapy has been reported to have a lower incidence of new infections at calving than antibiotic dry cow therapy alone (Berry and Hillerton, 2007). Here, the only treatment that had a lower rate of new infections than no treatment was the combination of Phyto-Mast and Orbeseal. Phyto-Mast contains plant-

derived oils with known antibacterial and anti-inflammatory effects (Mullen et al., in review [b]), but only remains in milk for up to 24 hr post-infusion (McPhee et al., 2011). Perhaps the teat sealant action of Orbeseal was enough to keep the Phyto-Mast in the udder and prevent infections from occurring, assuming that Phyto-Mast's short duration in the udder is due to it leaking from the udder rather than being internalized and processed by the cow. Further research comparing the combination of Phyto-Mast and Orbeseal to conventional antibiotic and teat sealant therapy is recommended to determine how the herbal treatment combined with an internal teat sealant compares with a more frequently used antibiotic and internal teat sealant therapy. In addition, research on more dairy farms will evaluate the efficacy of these treatments on a variety of dairies with variable mastitis levels and have greater power to detect differences among treatments.

CONCLUSIONS

Orbeseal was more successful than Phyto-Mast or no treatment at reducing SCS during the dry period. All three dry cow therapies did not significantly change SCS relative to no treatment. Ability of treatments to cure infections during the dry period was not significantly better than no treatment, but this analysis was limited by the low initial infection rate. The combination of Phyto-Mast and Orbeseal was significantly more effective than no treatment at preventing new infections, despite neither treatment alone being more effective than no treatment. Further research is recommended to establish the efficacy of these treatments in multiple herds with varying rates of infection.

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Table 5.1: Somatic cell score difference between freshening and dry off for quarters receiving no treatment, teat sealant, an herbal intramammary product, or the combination of the herbal product and teat sealant

Treatment ¹	Fresh Month ²	SCS Difference	Significance ³
No Treatment	OCT (n = 30)	0.08 ± 0.75	AB
No Treatment	NOV (n = 24)	-0.95 ± 0.84	ABC
No Treatment	DEC (n = 14)	-1.10 ± 0.96	ABC
Orbeseal	OCT (n = 35)	-1.65 ± 0.77*	BC
Orbeseal	NOV (n = 24)	-0.94 ± 0.90	ABC
Orbeseal	DEC (n = 12)	-2.70 ± 1.11*	BC
Phyto-Mast	OCT (n = 36)	-0.09 ± 0.75	AB
Phyto-Mast	NOV (n = 20)	0.84 ± 0.90	A
Phyto-Mast	DEC (n = 12)	-1.23 ± 1.04	ABC
Phyto-Mast	JAN (n = 4)	-1.32 ± 1.63	ABC
Phyto-Mast and Orbeseal	OCT (n = 8)	1.41 ± 1.29	AB
Phyto-Mast and Orbeseal	NOV (n = 24)	-0.60 ± 0.88	AB
Phyto-Mast and Orbeseal	DEC (n = 25)	-2.84 ± 0.82*	C
Phyto-Mast and Orbeseal	JAN (n = 11)	0.16 ± 1.07	AB

¹No Treatment = cows received no dry cow therapy; Orbeseal = 65% bismuth subnitrate (Zoetis, Kalamazoo, MI); Phyto-Mast = an herbal intramammary product for improving milk quality (Penn Dutch Cow Care, Narvon, PA); Phyto-Mast and Orbeseal = cows received Phyto-Mast first, then their teats were sealed using Orbeseal.

²Fresh Month = month in which samples were collected 5 to 7 days postpartum.

³Treatment and month combinations with different letters differ significantly ($P < 0.01$).

*SCS difference means different from zero ($P < 0.05$).

Table 5.2. Frequency of IMI in quarters infected at dry off and at 5 to 7 DIM in the subsequent lactation in a comparison of three non-antibiotic dry cow therapies and no dry cow therapy on a pasture-based research herd in North Carolina¹

	Infection present at dry off				Infection present 5 to 7 d postpartum			
	NONE	ORB	P-M	P-M+ORB	NONE	ORB	P-M	P-M+ORB
Gram-positive								
CNS	6	1	4	3	13	1	6	1
<i>Enterococcus</i> spp.	0	0	0	0	1	0	0	0
<i>Staph. aureus</i>	3	3	1	2	3	3	1	2
<i>Strep.</i> spp. (not <i>agalactiae</i>)	0	1	0	4	4	3	0	2
<i>Nocardia</i> spp.	1	0	0	0	0	0	0	0
Total gram-positives	10	5	5	9	21	7	7	5
Gram-negative								
<i>Serratia marcescens</i>	0	0	0	0	1	0	0	0
Other								
Mixed infections	1	0	0	0	2	0	4	0
Total infected quarters	11	5	5	9	24	7	11	5
Uninfected quarters	57	66	67	59	44	64	61	63
Total of all quarters	68	71	72	68	68	71	72	68

¹ NONE = No treatment; ORB = Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); P-M = Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), an herbal intramammary product for improving milk quality; P-M+ORB = Phyto-Mast and Orbeseal. Numbers presented are frequencies of all observed quarters receiving that specific treatment that were infected with that specific organism.

Table 5.3: Proportion of IMI cured by an herbal product, teat sealant, herbal product + teat sealant, or no dry cow therapy during the dry period in a pasture-based research herd in North Carolina¹

Organism	Treatment			
	NONE ²	ORB	P-M	P-M+ORB
Gram-positive				
CNS	1/6	1/1	2/4	3/3
<i>Nocardia</i> spp.	0/1	0/0	0/0	0/0
<i>Staphylococcus aureus</i>	0/3	0/3	0/1	1/2
<i>Streptococcus</i> spp. (not <i>agalactiae</i>)	0/0	1/1	0/0	3/4
Other				
Mixed infections	0/1	0/0	0/0	0/0
Total	1/11	2/5	2/5	7/9

¹Infections are recorded as number of quarters cured/number of quarters infected at dry off.

²NONE = No treatment; ORB = Orbeseal (65% bismuth subnitrate; Zoetis, Kalamazoo, MI); P-M = Phyto-Mast (Penn Dutch Cow Care, Narvon, PA), an herbal intramammary product for improving milk quality; P-M+ORB = Phyto-Mast and Orbeseal.

CHAPTER 6: CONCLUDING REMARKS AND FUTURE RESEARCH

The research included in this dissertation has expanded the scientific literature pertaining to organic dairies. The comparison of milk quality between organic and conventional dairies in North Carolina established the need for organic treatments for mastitis by reporting similar SCS and subclinical mastitis prevalence, as well as similar prevalence of mastitis-causing organisms, between the herd management types. Two studies in the dissertation examined the effect of herbal intramammary treatments on milk production and milk quality (as measured by SCC and presence of infection) compared with conventional antibiotic plus teat sealant or just teat sealant dry cow therapy. These studies established the two herbal treatments, Phyto-Mast and Cinnatube, as potential dry cow therapies in cattle because they had no apparent adverse effects on milk production or SCS. However, the herbal treatments alone or in combination were not significantly better or worse than conventional or no therapy at curing infections. With regards to preventing infections, either Cinnatube or the combination of Phyto-Mast and Orbeseal were significantly better than no treatment. The efficacy of one herbal product was investigated in greater detail by looking at the antibacterial efficacy of its ingredients in vitro. Only one ingredient, essential oil of thyme, had consistent antibacterial activity. The culmination of these research projects has resulted in greater knowledge of the organic dairy industry in the southeastern United States and a brief glimpse into the possible application of plant-derived

oils as mastitis therapy.

Though this research into the use of plant-derived oils for dry cow therapy is novel, it is not without its shortcomings. One major challenge faced during the studies involving testing the efficacy of herbal products was having enough statistical power. Several factors dictate the maximum statistical power that can be achieved; these factors include financial resources, experimental design, actual amount of cattle that were enrolled at the completion of the study, and quality of the samples taken. Finances dictated the size of several of the studies in this dissertation. Experimental design was challenging because of finances and because, even if the only published infection rate in U. S. organic herds (55.6%; Pol and Ruegg, 2007a) was used, the power of the final experiment could be reduced if the actual infection rate was lower than this reference rate, as was the case with all in vivo studies in this dissertation except the survey. The challenge of having sufficient cattle numbers is related to the enrollment of commercial herds as well as research herds. Dairy managers will choose to cull animals when they feel it is necessary; unfortunately, this can occur during a research trial. Finally, the quality of samples taken dictates the actual number of data points available for analysis. High contamination rates can be detrimental to the power of an experiment. The research in this dissertation should be repeated using either a larger number of animals, fewer treatment combinations, or a combination of the two to ensure high statistical power.

The in vitro study evaluating the antibacterial activity of the ingredients of Phyto-Mast established that the known antibacterial activity of thyme essential oil (Helander et al.,

1998; Cowan, 1999) is also effective when using milk as the culture medium. However, future research should examine the antibacterial activity of thyme essential oil when combined with each of the other plant-derived oils. Developing a standardized method to inoculate the same amount of bacteria during each replication would also make analysis of such an experiment more straightforward. The standard method for determining amount of bacteria present in solution uses a spectrophotometer and bacteria in solution. Using this method and inoculating a set volume of standardized bacteria-filled broth into the milk sample vials would likely have resulted in more straightforward results.

Despite these shortcomings, the research contained herein shows that herbal treatments can be competitive with conventional therapy at preventing infections during the dry period. Further research should examine the pharmacokinetics and pharmacodynamics of the herbal treatments tested here and of individual plant-derived oils in cattle. Initial work to this effect has been completed in goats (McPhee et al., 2011). Further research should also examine other treatments commonly used on organic farms. Though there is anecdotal evidence of efficacy of many different treatments, not many of these treatments have been scientifically evaluated using controlled studies in cattle. This is especially a problem for mastitis treatments (Ruegg, 2009), given the similar prevalence of mastitis-causing organisms on organic and conventional dairies. It would be very ironic to me if the research that I have performed in my dissertation leads into a “new direction” of pharmaceutical research into plant-based treatments, given that plant-based treatments were used for thousands of years prior to the advent of modern synthetic medicine.

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