EVALUATION OF HYDRATED LIME TREATMENT OF FREE-STALL BEDDING

AND EFFICACY OF TEAT SEALANT ON INCIDENCE OF DAIRY COW

MASTITIS

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Rekha Sutar Chettri

Certificate of Approval:

_____	_____
Daryl Kuhlers Professor Animal Sciences	Thomas A. McCaskey, Chair Professor Animal Sciences
_____	_____
Christine A. Sundermann Professor Biological Sciences	Donald E. Conner Professor Poultry Science

_____

Stephen L. McFarland
Dean
Graduate School

EVALUATION OF HYDRATED LIME TREATMENT OF FREE-STALL BEDDING AND EFFICACY OF TEAT SEALANT ON INCIDENCE OF DAIRY COW MASTITIS

Rekha Sutar Chettri

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Rekha Sutar Chettri

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Signature of Author

_____

Date of Graduation

VITA

Rekha Sutar Chettri, daughter of Padma Chettri and Bishnu Chettri, was born August 1, 1977, in Assam, India. She graduated from Chariali Girl's High School in 1993 and completed her pre-university schooling at Bishwanath College, Assam in 1995. She attended the College of Veterinary Science in Guwahati (affiliated with Assam Agricultural University, India) for five and half years and graduated to become a veterinarian in 2001. She was married to Hemraj Sutar on April 23, 2001 and started practicing as veterinarian for six months in India before joining to her husband in Florida, USA in 2002. She entered Graduate school at Auburn University in January 2003 under the guidance of Dr. Thomas McCaskey.

THESIS ABSTRACT

EVALUATION OF HYDRATED LIME TREATMENT OF FREE-STALL BEDDING AND EFFICACY OF TEAT SEALANT ON INCIDENCE OF DAIRY COW MASTITIS

Rekha Sutar Chettri
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Dairy cow mastitis is the inflammation of the mammary gland caused by trauma, chemical irritations and by infection caused by microorganisms. Mastitis is estimated to cost the US dairy industry about $1.8 billion annually. Infectious mastitis can be contracted from several sources including the cow's environment such as wet, muddy, and manure-soiled paddocks and stalls where the cow lies down. The cow spends most of the time lying in a stall between milking periods where the teat ends can come in contact with contaminated bedding resulting in environmentally-acquired mastitis.

A 12-month study was conducted to evaluate the use of hydrated lime as a germicidal treatment for dairy cow bedding to control mastitis. All Holstein cows at the E.V. Smith Research Center Dairy were evaluated by Dairy Herd Improvement Association (DHIA) records, and by analysis of the quarter, foremilk for somatic cell

count (SCC), and for microbes by blood agar culture. These analyses were used to determine the udder health status of the cows and to allocate the animals to two study groups of 16 cows each. To each stall of both groups of cows, 0.77 kg of peanut hulls was spread over the stall. Fifty grams of hydrated lime was applied to the back 1/3 of the stalls for the lime bedding treatment group of cows. Fresh bedding was applied daily to the stalls of both groups of cows and new lime was added daily to the stalls of the treatment cows. Udder quarter, foremilk, collected prior to afternoon milking, was collected monthly from each of the cows in the two groups. The quarter milk was analyzed for somatic cell count (SCC) by the Wisconsin mastitis test and for *Staphylococcus, Streptococcus, coliforms,* and other bacteria by blood agar culture. Also on a monthly basis, the DHIA records of the study cows were used to collect composite SCC data.

The SCC in milk increases in response to infection in the mammary gland, and according to published reports, a SCC in milk of >200,000/ml is indicative of an intra-mammary infection. Four levels of SCC were selected to compare the number of quarters in each cow group (control and lime-treated bedding) to determine the number of quarters that exceeded each SCC level. There were 43.65% and 38.04% fewer infected quarters in the lime-treated cow group compared to the control cow group at the >165,000/ml and > 200,000/ml SCC levels during 12-month study period, respectively. Based on DHIA composite milk data at the >165,000 SCC/ml and >200,000 SCC/ml levels, there were 9.96% and 32.88% fewer infected cows in the lime treatment group compared to the control group, respectively. The SCC legal cut off limit for sale of raw

milk is ≥750,000/ml. There was a 47.08% reduction (P<0.03) in the number of quarters with >750,000 SCC/ml for the lime treatment cow group.

Cows in the lime-treated bedding group had 73.2% fewer quarters infected with *Staphylococcus*, 72.84% fewer *coliforms*, and 73.95% fewer other bacteria compared to quarters of cows in the control group. The reduction rates for *Staphylococcus* (P<0.01) and coliform (P<0.01) infected quarters were significant for the cows bedded on the lime treated bedding. The study revealed that the application of 50 grams of hydrated lime daily to the back 1/3 of dairy cow free-stalls bedded daily with 0.77 kg of fresh peanut hulls reduced the incidence of mastitis in quarters of the cows using the lime-bedded stalls by approximately 45%. The cost to treat one lime stall was calculated to be $.011/day, or $4.02/cow/year. The economic benefit of the lime treatment was estimated to improve milk production by $42.51 ($46.53 - $4.02) /cow/year.

Corollary to the lime study the efficacy of Orbeseal (a teat sealant) was also evaluated to reduce the incidence of mastitis. Orbeseal was administered with the antibiotic Cephapirin at the time of cow dry-off in all cows at the farm except the non-lime and lime-treatment study cows which were administered only the antibiotic. Two consecutive quarter milk samples were collected before dry-off and after the cows freshened. Similar laboratory analyses were on the milk samples as was conducted for the lime study. Within the sealant-treated cow group, there were significant reductions of 65.82% (P<0.0001) and 60.99% (P<0.0002) for quarters with >165,000 SCC/ml and >200,000 SCC/ml. The number of quarters infected with *Staphylococcus* were reduced by 71.12% (P<.0007) in the sealant-treatment cow group.

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I. INTRODUCTION

Mastitis is the inflammation of the mammary gland caused by traumas, chemical irritations and by microorganisms, usually bacteria that invade the udder, multiply, and produce toxins that are harmful to the mammary gland. Mastitis is a costly disease of dairy cattle resulting in reductions in milk yield and milk quality (Makovec and Ruegg, 2003). Bovine mastitis is a disease and exhibits a complex epidemiological pattern that causes serious depression of productivity in dairy cows. Losses attributed to mastitis include reduced milk yield, milk losses, premature culling, treatment costs and increased labor (Fetrow, 2000). To simplify understanding the mastitis complex, it is useful to consider that three major factors are involved in this disease: the microorganisms as the causative agent, the cow as host, and the environment, which can influence both the cow and the microorganisms. Mastitis is the most costly dairy cattle disease to animal agriculture in the United States and throughout much of the world (NMC, 1998). In the absence of an effective mastitis control program, approximately 40 percent of cows are infected in an average of two udder quarters. It has been estimated that mastitis costs about $185 per cow per year (Schroeder, 1997). Mastitis can be controlled with a management program that includes a clean, stress-free environment, proper maintenance and operation of milking equipment, good milking

procedures including teat dipping, a dry cow treatment program, and a program for monitoring udder health status (National Mastitis Council, 1996).

A number of tests have been developed for detecting mastitis. Most tests estimate the somatic cell count (SCC) of milk. The cell count for normal milk is nearly always less than 200,000 cells/ml (Laevens et al., 1997; DeHaas et al., 2004; Schepers et al., 1997; Itavo et al., 2001; Smith, 1996). Counts higher than this are considered abnormal and indicate probable infection. The term "somatic" means "derived from the body". All milk contains white blood cells known as leukocytes which constitute the majority of somatic cells.

Traditional mastitis treatment relies heavily on the use of antibiotics to treat lactating and dry cows. This practice is not only costly, but is criticized for contributing to antibiotic resistant microbes that make the mastitis treatment less effective and also can potentially contribute to the pool of antibiotic resistant microbes affecting human health. Therefore, effective and economic mastitis control measures that can mitigate the need for expensive treatments are needed. Lime is a natural, inorganic compound and several calcium compounds are used as therapeutic agents for humans. It is therefore a natural, safe compound, that if demonstrated to be effective in the control of dairy cow mastitis, would economically benefit the dairy industry and reduce the opportunity for the development of antibiotic resistant microbes.

Previous research indicated that the use of hydrated lime for treating bedding in dairy cow stalls reduced coliform bacterial counts in the bedding and on teats of cows in lime-treated stalls (Fairchild et al., 1982; Hogan and Smith, 1997a). Other studies

indicated that hydrated lime was an effective germicide against *Escherichia coli* and *Salmonella* bacteria in bovine manure (McCaskey, personal communication, 2003).

The present thesis reports project that was developed based on a field study of the effects of a set of potential mastitis control procedures. Two mastitis control measures were selected for the study, 1) lime treatment of bedding in the freestall barn and 2) the use of Orbeseal a teat-end sealant at the beginning of the cow dry-off period. The project was not intended as a definitive evaluation of the procedures, but as a data source from which the nature of the biological effects and economic consequences of a control program might be deduced.

The fieldwork for this project was conducted with a research dairy herd at the E.V. Smith Research Center Dairy at Shorter, Alabama over a period of 12 months. An assessment of the benefits of the lime bedding treatment and the use of a teat-end sealant at dry-off was obtained from laboratory analyses of monthly quarter milk samples collected from the study cows and from Dairy Herd Improvement Association (DHIA) monthly records. The laboratory analyses utilized quarter, foremilk sample for bacterial isolation identification of major mastitis pathogens, and determination of the milk somatic cell count (SCC) by the Wisconsin Mastitis Test (WMT).

II. LITERATURE REVIEW

Historical Introduction:

Mastitis is generally considered to be the most costly disease of dairy cows (DeGraves and Fetrow, 1993). Dairy cow mastitis has major significance to mankind because milk is a primary food source. The extreme degree of adaptation of causative microorganisms to its particular ecological niche indicates that they have long been associated with the bovine udder. According to Heidrich and Renk (1967), the disease was primarily associated with chilling of milk cows. Nocard and Mollereau (1884) were the first authors to assign a microbial etiology to the disease, and this provided the starting point for modern scientific investigations of the disease (Morris, 1971).

Definition of Mastitis:

The word mastitis is derived from the Greek words *mastos* meaning "breast" and *itis* meaning "inflammation". Trauma or injury to the udder, chemical irritation, and microorganisms, mostly bacteria, are the major causes of mastitis. Bacterial pathogens that cause mastitis are classified as either contagious or environmental based on their primary reservoir and mode of transmission (Makovec and Ruegg, 2003). The inflammatory reaction of the mammary gland is a protective mechanism designed to eliminate the infecting microorganisms by neutralizing their toxins and assisting in the repair of damaged milk-producing tissues (Philpot and Nickerson, 2000).

Forms of Mastitis:

There are two major forms of mastitis, clinical and subclinical. Below is an overview of each form.

Clinical mastitis:

Clinical mastitis is usually caused by one of the major pathogens i.e. *Staphylococcus, Streptococcus,* or coliforms (Philpot and Nickerson, 2000). Clinical mastitis may be categorized by the severity of the inflammatory response such as peracute, acute, subacute and chronic. Symptoms include redness of the udder, swelling, heat, pain, and loss or change in milk production including decreased production and change in milk composition and appearance (Philpot and Nickerson, 2000).

a) Peracute: Peracute mastitis is characterized by sudden onset, severe inflammation of the udder and serous (watery appearance) milk (Philpot and Nickerson, 2000). This can lead to agalactia, which is the cession of milk production. The systemic illness is due to septicemia or toxemia, which characterized by fever, anorexia, depression, decreased rumen motility and dehydration of the animal. Despite systemic and parenteral therapy, many cows do not respond to treatment succumb to the death.

b) Acute: This form of mastitis is characterized by sudden onset and moderate to severe inflammation of the udder (Philpot and Nickerson, 2000). Milk appears grossly abnormal such as purulent, serum-like, watery or bloody. Systemic signs are similar but less severe than the peracute form.

c) Subacute: This type of mastitis is characterized by mild inflammation, and although there may be no visible changes in the udder, there generally are small flakes or clots in

the milk, and the milk may have an off-color. There are no systemic signs of illness evident with this type of mastitis (Philpot and Nickerson, 2000).

d) Chronic: Chronic mastitis may persist in a subclinical form for months or years with occasional clinical flare-ups. Symptoms include progressive development of scar tissue, changes in the size and shape of the affected quarter and reduction in milk yield (Philpot and Nickerson, 2000).

Subclinical mastitis

This form of mastitis can not be detected by visual observation of the udder or the appearance of the milk. Subclinical mastitis is responsible for the most prevalent type of intramammary infection (Philpot and Nickerson, 2000). This is the most important form of mastitis because it causes the greatest economic lose due to decreased milk production, reduced milk quality and it serves as a reservoir for infecting herdmates.

Economic Importance of Mastitis:

Mastitis has a major impact on the dairy industry and economic losses from mastitis are twice as high as losses from infertility and reproductive diseases (Philpot and Nickerson, 2000). Mastitis ranks at the top of the list among such dairy issues as disease risk, international trade and animal welfare. Economic loss due to mastitis in the United States is estimated to be approximately $185/cow annually (Schroeder, 1997). The calculation of the total annual loss is summarized in Table 1. If it is assumed that the same milk price prevailed across the United States and this value is multiplied by the total number of cows in the United States, which is about 9.5 million head, the total cost of mastitis in the United States would be about $1.8 billion. The average production loss per lactation for one infected quarter is reported to be about 1,600 lb of milk (Schroeder,

1997). Bray and Schearer (2003) reported that mastitis can decrease total milk production by 15 to 20%. Worldwide, annual milk losses caused by mastitis are reported to be nearly $35 billion (Ratafia, 1987).

Onset of Mastitis:

It is essential to know the anatomy and physiology of the mammary gland to understand how mastitis develops. The interior of each quarter is composed of a teat cistern, gland cistern, milk duct and glandular tissue. The mammary gland is a complex open self-regulatory system with a continuous flow of matter, energy and information (Burvenich et al., 2004). The glandular portion of the quarter contains millions of microscopic sacs or alveoli. Alveoli are surrounded by muscle cells that contract and squeeze milk from the alveolus during milking. Blood vessels are the major nutrient supplier to each alveolus and epithelial cells convert the nutrients into milk. Milk accumulates in the alveolar spaces, milk ducts and cisterns in between milkings. During milking the accumulated fluid is removed through the teat ducts (Schroeder, 1997).

Invasion of the udder occurs when bacteria pass through the teat canal and multiply inside the infected udder quarter. During milking bacteria may move up into the teat canal. They are propelled through the teat canal and into the teat cistern via droplet impacts against the teat orifice caused by milking machines vacuum fluctuations (Philpot and Nickerson, 2000).

Initially bacteria infect the tissue lining then enter the smaller ducts and milk producing areas of the lower portions of the affected gland and localize in the alveoli (Philpot and Nickerson, 2000). Bacteria also induce damage to the mammary tissues by producing toxins that cause swelling and death of the milk producing cells. This results in

inflammation. Fluids and blood clotting factors excreted by the tissues dilute the bacterial toxins which helps to repair tissue damage. The ability of bacteria to adhere to the tissues lining the interior of the mammary gland may be the cause of establishment of infection. (Philpot and Nickerson, 2000). Bacteria such as *Streptococcus agalactiae* and *Staphylococcus* spp can attach well to the tissue lining whereas *Escherichia coli* does not adhere as well. A specific toxin elaborated by *Mycoplasma bovis* also contributes to tissue damage (Anderson, 1993). The interaction between bacteria and leukocytes also influence the establishment of infection. The function of the leukocytes is to engulf and kill the bacteria but if any bacteria in the affected quarter survive, a chronic inflammation follows along with an elevated somatic cell count (Philpot and Nickerson, 2000).

Causative Organisms:

Mastitis can be caused by both contagious and environmental pathogens (disease-causing organisms). These two types of mastitis can occur separately or simultaneously. The most common contagious pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma* spp. Molenda et al. (2003) investigated the microbiological contamination on dairy farms with special regard to pathogenic bacteria. Studies were carried out to determine the prevalence of bacteria that cause mastitis on the farm in different seasons (Table 2). They reported that *S.aureus* a contagious cause, was the most common cause of mammary gland infections (Molenda et al., 2003).

Common environmental pathogens include *Escherichia coli*, *Nocardia* spp, *Klebsiella* spp., *Enterobacter* spp., non-agalactiae *Streptococcus* spp., *Citrobacter* spp, *Proteus* spp, *Pseudomonas* spp, *Serratia* spp.(Bodman and Rice, 1995). *Mycoplasma* spp., *Arcanobacterium pyogens*, *Pasteurella* spp., *Klebsiella* spp. and *Enterobacter* spp.

caused major milk production losses (Wilson et al., 1997). Some types of mastitis are more severe than others, depending on which pathogen has caused the infection. Below is a discussion on the primary causative agents of mastitis.

Streptococcus agalactiae

Streptococcus agalactiae is a Gram-positive pathogen that affects pre-milking heifers as well as older cows in dairy herds. It is considered one of the major causes of economic loss to dairy producers without a mastitis control program (James, 2002). These bacteria primarily infect the duct system of the lower portion of the affected quarter. Tissue debris and leukocytes occlude the ducts and block the milk secretion. Accumulated milk and bacteria cause involution, scar tissue formation and lowered production of milk. The most commonly identified other streptococcal species are *Streptococcus uberis, Streptococcus dysgalactiae* and *Aerococcus viridans* (Zadoks et al., 2004).

Staphylococcus aureus

The principle source of *Staphylococcus aureus* is the infected udder of cows (Sears and McCarthy, 2003). *Staphylococcus aureus* is more damaging to milk-producing tissues than *Streptococcus agalactiae* because it releases more injurious toxin. Roberson et al. (1994) reported that *S. aureus* is also found "free living" in bedding, but milk and skin injuries are the major sources for infections. These bacteria form deep-seated pockets of infection in glandular tissue. The cow's immune system attempts to keep bacteria in one place by walling-off these areas with leukocytes and scar tissue (Biggs, 1998). Some strains of *S. aureus* produce a toxin that cause blood vessel constriction, clotting and cutting-off the blood supply to the affected quarter, which can lead to

gangrene formation. *Staphylococcus aureus* can also cause intramammary infection (IMI) in prepartum (prior to first calving) heifers. The prevalence of *S. aureus* IMI in prepartum heifers varies considerably among different regions and herds. Some studies reported a very low prevalence and others reported a relatively high prevalence. Wagge et al. (1999) reported that in heifers where mastitis is associated with increased rectal temperature or other systemic signs, the proportion of udder quarters infected with *S. aureus* was larger than for heifers without a systemic reaction. In some herds, the overall mastitis level involved 97% of the animals and *S. aureus* might be responsible for 37% of the infections (Trinidad et al., 1990).

The coagulase negative S*taphylococcus* (CNS) that are associated with mastitis are mainly *S. hyicus* and *S. epidermidis*. Most CNS mastitis cases are subclinical or teat duct colonizations that slightly increase milk SCC, but CNS also can provoke a clinical disease that may result in persistent IMI that is difficult to cure (Myllys et al., 1994).

Coliforms

Coliform bacteria include the genera *Escherichia, Klebsiella, Citrobacter* and *Enterobacter* (Jay, 2000). These microorganisms are associated with manure, bedding materials, soil and polluted water -the cow's environment (Philpot and Nickerson, 2000). *Escherichia coli* produces an endotoxin, which is released upon death of the bacterial cells, that causes rapid movement and accumulation of somatic cells into the milk. Coliform mastitis can range in severity from peracute mastitis to subclinical mastitis. *Escherichia coli* has been classified as an environmental pathogen (Nemeth et al., 1994). The host defense of the bovine mammary gland has been shown to be efficient in controlling and eliminating *E. coli* infection (Hill et al., 1978). Although this ability has

been shown to be less effective in early lactation due to deficiencies in polymorphoneutrophil functions, a type of white blood cell, and somatic cell count numbers (Hill et al., 1979 and Hogan et al., 1989a).

Mastitis resulting from *Klebsiella* infection is often severe and can result in markedly decreased milk production (Newman, 1975). The mammary glands of cows that have died as a result of *Klebsiella* mastitis show sign of massive inflammation and widespread tissue necrosis (Bannerman et al., 2004). Sawdust bedding has been implicated in cases of coliform mastitis, especially *Klebsiella* that is often associated with sawdust (Fairchild et al.1982; Newman and Kowalski,1973). Hogan and Smith (1997a) demonstrated that the addition of 1kg of lime to 10 kg of sawdust cow bedding reduced the incidence of Gram-negative bacteria including *Klebsiella* spp. in the bedding.

Mycoplasma bovis

Mycoplasma are intermediate in size between bacteria and viruses and do not have cell walls. *Mycoplasma bovis* live naturally in the respiratory tract of cattle throughout the world (Jasper, 1981). In contrast to other forms of contagious mastitis, mycoplasmal infection can spread from the respiratory system to the udder. The spread can occur through air and the blood stream. The symptoms of mycoplasma mastitis are multiple quarter involvement, and decrease in milk production cows appear otherwise healthy but have severe mastitis and the milk has a sandy or flaky sediment in a watery or serous fluid (Ruegg, 2000).

Corynebacterium bovis

Coryneform bacteria are frequently isolated from bovine mastitis with *Corynebacterium bov*is as the most frequently isolated organism of this group

(Watts et al., 2001). Mastitis outbreaks caused by *C. bovis* have been reported most commonly in herds that do not practice post-milking teat dipping and dry cow therapy. Mammary glands infected with *C. bovis* have a decreased rate of concurrent infection with either *S. aureus* or *S. agalactiae* (Watts et al., 2000).

Diagnosis of Bovine Mastitis:

The New York State Cattle Health Assurance Program (Schukken and Frank, 2001) reported that the diagnosis of clinical mastitis is based on the appearance of abnormally appearing milk such as off-color, watery, bloody or a serum- like appearance. However, diagnosis of subclinical mastitis is problematic because the milk appears normal. Because mastitis is the inflammation of the mammary gland usually caused by microorganisms, the detection of the disease can therefore be performed by monitoring the inflammatory status with various cowside and laboratory tests (Philpot and Nickerson, 2000).

Cowside Mastitis Tests:

Physical examination

Mastitis can be detected by a physical examination of the udder. A physical examination for mastitis should be done on the empty udder. Mastitis indicates a hard, hot, swollen udder (Schukken and Frank, 2001). The mammary gland should also be examined for the presence of scar tissue and atrophied quarters. The amount of swelling, severity of pain and the overall appearance such as flakes, clots, stringiness and watery milk secretion of the cow will indicate the severity of infection and serve as a guide for diagnosis of mastitis (Schukken and Frank, 2001).

The strip test

According to Gilson (1995), the foremilk should be examined during preparation of the udder for milking. The strip cup or strip plate is indispensable in the milking parlor for determining the presence of clinical mastitis. Foremilk can be observed by squirting a few streams of milk onto a special strip cup. Abnormalities of the milk may show up as blood, flakes, clots and wateriness of the milk.

California mastitis test

The California mastitis test (CMT) is a simple, inexpensive, rapid screening test for mastitis. The test is based on the amount of cellular nuclear protein present in milk (Schukken and Frank, 2001). The CMT is conducted by mixing a CMT reagent with an equal quantity of milk. The reagent with material from the nuclei of the somatic cells in the milk react to form a gel. The reaction is then visually scored (Table 3) as 0, T (Trace), 1, 2, or 3, depending upon the amount of gel that forms (Jasper, 1967). The somatic cell count score (SCC) increases with an increase in gelation of the milk (Gilson, 1995).

Electrical conductivity

According to New York State Cattle Health Assurance Program (NYSCHAP), mastitic milk has a higher electrical conductivity (EC) than normal milk, which is caused by tissue damage resulting in an increase in sodium and chloride ions in the milk (Schukken and Frank, 2001). This method of detecting mastitis relies on the differences in salt concentrations that occur between infected and uninfected quarters of the same cow. The EC of milk is determined by the concentration of anions and cations in the milk, and when the cow suffers from mastitis, the concentration of Na^+ and Cl^- in the milk increases (Kitchen, 1981). The presence of bacterial infection in the affected quarter

increases the sodium and chloride ions in milk and decreases potassium ions and lactose, while the pH increases. Milner et al. (1996) reported that developing clinical mastitis could be predicted accurately by changes in the EC of foremilk. Most automatic milking systems have in-line EC monitoring devices (Norberg et al., 2004). Sensitivity of detection can be very high using a hand-held meter; however, this procedure is not practical for routine use in milking parlors. Automated systems used in milking parlors have lower sensitivity and specificity of detection because milk from one quarter with an elevated EC is diluted with milk from the other quarters (Maatje et al., 1992 and Nielen et al., 1995).

Laboratory Mastitis Tests:

Bulk tank somatic cell count

In February 2002, the National Mastitis Council Committee (2002) published guidelines for accurate sampling and reporting of bulk milk somatic cell counts. The bulk tank somatic cell count (BTSCC) is an internationally accepted measure of the quality and suitability of milk sold from the farm for human consumption (NMC, 2002). A BTSCC less than 200,000 cells/ml indicates a minimal level of infection; however, a series of BTSCC's over 500,000 cells/ml indicates a problem with subclinical or chronic infection. The most important factor affecting BTSCC is infection status. *S. agalactiae* produces higher somatic cell counts in milk than *S. aureus* and other minor pathogens such as CNS and *C. bovis* (Schukken and Frank, 2001).

Individual cow somatic cell count

The term somatic cell is a general term referring to the white blood cell. The individual cow SCC is the product of the SCC measurement of milk from all four

quarters and the level of production of the individual quarters. The occurrence of a clinical case of mastitis can be determined by an average SCC of more than 200,000 cells/ml of milk (DeHaas et al., 2004). An individual cow somatic cell count of <200,000 cells/ml is typical of an uninfected udder (Laevens et al., 1997). According to New York State Cattle Health Assurance Program researchers in Canada reported that the ability to correctly classify cows as infected or uninfected by SCC increased from 77.9% to 92.7% as the number of infected quarters rose from one to four quarters (Schukken and Frank, 2001). The chance of isolating a major pathogen increased when the quarter had SCC's above 200,000 cells/ml. For composite quarter milk, a threshold of approximately 250,000 cells/ml is reasonable for differentiating infected from non-infected quarters (Schukken and Frank, 2001). The DHIA records report both SCC/ml and log linear SCC scores for composite quarter milk of individual cows in a herd. An increase in one linear score unit is reported to equal a loss of 1.5 pounds of milk per cow per day or 400 pounds per lactation (Schukken and Frank, 2001). The current legal limit for bulk tank SCC is 750,000 cells/ml for Grade A producers (Miller et al., 2003). The relationship between SCC Linear Score (LS), Somatic Cell Count (SCC) and Estimated Daily Milk Losses (EDML) is shown in Table 4 (Shook and Seaman, 1983).

Electronic somatic cell count

A computer module was developed for electronically counting SCC. The module was tested using field survey and DHIA data from several dairy herds (Heald et al., 2000). Chronic infection can often be identified inexpensively by using monthly DHIA data such as lactation number, days in milk, SCC, and milk production (Berning et al., 1992). Electronic counting of SCC usually is performed on the total

mixed milk from all quarters of each cow. Warren (1995) stated that the advantages of the electronic counting procedures are, it can be automated thus allowing centralization of laboratory procedures, the SCC on preserved samples can be counted, and the procedure is more precise than the WMT and the CMT methods of estimating SCC. The disadvantages of electronic SCC procedures are that the equipment is expensive and require constant monitoring (Gilson, 1995).

Wisconsin mastitis test

The Wisconsin mastitis test is similar to the CMT for estimating SCC, but it is more precise than the CMT. The test is conducted by combining 2 ml of milk with 2 ml of WMT reagent in a WMT tube. The milk and reagent are mixed for 8 to 10 seconds in the WMT tube, and the mixture is drained through the hole in the tube cap for a period of exactly 15 seconds, and the tube is returned to the upright position. After waiting one minute, the amount of fluid remaining in the tube is measured in millimeter (mm). The relationship between WMT scores and SCC is shown in Table 5 (Philpot, 1978).

Bacterial culture of milk samples

Major pathogens are considered of relevance even when a few colony-forming units (cfu) are found in the milk (Hariharan et al., 2004). The common diagnostic schemes for collecting milk samples, and for isolating and identifying mastitis pathogens have been published by the National Mastitis Council (Hogan et al., 1999). Environmental streptococci can be reliably cultured from milk of infected quarters by plating 0.01 ml of milk on Esculin Blood agar (EBA). Milk from infected quarters generally contain greater than 100 cfu/ml (Hogan and Smith, 1987). Counts of coliforms are frequently < 100 cfu/ml when 0.01 ml of milk is streaked to EBA, which indicates

that coliform bacteria are not reliably cultured from milk of infected udder quarters. However, streaking 0.01 ml of milk on EBA and 0.1 ml on MacConkey agar facilitates the diagnoses (Hogan and Smith, 1987). The National Mastitis Council (1987) guidelines consider a single colony of *S. aureus* or *S. agalactiae* significant in culture. Isolation of two to nine colonies of environmental streptococci is considered significant, whereas, counts of CNS must be > 10/cfu (NMC, 1987). To isolate environmental S*treptococcus* spp. and *E. coli* predominant causes of mastitis (Dingwell et al., 2004), one standard loop (0.01mL) of milk sample is plated on the surface of a blood agar plate. Bacterial colonies on blood agar plates can be examined after 24-48 hours of incubation at 37C. Blood agar allows most organisms to grow but *Mycoplasma*-suspect samples should be plated on mycoplasma medium (Hogan et al., 1999). Hemolysis may be helpful in identifying *Streptococcus agalactiae* by the clearing zone around the colonies. *Corynebacterium* and yeast may need 48-96 hours of incubation for significant growth to occur (Sears and McCarthy, 2003).

Polymerase chain reaction

Due to limitations of cultural methods, polymerase chain reaction (PCR) has been developed to identify various mastitis pathogens (Khan et al., 1998). The PCR-based methodology is a very promising method for the rapid identification of bacteria. Identification of bacterial pathogens can be made in hours, rather in days required for conventional cultural methods. Very few cells are necessary to yield a positive diagnosis. Mastitis pathogens can be detected and identified at an earlier stage of mastitis. One of the major disadvantages of the PCR methodology is that it is very sensitive and minor contamination in the sample can lead to misdiagnosis (Phuektes et al., 2001).

Control of Mastitis:

Eliminating infection

Most contagious mastitis is transmitted by fomites from infected cows to non-infected herdmates during the milking process. Milker's hands, milking unit, and udder wash cloths are the primary sources of the infections. A full milking hygiene program such as single service towels to wash and dry udders, use of disinfectant for udder wash, rubber gloves worn by milkers, teat dipping with disinfectant, and disinfections of the milking unit between cow milking helps to eliminate infections (Anderson, 1993). There is some evidence that flies may be vectors for intra mammary infection (IMI) due to *S. aureus* (Fox and Gay, 1993). *Mycoplasma* control practices on the dairy farm are essential. *Mycoplasma* bovine mastitis is potentially a highly contagious disease that can cause a severe economic problem in affected herds (Gonzalez and Wilson, 2003). The testing of all fresh cows for *Mycoplasma* is an important control measure. In addition, culling or permanently segregating infected cows and milking them last, strict asepsis and the use of single-use materials are some major steps in the treatment of *Mycoplasma* mastitis cases (Jasper, 1979). Routine monitoring of bulk tank milk is necessary to detect the presence of pathogenic *Mycoplasma* (Ruegg, 2000). To avoid increasing this potential reservoir of infection, it is suggested that milk potentially containing *Mycoplasma* should not be fed to calves without pasteurization (Walz et al., 1997 and Bray et al., 2001).

Mastitis prevention

Prevention of mastitis can be accomplished by improving milking procedures such as disinfecting and drying teats before milking, keep milking cupliner slips to a minimum, teat dip with an effective germicidal and maintaining the milking system in

proper working order (Schroeder, 1997). Mastitis prevention also has been attempted by using immunization. Sears et al.(1990a) reported that immunized heifers were 2.8 times less likely to develop *S. aureus* IMI than controls when both groups were exposed to an experimental challenge of *S. aureus*. Watson and Schwartzkoff (1990) also reported that use of *S. aureus* bacterin was also successful in reducing *S. aureus* IMI in some herd. A point to consider is that a good immunization program will not overcome poor management (Fox and Gay, 1993). Immunization will not be a stand-alone mastitis control program, but eventually may become a component of a mastitis prevention program.

Environment

The cow environment should be clean and dry. Cows should not have access to manure, mud, or pools of stagnant water. Both the dry cow and lactating cow areas are very important in mastitis control, and stalls for dairy cows should be properly designed to promote comfort and cleanliness (Hogan and Smith, 1998). The udder is very susceptible to infection at calving, and many infections detected in early lactation occur at calving (Hogan and Smith, 1998). Calving pads for pregnant cows can be beneficial especially for cows calving during wet and cold months. The drainage system for the calving pads should have underground slotted polyvinyl chloride pipes (Davison and Andrews, 1997). If possible, heifers should be calved separately from the adult herd.

Teat dipping

A germicidal pre-milking and post-milking teat dipping program is very important in maintaining udder sanitation and in controlling mastitis.

Pre-milking teat sanitation was first discussed by Bushnell (1984), reported on immersing teats into a 1% iodine pre-milking teat dip followed by drying with paper towels prior to milking machine attachment. Bushnell (1984) reported an 80% reduction in new cases of clinical mastitis by dipping teats into a low-iodine dip. Pre-milking teat dipping is a relatively new concept that was developed to control environmental mastitis. Pre-milking and post-milking teat disinfections in combination with good udder preparation has been reported to be significantly more effective in controlling environmental pathogen IMI than just good udder preparation and post-milking teat dipping (Oliver et al., 1993). Phenol and phenolic compounds have been studied extensively as disinfectants and have been shown to have a wide spectrum of antibacterial activity against Gram-positive and Gram-negative pathogens (Oliver et al., 2001). Pre-milking teat disinfections in combination with a phenolic compound in association with good udder preparation and post-milking teat disinfections was reported to further reduce the occurrence of new IMI by numerous mastitis pathogens (Oliver et al., 2001). Chlorine, iodine, and chlorohexidine teat dips have been reported to be very effective against *M. bovis* on teat skin (Boddie et al., 2002). Post-milking teat sanitation was reviewed thoroughly by Pankey, (1984). Sanitation of teats after milking with an effective germicide consistently reduced the incidence of IMI caused by contagious pathogens such as *S. aureus* and *S. agalactiae* (Pankey et al., 1984). According to Pankey (1984) dipping teats with an effective germicide after milking reduced new IMI by 50% or more for most Gram-positive organisms. A 0.1% iodine teat dip significantly reduced the IMI caused by *S. aureus* by 87.9% and *S. agalactiae* by 66.5%. This indicates that 0.1% iodine has sufficient germicidal activity to be efficacious in preventing new IMIs

(Boddie et al., 2004). In another study to determine the efficacies of chlorine dioxide and iodophor teat dips, chlorine dioxide teat dip significantly reduced new IMI caused by *S. aureus* and *S. agalactiae* under experimental exposure to these pathogens. Boddie et al. (2000) reported that a 0.5% iodophor teat dip reduced new *S. aureus* IMIs by 92.9% and *S. agalactiae* by 43.4%. Galton (2004) reported on an automatic post-milking teat dipping system incorporated onto milking machines lines that significantly reduced new IMI's caused by *S. agalactiae*, *S. uberis* and *S. aureus*. New IMI were reduced 88.2% for *S. aureus*, 94.4% for *S. agalactiae* and 93.8% for *S. uberis*.

Antibiotic therapy

Antibiotic therapy is the most common use of mastitis treatment in adult dairy cows (Moore and Heider, 1984; Kaneene and Miller, 1992). Bacteria involved in bovine mastitis are broadly classified as either contagious or environmental pathogens based on this epidemiological association with the disease (Sandholm et al., 1990). Application of mastitis control programs implemented in the 1960s, included teat disinfection, antibitic application and culling of chronically infected cows. Mastitis pathogens have been brought under control in dairy herds through the use of management practices that utilize postmilking teat dipping, dry cow therapy, culling, maintenance of milking equipment and antibiotic therapy (Oliver et al., 2004). Oliver et al. (2004) reported that a high percentage of pregnant heifer mammary glands were infected during late gestation, at calving and during early lactation. The scientists demonstrated that prepartum intramammary infusion with cephalosporin sodium approximately 1 to 2 weeks before expected calving was an effective procedure for eliminating many infections in heifers during late gestation, and for reducing the prevalence of mastitis in heifers both during

early lactation and through out lactation. They reported that penicillin-novobiocin or pirlimycin hydrochloride was also effective in eliminating infections observed during the prepartum period and early lactation. Among the infected quarters 14 days before the parturition, 76% were uninfected following treatment with penicillin-novobiocin; 59% were uninfected following treatment with pirlimycin and only 26% were uninfected in the untreated negative control group (Oliver et al., 2004). In another study Oliver et al. (2004) evaluated the efficacy of the broad-spectrum antibiotic ceftifour. Ceftifour is a new broad-spectrum third-generation cephalosporin antibiotic. They reported that ceftifour therapy was effective for eliminating *S. uberis* experimental IMIs. Ceftifour therapy extended over 5 to 8 days was more effective than the standard 2 days treatment. Diarra et al. (2002) evaluated the therapeutic potential of bovine lactoferrin in combination with Penicillin G against *S. aureus.* Lactoferrin is an 80-kDa, iron-binding glycoprotein found in milk, bile, saliva, tear and in specific granules of neutrophil leucocytes (Schanbacher et al., 1993). The combination of lactoferrin with penicillin increased the inhibitory activity of penicillin by 2-to 4-fold, and reduced the growth rate of *S. aureus* strains tested, whereas the increase in the inhibitory activity of lactoferrin by penicillin was 16-to 64-fold. This finding suggests that bovine lactoferrin in combination with beta-lactam antibiotics could increase the antibacterial activity of these antibiotics against antibiotic resistant strains of *S. aureus* (Diarra et al., 2002).

The question about development of antibiotic resistance bacteria has caused major concern in the dairy industry during recent years. There have been attempts to develop guidelines for a more “rational use of antibiotics” and to minimize the risk of antibiotic resistance (Aarestrup, 2000).

Vaccines

Research on vaccines to reduce *Escherichia coli* and staphylococcal mastitis infections has shown promise. Studies have been conducted on a mutant strain of *E. coli* 0111:B4 (J5) which has a unique characteristic of core and lipid A antigens of lipopolysaccharide (LPS). Immunization with the J5 bacterin produces antibodies that are cross reactive with other coliforms, and increases antibody titers to the *E.coli* J5 core antigens in serum and mammary secretions (Tomita et al., 2000). Immunization with *E. coli* J5 reduced the severity and lowered the rates of clinical coliform mastitis. Tomita et al. (2000) investigated two commercially available *E. coli* J5 bacterins (JVAC and J5). They found that immunization with the J-VAC or J5 bacterin elicited a similar immune response to *E. coli* J5 whole cell antigens. Crist et al.(1997) reported that vaccination of cows with *E. coli* J5 vaccine at dry-off, 30 days before calving and at calving resulted in a 70% to 80% reduction in clinical coliform cases of mastitis.

Development of vaccines against *S. aureus* bacteria has been an area of active research with some promising developments (Crist et al., 1997). Giraudo et al (1997) developed vaccines with encapsulated *S. aureus* cells. They tested 30 heifers during a 7-month period. The 30 heifers were randomly assigned to 3 groups of 10 heifers each. The prepartum group received two injections of the vaccine at 8- and 4-weeks before calving. The postpartum group received two injections at 1- and 5- weeks after calving, and the control group received two injections of placebo at 8- and 4- weeks before calving. The prepartum group infections were reduced from 18.8% (control group) to 6.7% (treatment group) and the postpartum group infection rate was reduced from 18.8% to 6.0% (Giraudo et al., 1997).

Proper milking procedure

Stoltenow and Schroeder (1997) prepared fact sheets based on the National Mastitis Council recommended milking procedures for mastitis control. These milking procedures are:

a) provide a clean stress-free environment for the cows,

b) check foremilk and udder for mastitis,

c) wash teats with an udder wash sanitizing solution,

d) dry teats completely with individual paper towels,

e) attach the milking unit within one minute after the start of stimulation,

f) adjust units as necessary for proper alignment,

g) shut off vacuum before removing unit, and

h) dip teats with an effective germicide immediately after unit removal.

To minimize loss and achieve maximum milk yield, a practical milking management scheme should be followed (Bray and Shearer, 1986). The dairy cow should have a clean dry environment, which helps reduce mastitis and increases milking efficiency by reducing time and labor to clean udders before the milking process.

Backflushing milker claws between cows

Backflush is the process of rinsing and sanitizing the milker unit between uses from one cow to the next. Day (1998) reported that the basic concept of backflushing is to rinse the milk from the claw and liner and disinfect the unit. This can help to improve herd udder health by decreasing the amount of contagious pathogens that are transported by the milker to subsequent cows.

Automated backflushers have been developed to sanitize the liners and claws between milking. Bray and Schearer, (1993) reported that four or five cycles are generally used and those cycles consist of a water rinse, an iodine rinse, clear water rinse and finally a positive air-dry cycle.

The backflushing process usually reduces the number of bacteria on the teat liners between cows, but does not reduce the number of bacteria on the teats (Bray and Schearer, 1986).

Milking machine

The most important points of a milking system are vacuum, inflations, and pulsation (Dahl et al.,1993). For proper milk ejection and to open the sphincter muscle, the milking machine usually applies 12.5 to 15 inches psi mercury vacuum to the outside of the teat. This produces a pressure differential of 12.5 to 15 inches of negative vacuum pressure on the outside of the teat compared with the 0.8 psi positive pressure inside the teat (Bray and Shearer, 1993). The milking machine should be maintained and operated properly otherwise milking at a low vacuum increases the machine-on time, the frequency of liner slips, and a decreased milk flow rate (Rasmussen and Madsen, 2000).

Nutrition

No direct link between nutrition and mastitis is known to exist other than deficiencies severe enough to suppress overall health by limiting immune system function (Bodman and Rice, 1995). Selenium, vitamin A and beta-carotene are reported to be essential dietary nutrients to prevent udder infection (NMC, 1993). These nutrients are essential to body tissues, the mammary gland and needed by white blood cells to fight mammary gland infections. These nutrients maintain milk secretory cell health and

promote antibody transport and white blood cell movement into milk (NMC, 1993). Ongoing research at University of Kentucky indicates that copper may also play a role in maintaining the immune system in dairy cattle (Schroeder, 1997).

Bedding

Bedding and bedding management contribute to cow comfort, udder health and milk quality. Bedding materials have been implicated as primary sources of environmental pathogens during intermilking periods (Hogan et al., 1989b). Clean and dry bedding material in the cow stalls is essential for cow cleanliness. The cow's teats are susceptible to infection from soiled bedding materials and effective bedding management should ensure that teats contact a clean, well-bedded surface each time a cow lies down. Ideal bedding should be dry, inert, cost effective, contribute to cow comfort and cleanliness and should be easily managed. Bedding materials fall into two basic categories, which are organic and inorganic. Hogan et al. (1989) reported that organic bedding materials contained significantly higher bacterial counts than inorganic materials. Organic bedding materials they studied consisted of straw, hay, sawdust, wood shavings, crop residue, shredded paper, paper pulp residue, composted or dried manure and similar materials. These materials are used as bedding because they absorb moisture and are readily available. The major disadvantage of this type of organic bedding material is that it supports rapid growth of bacteria (Smith and Hogan, 2000; Hogan et al., 1990; Zehner et al., 1986; Hogan et al., 1989b). The major mastitis pathogens associated with bedding are environmental streptococci and coliforms such as *E.coli* and *Klebsiella spp* (Smith and Hogan, 2000; Zehner et al., 1986; Newman and Kowalski, 1973; Hogan et al., 1989b). Some environmental mastitis pathogens have been associated with specific types

of bedding materials. Straw bedding is often associated with increased levels of mastitis from *Streptococcus uberis* infection, and the use of green, hardwood sawdust containing bark material has been associated with a high incidence of *Klebsiella* mastitis (Newman and Kowalski, 1973). Particle size can become an important factor when developing management schemes for bedding materials. Small particle size of sawdust may support very rapid growth of bacteria. Material of fine particle size can easily cover the teat skin and lead to high populations of bacteria on the teats which creates an environment for IMI (Smith and Hogan, 2000). Wood products such as shavings have a larger particle size which supports slower growth of bacteria. Composted or dried manure solids also can be used as bedding material. These materials are usually free of major pathogens, however, when dried manure becomes mixed with urine and feces, high microbial populations are rapidly established in the bedding. Composting is effective in eliminating most enteric pathogens from manure solids (Mote et al., 1988).

Inorganic bedding material can consist of sand or crushed limestone. Sand is inert and does not support bacterial growth. Hogan et al. (1989) reported that the use of inorganic bedding materials appears to be most advantageous during summer and fall when Gram-negative bacterial populations are greatest in bedding for lactating cows. Smith and Hogan (2000), and Stowell and Inglis (2000) reported that bacterial counts in sand bedding were much lower than for organic bedding material and numbers of coliform bacteria and environmental streptococci in sand were nearly always lower than the numbers found in organic bedding.

Many other factors can affect the microbial load of bedding materials such as cleanliness, the frequency of changing the bedding materials and the amount of

application. Organic bedding materials can reach maximum bacterial populations in 24 hours after the material is laid down. Accumulation of excessive amount of urine, mud and manure cause rapid detoriation of the bedding.

The number and type of bacteria in bedding are related to the microbial load on teat ends and the rates of clinical mastitis in lactating dairy cows (Hogan et al., 1989b). Bacteria numbers in bedding depend on available nutrients, amount of contamination, moisture and temperature. Inorganic materials such as crushed limestone and sand are low in nutrients and moisture, and generally have lower microbial populations than organic bedding such as sawdust, shavings, recycled manure, pelleted corncobs, various types of seed hulls and chopped straws. All organic and inorganic bedding materials support high pathogen counts after becoming contaminated with manure (Blowey and Edmonson, 1995). Daily replacement of sawdust in the back one-third of dairy cow stalls helps to reduce microbial contamination of the bedding (Nickerson, 2002).

Additives can be used to lower the bacterial counts of bedding material (Godkin, 1999 and Hogan et al., 1999). A practice on some farms that use wood products for bedding is to add hydrated lime to stalls to control bacterial populations (Fairchild et al., 1982). Hydrated lime added to sawdust or shavings has been shown to increase bedding pH and to reduce the water content. Research indicates that adding lime to bedding prior to application and mixing just prior to use is the most effective means of reducing the bacterial population in bedding material. Alkalizing and acidifying agents have been used to lower bacterial counts in sawdust and recycled manure (Hogan et al., 1999 and Hogan and Smith, 1997a). Treatment of recycled manure with an alkaline conditioner or hydrated lime has been reported to lowered the total bacterial count 100-fold for

approximately 24 hours (Hogan et al., 1999). Differences in housing and management practices might circumvent the beneficial effect of adding lime, or making more frequent bedding changes might affect bacteria levels on the cow's teat end (Zehner et al., 1986; Hogan and Smith, 1997a; Godkin, 1999).

Housing conditions also affect the conditions of the bedding. Barns with inadequate ventilation will often have a microclimate at the barn floor level that will increase the moisture level of bedding. Lactating cow, dry cow, heifer housing, maternity, and calving areas are housing areas where teat-end exposure to environmental pathogens associated with bedding can occur (Smith et al., 2000). Differential bacterial counts such as *E.coli*, *Klebsiella*, *Staphylococcus* and *Streptococcus* can be useful for evaluating bedding quality and management. Barn design, ventilation, cleaning frequency, and animal population density are some of the factors that can influence microbial populations in bedding and the exposure of cows to potential infection (Godkin,1999). From the above discussion on the bedding materials, proper bedding management for all production groups of cows is critical for the effective control of mastitis.

Dry cow treatment

Dry cow therapy is the use of intramammary antibiotic therapy immediately after the last milking of the cow's lactation. The use of Food and Drug Administration approved infusion products at drying-off, when cows do not produce milk for a certain period of time, can decrease the number of existing infections and prevent new infections during the early weeks of the dry period. Dry cow therapy is recommended for all quarters of all cows at drying off which helps control environmental streptococci during

the early dry period (Crist et al., 1997). The nonlactating mammary gland has been considered refractory to enterobacterial infection, but research in the United States from 1943 has suggested that the dry period is the time of greatest risk for the acquisition of new Gram-negative IMIs (Bradley and Green, 2000). Intramammary infection during the dry period has an important influence on the subsequent clinical mastitis that occurs at calving. Intramammary infection with major pathogens in the late, dry and post-calving period increase the risk of clinical mastitis, and this mastitis occurs at a greater rate after calving than mastitis not associated with dry period infections (Green et al., 2002). According to Smith et al, (1966) antibiotic dry cow therapy has two functions, removal of preexisting IMIs present at drying off, and to prevent new IMIs during the nonlactating period. Antibiotic dry cow therapy was adopted as a cornerstone of mastitis control strategies in 1960s and was directed against the contagious pathogens (Smith et al., 1966). Therefore, dry cow intramammary infusion products typically contain antibiotics. One of the first applications of the use of antibiotics in the dry or freshening period of dairy cows was to reduce the incidence of summer mastitis (Pearson, 1950). Dry cow antibiotic therapy with cloxacillin is well established as a means of controlling and eliminating new and existing mammary infections. It was suggested that multiple infusions of cloxacillin might eliminate more infections in the dry period. According to Cummins and McCaskey (1987), multiple dry cow treatment with cloxacillin did not offer any advantage over a single cloxacillin treatment. They reported that with two treatments during the dry period, 75.5% of new infections were eliminated and with a single treatment 73.2% of new infections were eliminated. Berry and Hillerton (2002) reported that treatment with cepravin antibiotic during the dry period reduced the rate of

new infections by approximately 80%, and eliminated more existing infections than by spontaneous cure. Tilmicosin phosphate is a semi-synthetic macrolide antibiotic, which has been shown to have important interactions with bovine phagocytes and epithelial cells, and has a significant role in its clinical efficacy against intracellular organisms. Dingwell et al. (2003) conducted field trials at 75 commercial and two research dairy farms where the efficacy of tilmicosin was evaluated along with risk factors associated with *S. aureus* infections during the dry period. The researchers reported that *S. aureus* IMIs were eliminated from 62% of the cows and 67.5% of the quarters during the dry period. The cure rate following the administration of tilmicosin was 67.3% and 72.5% for cows and quarters, respectively. By comparison, the cure achieved with cloxacillin was 56.9% and 62.9% for cows and quarters, respectively (Dingwell et al., 2003).

In the longer term, the prophylactic use of antibiotics in food-producing animals and the use of alternative mastitis control approaches such as the use of teat sealants to minimize IMIs during the dry period should to be investigated (Bradley and Green, 2000). The efficacy of internal teat sealants containing between 25 and 37%, wt/wt, bismuth subnitrate in preventing new dry period IMIs was demonstrated by Meaney (1976). Woolford et al. (1998) further demonstrated the efficacy of a reformulation of the teat sealant containing 65% wt/wt bismuth subnitrate in a paraffin base without antibiotic. They reported the sealant was better than a negative control and equivalent to a positive antibiotic dry cow therapy control in preventing new IMIs during the dry period and during the following lactation. Huxley et al. (2002) also evaluated the efficacy of the 65%, wt/wt, bismuth subnitrate sealant in preventing new IMIs during the dry-off period. They compared the efficacy with the antibiotic cephalosporin by assessing the number of

new IMIs acquired during the dry period. They found the number of quarters with major pathogens at drying off was 60, with the use of teat sealant 27, and with antibiotic 33. The cure rate was better with the sealant than with the antibiotic. Godden et al. (2003) conducted a study on the teat sealant, Orbeseal, containing 65% wt/wt, bismuth subnitrate in a paraffin base, that was used in combination with a dry cow intramammary antibiotic (orbenin-DC; cloxacillin 500 mg). This combination was compared to the use of the antibiotic alone. They suggested that quarters treated with Orbeseal and antibiotic at dry-off would be at lower risk for acquiring a new IMI between dry-off and calving compared with quarters treated with antibiotic alone. Godden et al. (2003) reported that quarters treated with Orbeseal and antibiotic were 30% less likely to develop new IMI between dry-off and 1 to 3 Days In Milk (DIM), 31% less likely to have an IMI present at 1 to 3 DIM, 33% less likely to experience a clinical mastitis event between dry-off and 60 DIM, with a significantly lower somatic cell linear score at both 1 to 3 and 6 to 8 DIM compared to quarters treated with antibiotic alone.

III. MATERIALS AND METHODS

Selection and Management of Cows for the Study:

To evaluate the long term use of hydrated lime for the control of environmental, mastitis pathogens in free stall bedding for lactating dairy cows, a 12-month study was conducted at the E.V.Smith Research Center Dairy at Shorter, Alabama. The cows were selected for the study based on DHIA somatic cell count records and two consecutive analyses of quarter, foremilk for SCC by the WMT test, and for microorganisms by blood agar culture. All 101 cows in the Holstein milking herd were evaluated for summary health status by DHIA records and by WMT and blood agar culture of their foremilk. For 12 months prior to initiation of the study the production records for the herd was 22,169 lbs of milk, 773 lbs of butterfat (3.5%) and 653 lbs of protein (2.9%). Thirty-two cows were selected from the herd of 101 based on WMT somatic cell counts of 200,000/ml or less for quarter foremilk. The selection of animals for the study was based on an SCC of 200,000 cells/ml or less because an SCC of 200,000/ml or more is considered to indicate an intramammary infection (Laevens et al., 1997; DeHaas et al., 2004; Schepers et al., 1997; Itavo et al., 2001; Smith, 1996). Using udder health and not production records the 32 selected cows were divided into two groups of 16 cows, each which comprised the control and treatment groups. Each group of 16 cows was housed in a free stall farm in a bay of 44 free stalls. With the 16 study group cows in a bay, 26 additional lactating cows were also housed and treated similarly as the study group, but their milk was not analyzed

on a monthly schedule as was the study group animals. The additional animals were housed with the study animals because space was not available to house the study groups of animals separately into two groups of 16. Thus, the setup was 16 control animals plus 26 additional animals in one bay with 44 free stalls. The treatment group of animals was housed in an adjacent bay of 44 free stalls with 26 additional animals. The cows in the two study groups ranged in age from 3 to 6 years. The allocation of the additional animals to the two-study group was done to evenly distribute the animals among the two study groups based on DHIA records, and WMT and blood agar culture of the quarter foremilk collected twice during a 4-week period prior to initiation of the study.

All cows were bedded in free stalls measuring 1.77 m (69 inches) long (Brisket rail to curb) by 1.26 m (49 inches) wide. The floor of the stalls was concrete with a 3% grade to the curb, covered with a foam mat, and this was covered with a waterproof, rubberized cover. Approximately 0.77 kg (1.7 lbs) of dry peanut hulls were placed in each free stall for both groups of cows including all the stalls in the bay in which the study animals were housed. In addition the treatment group of cows and their herd mates in one bay received daily about 50 grams (0.11 lbs) of hydrated lime in the back one-third of each stall. The hydrated lime was supplied by the Cheney Lime and Cement Company in Allgood, Alabama. The lime met American Water Works Association Standard B-202 for potable water treatment.

Each morning at 0600 h the cows of both study groups were moved to Bermuda grass paddocks for about 5 hours. The cows of the two study groups were kept separate and not permitted to commingle when moved to or from the paddocks. When the cows were in the paddocks, the free stalls were manually raked clean and fresh peanut hulls

were placed in the stalls. Line was sprinkled manually on the back one-third of the stalls for the treatment group of animals.

The cows were milked daily at 0200 h and again at 1300 h. The cows in each group were milked separately, with the lime-treatment group being milked before the control group of cows. Prior to milking, each teat was stripped, pre-dipped in 0.5% iodine solution (Pre-post 5000, manufactured by Chemland, Kansas City, Missouri), and 30 seconds after dipping the teats were wiped dry with two consecutive paper towels. The cows were milked in a double four-herringbone parlor into a 3-inch (0.07m) low milk line under 14 inches (0.36m) vacuum. The milking equipment was manufactured by WestFalia Surge. After milking the teats were post-dipped with a 1% iodine barrier solution (Blockade) manufactured by West Agro Inc, Kansas City, Missouri. The antibiotic used to treat mastitis in lactating cows was PIRSUE (Pirlimycin hydrochloride, 50mg/10ml syringe manufactured by Pharmacia/Upjohn, Kalamazoo, Michigan). For mastitis control in dry-off cows the antibiotic TOMORROW (Cephapirin benzathine, 300mg/10ml syringe manufactured by Fort Dodge Animal Health, Fort Dodge, Iowa) was used. The cow dry-off period was 6 weeks. All aspects of management for the two study groups of animals were the same except for the use of hydrated lime in the stalls of the treatment group of cows. The cows were fed a corn silage based total mixed ration. The 3.69 m (145.23 inch) wide alleyway in each bay of free stalls was water-flushed three times a day, when the cows were being milked in the afternoon, in the morning and again when the cows were moved to the Bermuda grass paddocks.

Experimental Design:

The experiment was conducted with two groups of Holstein cows. Each group consisted of 16 cows. With 16 cows in the control group (no lime treated freestalls) and 16 cows in the treatment group (lime-treated freestalls). The study was conducted for 12 months during which time quarter, foremilk was collected monthly and analyzed for SCC by the WMT procedure and for microorganisms by blood agar culture. Also DHIA composite milk SCC scores were collected monthly. Another part of the study was to evaluate the use of a teat-end sealant (Orbeseal, manufactured by Pfizer Chemical Health, New York, New York) administered with the antibiotic cephapirin at the time of cow dry-off to determine its efficacy to reduce the incidence of mastitis in cows during the dry period. All cows were administered the antibiotic and teat-end sealant except the lime-treated and non-lime treated groups which were administered only the antibiotic dry cow therapy. The quarter, foremilk of each cow in the lime-treatment and control groups was collected at least once a month, when milk sampling was carried out at the afternoon milking. For the sealant and non-sealant treated cows, two consecutive samples were collected before dry-off and again within a few days after the cows freshened.

The collection of udder quarter, foremilk samples was done in the following manner according to NMC (1990) guidelines. Sterile glass vials with tight fitting screw caps were used for collection of quarter, foremilk. Sample vials were labeled with a waterproof marker to identify the cow and quarter (e.g 140/LF, 140/LR, 140/RF, 140/RR). The teats were stripped, dipped into a 0.5% iodine disinfectant solution then dried with two consecutive paper towels. The germicide was allowed to remain on the teats for 20 to 30 seconds before removal. To avoid contamination of the teat ends during

milk sample collection, the near teats were sampled first then the far ones. The collection tube was held horizontally near the teat end. Three to four ml of milk were collected per vial and placed on ice for transport to the laboratory.

The quarter milk samples were cultured on blood agar plates immediately in the laboratory, and then stored at 4 C over night. The Wisconsin Mastitis Test was performed within 24 hours of collecting the milk.

Wisconsin Mastitis Test:

The Wisconsin Mastitis Test was performed to determine the SCC of the milk samples and to determine if the SCC was greater than 200,000 cells/ml, which indicated intramammary infection (Laevens et al., 1997; DeHaas et al., 2004; Schepers et al., 1997; Itavo et al., 2001; Smith, 1996). The test was conducted by combining 2 ml of WMT reagent (Bromocresol purple) with 2 ml of milk in a WMT tube fitted with a cap that has a small calibrated hole. The milk and reagent were mixed for 10 seconds. The mixture was drained through the cap of the WMT tube for 15 seconds and the tube was returned to an upright position. After one minute, the amount of fluid remaining in the tube was measured in millimeters and recorded. Table 4 presents the relationship between the WMT scores (mm) and the SCC of milk (Philpot, 1978). This table was used to convert the WMT scores to the numbers of SCC/ml of milk.

Culture of Milk Samples:

The common diagnostic schemes for collecting samples, isolating and identification of mastitis pathogens have been published by the National Mastitis Council (1999) in the Laboratory Handbook on Bovine Mastitis. Isolation of organisms was performed by plating one standard loop (0.01ml) of milk sample on the surface of a blood

agar plate, which were observed after 48 hours of incubation at 37 C. Colonies were tentatively identified, and a presumptive diagnosis of *Staphylococcus, Streptococcus,* coliforms and other bacteria was made based on colony growth, morphology and appearance. Gram stain and culture characteristics were used for primary identification for all isolates. Catalase test has done to further differentiate gram positive colonies of *Staphylococcus* and *Streptococcus*. Catalase positive test confirmed identification of *Staphylococcus*. Hemolysis was also helpful in identifying *Streptococcus* by the clearing zone around the colonies. Coliform bacteria were identified by selecting suspect colonies from blood agar and streaking for isolation on MacConkey agar (Difco). Red to pink colonies on MacConkey agar, indicating the ability to ferment lactose, were confirmed by gas production in Brilliant Green Lactose Bile broth (Difco).

Statistical Analysis:

Two sets of statistical analysis were performed for selected major pathogens and somatic cell counts to compare the control and treatment cows in two separate experiments, namely lime-treated versus non-lime treated cow groups (lime study) and sealant versus non-sealant cow groups (sealant study). Data were analyzed by fitting a linear model that included isolation of specified pathogens *Staphylococcus, Streptococcus*, coliforms and other bacteria, WMT scores of the four quarters, SCC of the four quarters, and DHIA SCC of the composite cow milk sample as the dependent variables. Independent variables included in the model were the fixed effects of treatment (lime and non-lime treated for lime study and sealant and non-sealant for sealant study), lactation number, and days in milk as a covariate with repeated measurements within cows as random effect. In the preliminary analyses the Proc MEAN (SAS, 2003)

procedure was performed to check the averages, standard deviations and standard errors of the dependent variables for the control and treated cow's records collected over a 12-month period. After checking for the homogeneity of variances among groups within each experiment, simple standard t-tests were carried out. The Proc MIXED (SAS, 2003) procedure was used to account for the random effect of repeated measures in the analyses to compute the analysis of variance (ANOVA), least squares means and probability (p-values) for fixed effects in the model by analyzing all dependent variables investigated in this study. Level of statistical significance was evaluated at $p < 0.05$ for all effects considered in the model.

IV. RESULTS AND DISCUSSION

Lime Study:

Estimation of Least Square Mean and Standard Error:

Effect of hydrated lime on somatic cell count

Hydrated lime was evaluated as a stall bedding treatment for dairy cows to limit the incidence of mastitis in the dairy herd. According to the National Lime Association website (www.lime.org), lime inhibits pathogens by controlling the environment required for bacterial growth. Calcium hydroxide (hydrated lime) is an alkaline compound that can create pH levels as high as 12.4. At pH levels greater than 12, the cell membranes of pathogens are destroyed. The high pH also provides a vector attraction barrier (i.e., prevents flies and other insects from infecting the treated biological waste). Because lime has low solubility in water, lime molecules persist in biosolids. This helps to maintain the pH above12 and to prevent regrowth of pathogens. In addition, when quicklime (calcium oxide, or CaO) is used, an exothermic reaction occurs in the presence of water. This heat release can increase the temperature of the biological waste to 70°C, which provides pasteurization and helps dry out the solid waste. When the number of bacteria in bedding is reduced this generally results in a decrease in the incidence of clinical mastitis caused by Gram-negative bacteria (Hogan and Smith, 2003).

In a study conducted by Hogan et al. (1999), hydrated lime reduced the bacteria count in recycled manure for 1 day. Kupprion et al. (2002) reported that hydrated lime treatment suppressed bacteria growth on days 1 and 2 with the antibacterial effect diminished on day 3 . They reported that the antibacterial activities of bedding treatments were related to the pH of the bedding materials (Hogan et al., 1999).

In the present study, peanut hulls were chosen as bedding materials for our study because they were commercially available, cost effective, biodegradable, and usable in most manure handling systems. All bedding materials including peanut hulls will support bacteria growth after becoming contaminated with manure (Blowey and Edmonson, 1995) and high levels of bacteria can contribute to mastitis. Previous studies on the use of lime for bedding treatment focused on the pH effect of pathogen inactivation. In contrast to determining environmental microbial loads, the present study was designed as an evaluation of hydrated lime as a bedding treatment for the long term effect on the incidence of mastitis as monitored by quarter milk SCC and blood agar culture of microbes.

Researchers have reported that an SCC >200,000 cells/ml of milk indicates an intramammary infection (Laevens et al., 1997; DeHaas et al., 2004; Schepers et al., 1997; Itavo et al., 2001; Smith, 1996). Based on this premise the SCC of foremilk from the lime-treatment and the control groups of cows over the 12-month study period was analyzed to determine the percent of intrammmary infections in each group of cows. A depiction of the percentage of the udder quarters with SCC> 165,000/ ml for the two groups of cows is shown in the Figure 1. The percent of quarters with SCC >165,000 was less for the lime bedded cows each month of the study compared to the control cows. For

the lime-treatment cow group the percentage of quarters with > 165,000 SCC varied from a high of 16.67% in April 2004, at the beginning of the study, to a low of 3.92% in March 2005 at the end of 12-month study. The least square mean of quarters with > 165,000 SCC for the lime-treatment group was 0.5457 (SE ± 0.1804) compared to 0.9684 (SE ± 0.1709) for the control group and the difference was 0.4227 (SE ± 0.2207) (Table 6). Based on this difference the numbers of quarters with SCC>165,000/ml of milk was decreased 43.65% for the lime-treatment cow group compared to the control group for the 12-month study. However, this difference was not significant at the P< 0.05 level (Table 6).

The percentage of quarters with SCC >165,000 had a greater monthly variation for the control cows than for the treatment group of cows (Figure 1). For the control group the percentage varied from a high of 33.9% for August 2004 to a low of 12.7% for June 2004. There appeared to be a bimodal distribution in the percentage of quarters in the control group with >165,000 SCC. One concentration of higher percentage occurred from March to May and another from August to October. The higher percentage that occurred during March to May might be related to wet and muddy conditions that occur during spring rains, and the higher percentage during August to October might be influenced by the calving season when the majority of the cows calf and are in the early stage of lactation. This is the time when most intramammary infections occur (Hogan and Smith, 1987).

An increase in the percentage of quarters with SCC >200,000 (Figure 2), >400,000 (Figure 3) and >750,000 (Figure 4) for the control group of cows also occurred in the late summer to early fall. The least square mean of quarters with > 200,000 SCC

for the control group was 0.8025 (SE ± 0.1391) compared to 0.4972 (SE ± 0.1477) for the lime-treatment cow group (Table 6). The difference between the control and treatment least square means estimate is 0.3053 (SE ± 0.1783) which is a 38.04% decrease in the number of quarters with SCC > 200,000 in the lime-treatment cow group (Table 6). The difference was not significant at the P< 0.05 level. The percentage of quarters with >200,000 SCC in the control group varied from a high of 32.73% for September 2004 to a low of 6.35% for June 2004 (Figure 2). For the >400,000 SCC level the least square mean was 0.5113 (SE ± 0.0874) for the control and 0.3536 (SE ± 0.0926) for the treatment cows (Table 6). The percent decrease in quarters with SCC>400,000/ ml for cows in lime treatment over the control cows was 30.84% (P< 0.1705). The least square mean of the quarters with >750,000 SCC for the control cows was 0.3821 (SE ± 0.0628) compared to 0.2022 (SE ± 0.0667) for treatment group (Table 6). The percent decrease in quarters with SCC>750,000/ml for cows in the lime-treatment group compared to the control cows was 47.08%. The current legal limit for bulk tank SCC is 750,000 cells/ml for Grade A milk producers (Miller et al., 2003). There was a significant decrease (P<0.0330) in the numbers quarters with SCC> 750,000 (Table 6) in the lime-treatment cow group, but no significance was observed at the other SCC levels. A graphic representation of the least square means for the four levels of SCC for the control and treatment cows is shown in Figure 5. At each SCC level there were more infected quarters in the control cows than in the lime-treatment cow group.

Based on SCC of foremilk from the lime-bedded and the control cow groups collected monthly over a 12-month period, the following overall results were obtained.

There were 51.87%, 49.32%, 40.20% and 68.12% fewer quarters in the lime-bedded cow group than in the control group at the SCC levels of >165,000/ml, > 200,000/ml, >400,000/ml and >750,000/ml, respectively. Which was approximately a 50% reduction over the four SCC levels (Appendix Table 1). According to statistical analysis on the same results there was 43.65%, 38.04%, 30.84% and 47.08% reduction in the numbers of quarters with SCC>165,000/ml, > 200,000/ml, >400,000/ml and >750,000ml in the lime-treatment cow (Table 6). This study proved that there was significantly less SCC >750,000 cells/ml in lime-treated cows, and the study revealed that the daily application of hydrated lime with freshly bedded peanut hulls in dairy cow free-stalls reduced the incidence of mastitis by approximately 45% (Table 6).

Data collected monthly by the Dairy Herd Improvement Association (DHIA) for the control and treatment cows were also used to determine the benefits of the lime bedding treatment. The least square mean of cows with > 165,000 DHIA SCC for the lime-treatment group was 0.3147 (SE ± 0.0787) compared to 0.3495 (SE ± 0.0742) for the control group (Table 7). The difference between the control and treatment least square means estimate was 0.0348 (SE ± 0.0947). Therefore, the mean percent decrease in the numbers of cows with DHIA SCC>165,000/ml for cows in the lime-treatment group was 9.96% compared to 43.65% for our laboratory findings (Table 6 and 7). The least square mean of cows with > 200,000 DHIA SCC for the lime-treatment group was 0.2346 (SE ± 0.0715) compared to 0.3495 (SE ± 0.0675) for the control group, with a difference of 0.1149 (SE ± 0.0863) (Table 7). The percent decrease in cow composite milk samples with DHIA SCC>200,000/ ml for cows in the lime treatment study was 32.88% compared to 38.04% of our laboratory finding. For the >400,000 DHIA SCC level the

least square mean was 0.2385 (SE ± 0.0550) for the control and 0.1763 (SE ± 0.0583) for the treatment cows (Table 7). The difference in the least square means estimates was 0.0622 (SE ± 0.0702), which was a 26.08% decrease in the number of cows with SCC>400,000/ ml for the lime-treatment group compared to 30.84% for our laboratory findings (Table 6 and 7). At the >750,000 SCC level the least square mean for the lime-treatment group was 0.1064 (SE ± 0.0463) compared to 0.1400 (SE ± 0.0437) for the control group (Table 7). This was a 24.00% decrease in the number of cows with SCC>750,000/ml for cows in the lime-treatment group compared to 47.08% for our findings. Based on DHIA SCC data there were no significant differences between the two groups of cows at any of the four SCC levels (Table 7). The DHIA data for the four SCC levels for the control and treatment cows is shown in Figure 6. The DHIA SCC data (Figure 6) is about half the magnitude of our SCC determined levels (Figure 5).

Non-statistical data for the four categories of DHIA SCC analysis are shown in Appendix Table 2. The decrease in the number of composite cow milk samples at SCC levels of >165,000/ml, > 200,000/ml, >400,000/ml and >750,000/ml for the lime-treated cows were 18.21%, 36.92%, 33.82% and 22.93%, respectively. This was an average 28% reduction over the four SCC levels (Appendix Table 2) compared to 50% reduction in quarter milk SCC (Appendix Table 1).

This difference is likely due to the method of milk sample collection. Our sample analysis was based on cow quarter milk samples whereas DHIA data are based on a composite milk sample of all four quarters. Franken et al. (1995) and Lam et al. (1996) reported that the sensitivity for detecting *S. aureus* from culture of a single composite milk sample from subclinically infected cows is only 58% to 63%. The low sensitivity is

thought to be due to the dilution effect of milk from uninfected quarters. The sensitivity for detecting *S. aureus* from a single quarter sample increased to 75% (Sears et al., 1990b).

During the study the numbers of quarters with SCC >165,000/ml, >200,000/ml, and >750,000/ml were compared at the beginning and at the end of the study for the control and treatment groups of cows. During the first month of the study, the difference between the two cow groups was low whereas the difference was much higher in the last month of the study (Figure 7). The difference in the least square means between the control and treatment cows quarters with > 165,000 SCC for the first month of study was 0.1250 (SE ± 0.4263), which was a 8.33% difference. The difference in the least square means at the > 165,000 SCC level for the last month of study was 0.9125 (SE ± 0.4334), which was a 69.52 % difference (Table 8). At the >200,000 SCC level least square means difference for the first month of the study was 0.1875 (SE ± 0.3743), which was a 27.27% difference between the two cow groups, whereas the difference for the last month of the study was 0.8583 (SE ± 0.3805), and this was a 76.29% difference (Table 8). At the > 750,000 SCC level for the first month of study the least square mean was 0.06250 (SE ± 0.1352), which amounted to a 50% difference, and for the last month of the study the difference was 0.4375 (SE ± 0.1374), resulting in a 100 % reduction (Table 8). The decrease in quarters with SCC >165,000/ml, > 200,000/ml, and >750,000/ml in the first month of study were not significant. However, there was a significant decrease of 69.52%, 76.29% and 100% ($P<0.0330$, $P<0.02$, $P<0.002$) in quarters with SCC >165,000/ml, > 200,000/ml, and >750,000/ml, respectively, in the last month of the study in the number of quarters at the three SCC levels for quarter milk from cows bedded on

the lime-treated peanut hulls (Table 8). Based on these data the lime-treated bedding reduced the number of quarters with SCC>165,000, >200,000 and >750,000. The first month and last month results are based on one sample analysis in the first and last month of the 12-month study, and therefore these data are only speculative.

Researchers have reported that organic bedding material such as chopped straw and fine sawdust naturally have some level of bacteria associated with them. In addition, shortly after placement in freestalls, the bedding becomes soiled with manure, leaked milk and other body fluids such as uterine discharges etc. Plenty of bacteria, nutrients and moisture contaminate the bedding to quickly seed and promote bacterial growth (Ingalls, 2000). Inorganic bedding such as sand on the other hand offers nothing for bacteria to metabolize since it is an inert silica material. It also allows water, urine and milk to drain reasonably well through it reducing the surface moisture and nutrient supply for bacteria (Ingalls, 2000). Adding lime to stall bedding is reported to slow bacteria growth for about 24 hours by raising the pH beyond the optimal range for bacterial growth, and by tying up some of the moisture. Within 24 hours, the effect deteriorates and bacterial growth resumes (Ingalls, 2000).

Since an increase in SCCs in milk is in response to infection of the udder, usually due to bacterial infection, and the addition of lime to cow bedding has been reported (Ingalls, 2000) to inhibit the growth of bacteria, it is therefore deduced that the lime inhibited the growth of bacteria which were responsible for the IMIs. A similar analysis of SCC data for the first and last month of the study was performed using DHIA data (Figure 8). The data reveal that the difference between DHIA SCC for the two bedding treatments was greatest at the >200,000/ml SCC level in the last month of the study.

Although the data reveal that there were fewer composite milk samples with >200,000/ ml SCC for the lime cow treatment group, the difference was not significant (Table 9). The difference in the least square mean between the control and treatment composite milk cows with >165,000 DHIA SCC for the first month of study was -0.0750 (SE ± 0.1544) which indicated that 37.50% less of the cows in the control group had DHIA SCC >165,000/ml. The differences in the least square means for the last month of study was 0.0476 (SE ± 0.1597), which was a 35.70% reduction due to the lime-treatment (Table 9). The least square means difference at the >200,000 DHIA SCC for the first month of study was 0.0083 (SE ± 0.1455), which indicated that there were 14.28% fewer cows with DHIA SCC >200,000/ml in the control cow group. However, in the last month of the study the least square means difference was 0.1190 (SE ± 0.1504), which indicated there were 100% fewer cows with >200,000 SCC/ml in the lime-treatment group (Table 9). The difference in the least square means between control and treatment cows with > 400,000 DHIA SCC for the first month of study was -0.0666 (SE ± 0.1079) which revealed that 6.97% fewer of the cows in the control cow group had DHIA, SCC >400,000/ml (Table 9). In the last month of the study the difference was 0.0571 (SE ± 0.1116), which showed that there were 28.55% fewer cows in the treatment cow group with SCC >400,000/ ml (Table 9). Although the DHIA data indicate there were fewer cows with SCC's >165,000, >200,000 and >400,000/ml for the lime-treatment cow group compared to the control cows for the last month of the study, statistical analysis of the data revealed no significant difference between the two cow bedding treatments.

Effect of hydrated lime on the incidence of mastitis in each quarter

The distribution of the incidence of mastitis among the four quarters of the bovine udder may lead to a better understanding of how mastitis spreads within the cow (Adkinson et al., 1993). Researchers report that *Staphylococcus*, *Streptococcus* and coliforms are the major causative organisms of mastitis (Bodman and Rice, 1995). Since this study was based on the analysis of quarter milk samples, the bacteriological data of each quarter was analyzed to determine how many times each quarter was colonized with *Staphylococcus*, *Streptococcus,* coliforms and other bacteria. The least square mean of the number of times, the left front quarter was colonized with microbes in the control group was 0.1978 (SE ± 0.0589) compared to 0.1537 (SE ± 0.0630) for the lime-treatment group (Table 10). The difference between the control and treatment least square means estimate was 0.0441 (SE ± 0.0750), which was calculated to be 22.31% fewer colonizations of the left front quarters of the cows bedded on the lime-treated bedding compared to cows bedding on peanut hull bedding without lime. However, this reduction was determined not to be significant. The left rear quarters had a treatment difference of 0.1383 (SE ± 0.0635) and 22.03% reduction in the number of colonized quarters for the lime-treatment versus the control cows. The right front quarters had a treatment difference of 0.2507 (SE ± 0.0770) and a 41.68 % reduction in the number of colonized quarters, and the right rear quarters had a difference of 0.3020 (SE ± 0.0863) with a 76.45 % reduction. The right rear had the highest treatment difference of the four quarters .The overall reduction in the number of colonized quarters for all four quarters was 58.68% for the lime versus the control cows.

Based on the least square means of the number of colonized quarters, the left rear, right front, and right rear had the most colonized quarters for the control cows (Table 10). The lime bedding treatment cows had a higher incidence of colonization on the left-side quarters compared to the right-side quarters. These data are highly speculative because a number of factors could influence the incidence of microbes in each of the four quarters. One factor is on which side does the cow lie. A cow that lies on the left side is likely to have the teats on the left side in contact with the bedding. High-producing cows with rigid udders provide less opportunity for teats to contact the bedding. However, a few studies report that there is a lower incidence of IMI and lower SCC in the front quarters than in the rear quarters (Barkema et al., 1997; Adkinson et al., 1993; and Kikkers et al., 2004) This study did not corroborate with their results.

The reduction in the number of colonized quarters for the lime-treatment cow group was highly significant for all quarters except the left front. The significance level in the left rear, right front and right rear was $P<0.03$, $P< 0.002$ and $P< 0.0015$, respectively (Table 10). The number of colonizations in the left rear, right front and right rear was more prevalent in the control group than in the lime-treated cow group. There was a 22.03%, 41.68% and 76.45% reduction in the number of colonizations in the left rear, right front and right rear quarters, respectively, for the lime-treatment cows. The colonization rate was highest in the right rear among the four quarters in the control group. Kikkers et al. (2004) reported that the incidence of mastitis for the rear quarters was always higher than for the front quarters. A graphical representation of the incidence of infection in each of the four quarters for the control and lime-treatment cows is shown in Figure 9.

Effect of hydrated lime on incidence of pathogens in quarter milk

The most common contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae*. Common environmental pathogens include *Escherichia coli, Nocardia, Klebsiella spp., Enterobacter spp., non-agalactiae Streptococcus spp., Citrobacter, Proteus, Pseudomonas* and *Serratia spp* (Bodman and Rice, 1995). Quarter milk samples were analyzed monthly to determine the incidence of quarters infected with *Staphylococcus, Streptococcus*, coliforms and other bacteria. Among the 631 total quarters for the non-lime cow group, 209 (33.1%) of the quarters were colonized with these four categories of microbes (Appendix Table 3). There were 611 quarters in the lime-treated cow group and 106 (17.33%) were colonized with microbes. There were 46.16%, 37.15%, 89.87% and 48.31% fewer quarters in the lime bedded cow group infected with *Staphylococcus, Streptococcus*, coliforms and other bacteria, respectively, than the non-lime bedded cows (Appendix Table 3).

The percentage of udder quarters colonized with *Staphylococcus* for the control and lime-treatment cow groups is shown in Figure 10. In the beginning two months of the study the percent of quarters colonized with *Staphylococcus* was high in both the control (avg 25.8%) and the lime-bedded cows (avg 25.8%). For the lime-treatment cow group the percentage of quarters with *Staphylococcus* varied from a high of 35% in May 2004, at the beginning of the study, to a low of 0% in September and November 2004 (Figure 10). The least square mean of quarters colonized with *Staphylococcus* for the lime-treatment group was 0.1232 (SE ± 0.1100) compared to 0.4578 (SE ± 0.1024) for the control group. The least square means estimate difference was 0.3346 (SE ± 0.1298)

which was a 73.08% decrease in *Staphylococcus* colonized quarters for the lime-treatment cow quarters over the control cow quarters (Table 11).

The percent of quarters colonized with *Streptococcus* was 0% in the first month of the study, and by the end of the study, the colonized quarter percentage was 18.18% for the control cows. For the lime bedding treatment cows 3.33% of the quarters were colonized during the first month of the study, the highest infection rate was 21.43% during June 2004, and in the last two-months of study 0% quarters were colonized with *Streptococcus* (Figure 11). The least square mean of quarters colonized with *Streptococcus* for the lime-treatment group was 0.2968 (SE ± 0.1211) compared to 0.4566 (SE ± 0.1133) for the control group. The least square mean difference was 0.1598 (SE ± 0.1440) amounting to a 34.99% reduction in the *Streptococcus* colonized quarters for the lime-treatment cows (Table 11). The percentage of the udder quarters colonized with coliforms for the two groups of cows is shown in Figure 12. During the first month of study, the percent of quarters colonized with coliforms was 0% for both the control and the lime-bedded cows. For the lime-treatment cow group the highest percentage of quarters with coliforms was 9.8 % in December 2004 and the lowest was 0% in April, July, August, September and October 2004, and January, February and March of 2005 (Figure 12). The highest prevalence rate of coliforms in the control cow group was 14.29%, whereas a 0% prevalence occurred in the same month for the lime bedded cows. The least square mean of quarters colonized with coliforms for the lime-treatment group was 0.0446 (SE ± 0.0450) compared to 0.1646 (SE ± 0.0418) for the control group. The least square means estimate difference was 0.1199 (SE ± 0.0528) which was determined

to be a 72.84% reduction in coliforms colonized quarters for the lime-treated cow bedding group (Table 11).

An illustration of the percentage of quarters colonized with other bacteria (other than *Staphylococcus, Streptococcus* and coliforms) for the two groups of cows is shown in Figure 13. The highest prevalence rate of other bacteria was 18.18% in September for the control group cows, whereas in the treatment group the highest prevalence rate was 9.3% in the same month. The least square mean of the number of quarters colonized with other bacteria for the lime-treatment group was 0.0473(SE ± 0.0604) compared to 0.1816 (SE ± 0.0562) for the control group, and the difference was 0.1343 (SE ± 0.0707) which was a 73.95% decrease in other bacteria colonized quarters for the lime-treatment cow group (Table 11, Figure 14).

In our study *Staphylococcus* followed by *Streptococcus* were the principal mastitis pathogens isolated from the control cow group, whereas *Streptococcus* followed by *Staphylococcus* were the principal pathogens for the treatment cow group (Table 11). The infection rate for *Staphylococcus, Streptococcus,* coliforms and other bacteria was less in the lime-treated cow group than in the control group. Based on statistical analysis of microbe colonized quarters, there was a 73.08% differnce in *Staphylococcus* colonized quarters for the lime-treatment cow quarters over the control cow quarters (Table 11). *Streptococcus*, coliforms and other bacteria were 34.99%, 72.84% and 73.95%, respectively, lower in the lime-bedded cows (Table 11). Among the four categories of bacteria the decrease was significant for *Staphylococcus* ($P< 0.01$) and for coliforms ($P<0.03$) infected quarters.

During the study, the numbers of quarters colonized with *Staphylococcus, Streptococcus* and other bacteria were compared between the first month and the last month of the study for the control and treatment groups of cows (Table 12). Between the first and last month of the study, the reduction rate for *Staphylococcus* colonized quarters was high (Figure 15). Likewise the reduction rate for the *Streptococcus* infection was very high during the last month. The prevalence rate of other bacteria was lower in the first month than in the last month of the study. The differences in the least square mean between control and lime-treatment cows for *Staphylococcus* colonized quarters for the first month of study was 0.6250 (SE ± 0.2980) which indicated that there were 50% fewer quarters with *Staphylococcus* in the lime-treatment cow group. The difference in the least square means between control and treatment cows for *Staphylococcus* colonized quarters for the last month of study was 0.1792 (SE ± 0.3029) which indicated there were 57.34% fewer *Staphylococcus* colonized quarters in the lime-treatment cow group. The estimate mean difference of *Streptococcus* colonized cows in the control and treatment cow groups for the first month of study was -0.1250 (SE ± 0.2854) which means there were more *Streptococcus* colonized quarters in the lime-treatment group. The differences in the least square mean between the control and treatment cows for *Streptococcus* colonized quarters for the last month of study was 0.6083 (SE ± 0.2901) which means there were 69.52% fewer *Streptococcus* colonized quarters of the lime-treatment cow group. The differences in the least square mean between control and treatment cows for other bacteria colonized quarters for the first month of study was 0.1250 (SE ± 0.1123) which indicated that all of the infections were in the control cow group (Table12).

The difference in the *Staphylococcus* infection occurrence was significant (P<0.04) during the first month of the study but not during the last month of study. There was a significant (P<0.04) difference in the occurrence of *Streptococcus* in the last month of the study for the lime treatment. The effect of the lime bedding treatment on the incidence of mastitis pathogens in the quarters of the two groups of study cows is probably best determined at the end of the study when the bedding treatments have been imposed for 12-months. Although the data indicate a 57.3% lower incidence of *Staphylococcus* infections in the lime bedding cow groups, the reduction was not significant due to the high variation in the data. However, there was a 69.5% lower incidence of *Streptococcus* in the lime bedding cow group which was significant (P< 0.04).

The National Mastitis Council did a survey on 4,957 cows from 67 herds in 14 states in the USA (Hogan and Smith, 1997b). It was reported that the prevalence of environmental streptococci and *S. aureus* infections were equal. Environmental streptococci were found in 11.5% of cows and 3.9% of the quarters, and *S. aureus* was present in 11.5% of the cows and 4.0% of quarters. Coliforms were detected in 5.0% of the cows and 1.3% of the quarters and *Streptococcus agalactiae* was present in 6.5% of cows and 4.3% of quarters (Hogan and Smith, 1997b). Heifers in 28 herds from California, Washington, Louisiana, and Vermont were sampled around breeding time and within four days of calving. Infection rates caused by environmental pathogens increased from 1.5 to 7.7% of the quarters over this period, while *S. aureus* infections were 2.8% (Fox et al., 1995).

This study revealed higher levels of *S. aureus* infections in the two cow bedding treated groups than reported by Hogan and Smith (1997b). They reported that among

4,957 cows they surveyed 4% of the quarters were infected with *S. aureus* and 1.3% with coliforms. For this study 14.6% of the quarters were infected with *S. aureus* and 1.3% with coliforms. For this study 14.6% of the quarters of the non-lime cow bedding group were infected with S. aureus, 3.2% with coliforms, and for the lime bedding cow group 7.9% were infected with *S. aureus* and 0.3% with coliforms. Although this study reported higher levels of *S. aureus* infection, even in the lime bedding cow group, than reported by Hogan and Smith (1997b), the incidence of the coliforms in this study in the lime bedding cow group was lower. Perhaps the lime which is reported to control environmental pathogens such as coliforms (Hogan and Smith, 1987) accounted for the lower incidence of coliforms infections in the lime bedding cow group.

As with any business, economics in dairying is an important concern. The economic benefit of the lime treatment in terms of decreasing the incidence of mastitis and its impact on milk loss was calculated to be $42.50/cow/year (Table 21).

Sealant Study:

Effect of sealant on somatic cell count

Invasion of pathogens into the mammary gland during the dry period may occur before the formation of a complete keratin plug in the streak canal (Comalli et al., 1984; Williamson et al., 1995; Dingwell et al., 2003). Dingwell et al. (2003) reported that 50% and 23% of teat ends were still open after 1 and 6 wk into the dry period, respectively. The latter study reported that quarters that remained open and quarters that had cracked teat-ends were 1.7x more likely to develop new IMIs during the dry period compared to quarters that were closed and not cracked (Dingwell et al., 2003). The practice of dry cow therapy with a long-acting antibiotic at dry-off has been successful in curing many

existing subclinical infections as well as offering short-term protection against new IMIs when susceptible pathogens invade the gland during the early dry period (Natzke, 1981; Browning et al., 1990; Bradley and Green, 2001). However, new IMIs may still occur if invading pathogens are not sensitive to the active ingredients in the antibiotic preparations being used and/or the antibiotic does not persist at therapeutic levels throughout the entire dry period (Smith et al., 1985; Bradley and Green, 2001).

Corollary to the lime bedding treatment study, a study was conducted to determine the effectiveness of an internal teat sealant Orbeseal containing bismuth subnitrate in protecting quarters against new dry period IMI caused by major mastitis pathogens, particularly *Staphylococcus*, *Streptococcus* and coliforms. The teat sealant study explored differences in quarter foremilk of cows for SCC and blood agar culture prior to dry-off and immediately after freshening for cows administered the teat sealant compared to cows not receiving the sealant. The incidence of IMI was determined by monitoring the SCC of quarter milk and udder quarters with >200,000/ml was indicative of an intramammary infection (Laevens et al., 1997; DeHaas et al., 2004; Schepers et al., 1997; Itavo et al., 2001; Smith, 1996). *Staphylococcus*, *Streptococcus* and coliforms were the major pathogens of our interest.

The length of the dry period has been documented as an important factor in the development of new IMI (Enevoldsen and Sorensen, 1992). In a UK study, 12.8% of quarters acquired new enterobacterial infections during the dry period (Bradley and Green, 2000). The primary source of bacterial challenge during the dry period was from the environment (Eberhart, 1986). The early and late dry periods are the times of greatest

risk (Smith et al., 1985). Therefore, attention should be paid to minimizing the exposure of teat-ends to environmental organisms by maintaining dry cows in a clean and dry area. The efficacy of internal teat sealers containing between 25 and 37%, wt/wt, bismuth subnitrate in preventing the acquisition of new dry period IMI was demonstrated in the 1970s (Meaney, 1976). The efficacy of a reformulation of a teat sealant containing 65%, wt/wt, bismuth subnitrate in a paraffin base without antibiotic was significantly better than a negative control and equivalent to a positive antibiotic dry cow therapy in preventing new IMI during the dry period and in reducing new mastitis cases during the following lactation (Woolford et al., 1998). In another study to assess the efficacy of 65%, wt/wt, bismuth subnitrate compared with the antibiotic containing a cephalosporin for preventing new IMIs acquired during the dry period, 60 quarters were infected with major pathogens at drying off, 27 with the teat sealant and 33 treated with antibiotic (Huxley et al., 2002). In a combined study with the teat sealant Orbeseal (65%, wt/wt, bismuth subnitrate) and a dry cow intramammary antibiotic, researcher suggested that quarters treated with Orbeseal and DCT at dry-off would be at lower risk for acquiring new IMI between dry-off and calving compared with quarters treated with antibiotic alone. Radiographical examination of teats treated with a teat sealant has demonstrated the presence of the teat sealant after 100 days of infusion (Woolford et al., 1998).

An SCC >200,000 cells/ml of milk indicates an intramammary infection (Laevens et al., 1997; DeHaas et al., 2004; Schepers et al., 1997; Itavo et al., 2001; Smith, 1996). Based on this same premise as lime study the SCC of foremilk from the sealant-treatment and the non-sealant groups of cows over the 12-month study period was analyzed to determine the percentage of intrammmary infections in each group of cows. The teat

sealant was administered at the last milking prior to dry-off. Non-sealant cows consisted of both lime and non-lime treated cows, and the sealant-treated cows consisted of all other cows in the lime and non-lime bedding group of cows. There were 54 sealant-treated cows and 32 non-sealant treated cows. All quarters of cows in both groups were infused with the antibiotic TOMORROW (Cephapirin benzathin, 300mg, Fort Dodge animal Health) at dry-off.

The percent reduction in the number of quarters with SCCs >165,000/ml before and after the dry period are shown in Table 13. For the non-sealant, cow treatment group 103 quarter milk samples were analyzed for SCC by the WMT method prior to dry-off and 87 samples were analyzed after dry-off. The percent reduction in the number of quarters with >165,000 SCC before and after dry-off was 80.92% for the non-sealant cow group. For the sealant cow group 178 quarters were analyzed before dry-off and 177 quarters after dry-off. The percent reduction in the number of quarters with >165,000 SCC before and after dry-off in the sealant cow group was 66.05%. Therefore, the benefit of the non-sealant was a reduction of 18.37% in the number of infected quarters. At the >200,000 SCC level the reduction was 76.34% for the non-sealant cow group and 61.32% for the sealant group (Table 13). The overall reduction attributed to the non-sealant treatment was 19.67%. At the >400,000 SCC level the overall reduction was 22.38% and at the >750,000 SCC level the overall reduction was 25.34% in non-sealant cow group. These results suggest there was no benefit observed in reducing the SCC of foremilk by using the teat sealant (Table 13). A graphical representation of Table 13 is shown in Figure 16.

The least square mean of quarters with >165,000 SCC for the sealant-treatment group before dry-off was 1.7442(SE ± 0.1762) and after dry-off 0.5952 (SE ± 0.1781) compared to 1.0909 (SE ± 0.2461) before dry-off and 0.2500 (SE ± 0.2581) after dry-off for the non-sealant group (Table 14). The difference between the before and after dry-off least square mean in the sealant-treated group was 1.1481 (SE ± 0.2504) and in non-sealant group 0.8401 (SE ± 0.3566) .The percentage decrease in quarters with SCC>165,000/ ml for cows in sealant-treatment after the dry period was 65.88% compared to 77.08% for the non-sealant cow group. At the > 200,000 SCC level the difference between the before and after dry-off least square mean in the sealant-treated group was 0.8937 (SE ± 0.2352) and in the non-sealant group 0.6591 (SE ± 0.3349) (Table 14). The group percentage decrease in the number of quarters with SCC>200,000/ml for cows in the sealant-treatment group after dry period was 60.99% compared to 72.50% for the non-sealant cows. At the >400,000 SCC level the difference between the before and after dry-off least square mean in the sealant-treated group was 0.3383 (SE ± 0.1895) which was a 42.98% decrease in quarters. For the non-sealant group the difference was 0.1636 (SE ± 0.2698) (Table 14), which was a 44.99% decrease in quarters with SCC>400,000/ ml. The least square mean of quarters with >750,000 SCC for the sealant-treatment group before dry-off was 0.6279(SE ± 0.1181) and after dry-off 0.3333 (SE ± 0.1195) compared to 0.2273 (SE ± 0.1651) before dry-off and 0.1500 (SE ± 0.1732) after dry-off for the non-sealant group. The difference between the before and after dry-off least square mean for the sealant-treated group was 0.2946 (SE ± 0.1680) and in non-sealant group 0.0772 (SE ± 0.2393) (Table 14). The percentage decrease in the number of quarters with SCC>750,000/ ml for cows in the

sealant-treatment after dry period was 46.91% compared to 33.96% for the non-sealant cows. A graphical representation of the data in Table 14 is shown in Figure 17.

There was a slight improvement in lowering the SCC of cows on the teat sealant treatment. The difference (improvement) before and after dry-off for sealant cows compared to the non-sealant cows was less than 5% at each of the four SCC levels (Table 13). However, based on statistical data (Table 14), there was a slight improvement in favor of the non-sealant cow group. Both the sealant and non-sealant cows showed lowering of the SCC at calving compared to before dry-off. However, the reduction was greater for the non-sealant cows. The percent decrease in quarter SCC before and after dry-off for the non-sealant cows was significant ($P<0.0199$) at the >165,000 SCC level, but not at the higher SCC levels. Whereas the decrease was significant for the sealant cow group at both the >165,000 ($P<0.001$) and >200,000 ($P<0.0002$) SCC levels (Table 14). These data indicate that there was a decrease in the number of quarters with elevated SCC after dry-off for both groups of cows, but no statistical analysis was made across groups to determine whether the sealant treatment offered any benefit in reducing the incidence of mastitis.

Perhaps the assignment of all the lime bedding study cows to the non-sealant cow group confounded the study making an interpretation of the data more difficult. Due to constraints in housing the animals, it was not possible to avoid using some of the animals in both the lime and teat sealant studies.

The percent reductions in the number of cows with DHIA SCCs >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml are shown in Table 15. For the non-sealant, cow treatment group 26 cow DHIA records were examined for SCC data prior to dry-off

and 22 cow records were examined after dry-off. For the sealant cow group 46 cow DHIA records were examined before and after dry-off. The percent reduction in the number of cows with >165,000 SCC was 46.28% for the non-sealant cows, and 7.98% for the sealant cows. This shows there was a 82.75% greater decrease in the non-sealant cow group compared to the sealant group. At the >200,000 SCC level the reduction was 70.44% for the non-sealant cow group and 0% for the sealant group which was a 100% greater reduction in the non-sealant cow group (Table 15). These results indicate there was a higher percent reduction for the non-sealant cow groups at the SCC levels of >165,000 and >200,000 DHIA SCC/ml of milk. At the >400,000 SCC level there was a 40.89% and 30.00% reduction in the number of cows for the non-sealant and sealant-treatment cow group, respectively. At the >750,000 DHIA SCC level the sealant cow group showed a slight benefit with an overall 7.89% reduction associated with using the teat sealant (44.47% reduction) compared to the non-sealant treated cows (40.96% reduction). These results suggest there was a mix benefit in reducing the DHIA SCC of milk by using the teat sealant (Table 15). A graphical representation of Table 15 has shown in Figure 18.

The least square means and standard errors for the DHIA somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml for the non-sealant and sealant cow groups are shown in Table 16. A graphical representation of Table 16 is shown in the Figure 19. There was no significant effect between the sealant and non-sealant groups of cows based on the DHIA SCC data.

Effect of sealant on the incidence of mastitis in each quarter

Since this study was based on quarter milk samples, all four quarters were analyzed to determine how many times the quarters for the lime-treatment and non-lime treatment cow groups were colonized with microbes.

The least square mean difference for the left front (LF) quarter before and after dry-off for cows in the sealant group was 0.1368 (SE ± 0.0849) and in the non-sealant group 0.3539 (SE ± 0.1210) (Table 17). The percentage decrease in the number of colonized quarters of the sealant-treated cows after the dry period was 53.47% compared to 87.77% for the non-sealant cows. In the left rear (LR) quarter the difference in the least square mean between before and after dry-off in the sealant cow group was 0.1700 (SE ± 0.0922) with a 48.87% decrease and in the non-sealant group 0.1182 (SE ± 0.1360) with a 37.14% decrease (Table 17). For the right rear (RR) quarter the percentage decrease in colonized quarters for cows in the sealant-treatment after dry period was 50.00% compared to 44.72% in non-sealant cows. The difference in the least square mean for all four quarters before and after dry-off in the sealant cow group was 0.7076 (SE ± 0.2658) resulting in a 55.32% decrease and in the non-sealant group 0.8455 (SE ± 0.3785) which was a 54.70% decrease (Table 17).There was no significant reduction in the occurrence of infection in any of the four quarters of the sealant or non-sealant treated cow groups. A graphical representation of the data is shown in Figure 20.

Effect of sealant on incidence of pathogens in quarter milk

The percentage of the number of times quarters were colonized with *Staphylococcus* before dry-off for the non-sealant treatment group was 9.70% compared to 22.47% for the sealant group (Table 18). After dry-off *Staphylococcus* colonized

1.14% of the quarters of the non-sealant cow group compared to 16.94% for the sealant group, which is a 72.11% overall reduction in the non-sealant group. *Streptococcus* colonized 17.47% of the quarters before dry-off for the non-sealant treatment group compared to 6.74% for the sealant group, and after dry-off *Streptococcus* colonized 14.94% of the quarters of the non-sealant cow group compared to 5.08% for the sealant group, which is a 41.18% reduction due to the sealant treatment. Before dry-off 2.91% of the non-sealant cows had quarters colonized with coliforms compared to 0.56% for the sealant group. After dry-off 2.29% of quarters of the non-sealant cows had coliforms, and none of the sealant cow quarters were colonized with coliforms. The overall reduction due to the sealant treatment was 78.7%. Other bacteria colonized 8.73% of the quarter of the non-sealant cows and 3.37% of quarters of the sealant cow group before dry-off and 2.29% quarters were colonized after dry off in non-sealant treated cows and 0.56% cows were colonized after dry-off in sealant-treated cows. There was a 11.53% reduction due to the sealant treatment in the number of other bacteria colonized quarters. Based on the overall difference between the sealant and non-sealant cow groups, the percent reduction in the number of quarters harboring the four bacterial groups, excluding *Staphylococcus,* was higher in the sealant cow group (Table 18). A graphical representation of the data in Table 18 is shown in Figure 21.

The least square mean of the number of times the quarters were colonized with *Staphylococcus* for the sealant-treatment group before dry-off was 0.9073(SE ± 0.1306) and after dry-off 0.2619 (SE ± 0.1321) compared to 0.4545 (SE ± 0.1825) before dry-off and 0.0500 (SE ± 0.1914) after dry-off for the non-sealant group (Table 19). The difference between the before and after dry-off least square means for the sealant cow

group was 0.6451 (SE ± 1.1857) and 0.4045 (SE ± 0.2645) for the non-sealant group. The decrease in quarters colonized with *Staphylococcus* before and after dry-off was 71.12% for the sealant-treated cows and 88.99% for the non-sealant cows. The least square mean for quarters colonized with *Streptococcus* for the sealant-treatment group before dry-off was 0.2826(SE ± 0.1210) and after dry-off 0.2222 (SE ± 0.1223) compared to 0.6818 (SE ± 0.1749) before dry-off and 0.4500 (SE ± 0.1834) after dry-off for the non-sealant group. The difference between before and after dry-off least square mean for the sealant cow group was 0.0603 (SE ± 0.2535) and for non-sealant group 0.2318 (SE ± 0.1720) (Table 19). The percent decrease before and after dry-off for quarters colonized with *Streptococcus* was 21.33% for the sealant-treated cows and 33.99% for the non-sealant cows. The least square mean of the number of times quarters colonized with coliforms for the sealant-treatment group before dry-off was 0.0222(SE ± 0.0384) and after dry-off 0.0444 (SE ± 0.0384) which is a difference of - 0.0222 (SE ± 0.0543) compared to 0.0909 (SE ± 0.0549) before dry-off and 0.1000 (SE ± 0.0576) after dry-off with a difference of -0.0090 (SE ± 0.0796) for the non-sealant group (Table 19). The decrease in quarters colonized with coliforms was –50.00% in the sealant cow group, compared to –9.00% in the non-sealant cows. The least square mean of quarters colonized with other bacteria for the sealant cow group before dry-off was 0.1364(SE ± 0.0643) and after dry-off 0.0227 (SE ± 0.0643). For the non-sealant group the least square mean was 0.3333 (SE ± 0.0931) before dry-off and 0.1053 (SE ± 0.0979) after dry-off. The difference between the before and after dry-off least square mean in sealant treated group was 0.1136 (SE ± 0.0910) and for the non-sealant group 0.2281 (SE ± 0.1352) (Table 19). Based on these data the percent decrease in the number of quarters colonized by other

bacteria was 83.24% for the sealant cow group, compared to 68.43% for the non-sealant group. A graphical representation of Table 19 is shown in the Figure 22.

The statistical data verify the greater reduction for *Staphylococcus* and *Streptococcus* for the non-sealant cows compared to the sealant-treated cows. But the reduction in the incidence of *Staphylococcus* before and after the dry-off was highly significant ($P<0.0007$) for the sealant-treated cows and not for any of the bacterial groups in either the sealant or non-sealant groups. However, none of the other data involving the four groups of microbes were significantly related to the sealant and non-sealant treatments. Based on this study the benefit of using Orbeseal as a treatment to minimize intramammary infections during the dry period is not certain and needs further study. These data do not indicate a clear benefit for using the Orbeseal teat sealant. More studies over a longer period of time need to be conducted to evaluate the economic benefit of using the teat sealant to prevent new IMI during the dry period.

The benefit of Orbeseal teat sealant, which is a mixture of bismuth subnitrate in a paraffin base, to minimize the occurrence of new IMIs during the dry period has been reported with and without the use of intramammary dry cow antibiotics. One study compared the used of Orbeseal (n=197 cows) to a non-sealant group (n= 204 cows) that received no dry cow therapy (Berry and Hillerton, 2002). Only cows that had SCC of <200,000 cells/ml and no history of clinical mastitis were used in the trial. Orbeseal significantly reduced clinical mastitis during the dry period (0% for Orbeseal versus 3% for non-sealant cows) and reduced the rate of new subclinical infections detected at calving (12% for Orbeseal versus 45% for non-sealant cows).

V. CONCLUSIONS

The addition of 50 grams of hydrated lime daily to the back 1/3 of dairy cow free-stalls bedded daily with 0.77 kg of fresh peanut hulls reduced the incidence of mastitis in quarters of the cows using the lime-bedded stalls by approximately 45%. The cost to lime treat one stall was calculated to be $.011/day, or $4.02/cow/year. The economic benefit of the lime treatment was estimated to improve milk production by $42.51 ($46.53 - $4.02) /cow/year.

The outcome of the study to determine the benefit of using the teat sealant Orbeseal as a treatment to minimize intramammary infections during the dry period was not certain and needs further study. However, there was a significant reduction of 60.99% ($P< 0.0002$) in the number of infected quarters at 200,000 SCC/ml level within the sealant- treatment cow group. These data do not indicate a clear benefit for using the teat sealant. More studies need to be conducted over two or more dry periods to evaluate the economic benefit of using the teat sealant to prevent new intramammary infections during the dry period.

VI. TABLES AND FIGURES

Table 1. Estimated cost of annual losses due to mastitis. *

Sources of loss	Loss per Cow ($)	Percent of Total (%)
Reduced production	121.00	66.00
Discarded milk	10.45	5.70
Replacement cost	41.73	22.60
Extra labor	1.14	0 .10
Treatment	7.36	4.10
Veterinary services	2.72	1.50
Total	184.40	100.00

*Assumptions: One-third of cows infected in an average of 1.5 quarters; milk loss 856 lbs per infected quarter; milk price $12.07 per hundred weight.
Source: National Mastitis Council, 1996.

Table 2. The results of bacteriological examination samples of milk in farm B.

Season	Number of samples	Samples with			Pathogenic bacteria	%*
		No bacteria growth	Growth	Pathogenic		
Autumn	77	29	48 62.3%	46 59.7%	*S. aureus* *S. agalactiae* *A. pyogenes*	95.6%
Winter	61	41	30 49.2%	19 31.1%	*S. aureus* *E. coli*	63.3%
Spring	15	3	12 81.3%	6 40.0%	*S. aureus* *S. agalactiae*	50.0%
Summer	42	16	26 61.9%	21 50.0%	*S. aureus* *S. agalactiae* *A. pyogenes*	80.8%
All	195	89	116	92		

* Percent of samples with growth of pathogenic bacteria in all samples contaminated with microorganisms

Source: Molenda et al., 2003

Table 3. Approximate ranges in somatic cell counts for California mastitis test scores.

Score	Somatic Cell Range (SCC/ml)		
N	0	to	200,000
T	200,000	to	400,000
1	400,000	to	1,200,000
2	1,200,000	to	5,000,000
3	Over 5,000,000	to	

Source: Jasper, 1967

Table 4. Relationship between somatic cell count linear score, somatic cell counts and estimated daily milk losses.

SCC Linear Score	SCC range (SCC/ml)			Estimated Daily Milk Loss (lbs)
0	0	to	18,999	0
1	19,000	to	35,999	0
2	36,000	to	71,999	0
3	72,000	to	141,999	1.5
4	142,000	to	283,999	3
5	284,000	to	565,999	4.5
6	566,000	to	1,130,999	6
7	1,131,000	to	2,262,999	7.5
8	2,263,000	to	4,523,999	9
9	4,524,000	to	>9,999,999	10.5

Source: Shook and Seaman, 1983

Table 5. Relationship between Wisconsin mastitis test scores and somatic cell counts (1,000's/ml).

WMT (mm)	Somatic Cell Count	WMT (mm)	Somatic Cell Count
3	140	21	990
4	165	22	1050
5	195	23	1130
6	225	24	1200
7	260	25	1280
8	300	26	1360
9	340	27	1440
10	380	28	1525
11	420	29	1610
12	465	30	1700
13	515	31	1800
14	565	32	1930
15	620	33	2030
16	675	34	2180
17	730	35	2280
18	790		
19	855		
20	920		

Source: Philpot, 1978

Table 6. Least square means and standard errors for quarters with somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml in control and lime-treatment cow groups.

SCC/ml (x1000)	Control		Treatment		Difference		Reduc -tion	P value
	Estimate	± SE	Estimate	± SE	Estimate	± SE	%	Pr > t
>165	0.9684	0.1709	0.5457	0.1804	0.4227	0.2207	43.65	0.0650
>200	0.8025	0.1391	0.4972	0.1477	0.3053	0.1783	38.04	0.0971
>400	0.5113	0.0874	0.3536	0.0926	0.1577	0.1123	30.84	0.1705
>750	0.3821	0.0628	0.2022	0.0667	0.1799	0.0804	47.08	0.0330

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

Table 7. Least square means and standard errors for DHIA somatic cell count of milk for control and lime-treatment cow groups for the 12-month study.

SCC/ml (x1000)	Control		Treatment		Difference		Reduc -tion	P value Pr > t
	Estimate	± SE	Estimate	± SE	Estimate	± SE	%	
>165	0.3495	0.0742	0.3147	0.0787	0.0348	0.0947	9.96	0.3850
>200	0.3495	0.0675	0.2346	0.0715	0.1149	0.0863	32.88	0.1934
>400	0.2385	0.0550	0.1763	0.0583	0.0622	0.0702	26.08	0.3831
>750	0.1400	0.0437	0.1064	0.0463	0.0336	0.0559	24.00	0.5526

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

Table 8. Least square means and standard errors for quarters with somatic cell counts >165,000/ml, >200,000/ml and >750,000/ml for the control and lime-treatment cow groups in the first and last month of study.

SCC/ml (x1000)	Differences of Least Square Mean in the Control and Treatment cows in first month				Differences of Least Square Mean in the Control and Treatment cows in Last month			
	Estimate	± SE	%	P value Pr > t	Estimate	± SE	%	P value Pr > t
>165	0.1250	0.4263	8.33	0.7704	0.9125	0.4334	69.25	0.0395
>200	0.1875	0.3743	27.27	0.6183	0.8583	0.3805	76.29	0.0278
>750	0.0625	0.1352	50.00	0.6455	0.4375	0.1374	100.00	0.0023

Foot note: Table 7 derived from Appendix Table 4.

Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

Table 9. Least square means and standard errors for quarters with DHIA somatic cell counts >165,000/ml, >200,000/ml and >750,000/ml for the control and lime-treatment cow groups in the first and last month of study.

SCC/ml (x1000)	Differences of least square mean between the control and treatment cows in first month				Differences of least square mean between the control and treatment cows in last month			
	ED	± SE	%	P value Pr > t	ED	± SE	%	P value Pr > t
>165	-0.0750	0.1544	-37.50*	0.6291	0.0476	0.1597	35.70	0.7667
>200	-0.0083	0.1455	-14.28	0.9545	0.1190	0.1504	100.00	0.4320
>400	-0.0666	0.1079	-6.97	0.5392	0.0571	0.1116	28.55	0.6105

Foot note: Table 8 derived from Appendix Table 5.

ED stands for Estimated Difference.

* Negative percent indicates that there were fewer cows with SCC> 165,000, >200,000 and > 400,000 in the control cow group compared to the lime-treatment group.

Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

Table 10. Least square means and standard errors for number of quarters colonized with microbes for control and treatment cows during the 12-month study.

Quarter position	Control		Treatment		Difference		Reduction	P value
	Estimate	± SE	Estimate	± SE	Estimate	± SE	%	Pr > t
LF	0.1978	0.0589	0.1537	0.0630	0.0441	0.0750	22.31	0.5608
LR	0.3318	0.0503	0.1935	0.0541	0.1383	0.0635	22.03	0.0374
RF	0.3279	0.0603	0.0772	0.0642	0.2507	0.0770	41.68	0.0028
RR	0.3871	0.0682	0.0851	0.0733	0.3020	0.0863	76.45	0.0015
Total	1.1855	0.1811	0.4899	0.1926	0.6956	0.2316	58.68	0.0053

Foot note: LF stands for Left Front, LR stands for Left Rear, RF stands for Right Front and RR stands for Right Rear.

Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

To calculate least square mean, colonized quarters assume as “1” and non-colonized quarters as “0” for each cow udder quarter for 12-month.

Table 11. Least square means and standard errors for number of times quarters infected with *Staphylococcus, Streptococcus,* coliforms and other bacteria for control and treatment cows during the 12 months of study.

Microbe	Control		Treatment		Difference		Reduc -tion	P value
	Estimate	± SE	Estimate	± SE	Estimate	± SE	%	Pr > t
Staph	0.4578	0.1024	0.1232	0.1100	0.3346	0.1298	73.08	0.0151
Strep	0.4566	0.1133	0.2968	0.1211	0.1598	0.1440	34.99	0.2761
Coli	0.1646	0.0418	0.0446	0.0450	0.1199	0.0528	72.84	0.0307
O B	0.1816	0.0562	0.0473	0.0604	0.1343	0.0707	73.95	0.0670

Foot note: *Staph* stands for *Staphylococcus*, *Strep* stands for *Streptococcus*, Coli stands for coliforms OB stands for Other Bacteria.

Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

Table 12. Least square means and standard errors for number of times quarters infected with *Staphylococcus, Streptococcus,* and other bacteria for control and treatment cows between the first and last month of the study.

Microbe	Differences of Least Square Mean in the Control and Treatment cows in first month				Differences of Least Square Mean in the Control and Treatment cows in Last month			
	Estimate	± SE	%	P value Pr > t	Estimate	± SE	%	P value Pr > t
Staph	0.6250	0.2980	50.00	0.0403	0.1792	0.3029	57.34	0.5565
Strep	-0.1250	0.2854	-100.00	0.6629	0.6083	0.2901	69.52	0.0403
OB	0.1250	0.1123	100.00	0.2703	-0.1429	0.1161	-100.00	0.2236

Foot note: Staph stands for *Staphylococcus*, Strep stands for *Streptococcus*, and OB stands for Other Bacteria.

Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

Table 12 derived from appendix Table 6

Table 13. Number of quarters with somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml in sealant-treated and non-sealant treated cow groups before and after dry-off.

SCC/ml (x 1000)	No Sealant					Sealant					% Overall difference
	Number of quarters Before Dry-off	% Before Dry-off	Number of quarters After Dry-off	% after dry off	% Difference before and after dry-off	Number of quarters Before Dry-off	% before dry off	Number of quarters After Dry-off	% after dry off	% Difference before and after dry-off	
>165	31	30.09	5	5.74	80.92	77	43.25	26	14.68	66.05	-18.37
>200	25	24.27	5	5.74	76.34	65	36.51	25	14.12	61.32	-19.67
>400	10	9.70	4	4.59	52.68	34	19.10	20	11.29	40.89	-22.38
>750	6	5.82	3	3.44	59.10	27	15.16	15	8.47	44.12	-25.34

Foot note: 103 total quarters before dry-off in non-sealant cow group, 87 total quarters after dry-off in non-sealant cow group. 178 total quarters before dry-off in sealant cow group, 177 total quarters after dry-off in sealant cow group.

Table 14. Least square means and standard errors for quarters with somatic cell counts >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml for the non-sealant and sealant-treated cow groups.

SCC/ml (x1000)	No Sealant (NS)				Sealant (S)				Difference				% Decrease		P value Pr > t	
	Estimate		SE		Estimate		SE		Estimate		SE					
	Bd	Ad	Bd	Ad	Bd	Ad	Bd	Ad	NS	S	NS	S	NS	S	NS	S
>165	1.0909	0.2500	0.2461	0.2581	1.7442	0.5952	0.1762	0.1781	0.8401	1.1489	0.3566	0.2504	77.08	65.88	0.0199	0.0001
>200	0.9091	0.2500	0.2311	0.2424	1.4651	0.5714	0.1653	0.1673	0.6591	0.8937	0.3349	0.2352	72.50	60.99	0.0513	0.0002
>400	0.3636	0.2000	0.1862	0.1953	0.7907	0.4524	0.1332	0.1348	0.1636	0.3383	0.2698	0.1895	44.99	42.98	0.5453	0.0766
>750	0.2273	0.1500	0.1651	0.1732	0.6279	0.3333	0.1181	0.1195	0.0772	0.2946	0.2393	0.1680	33.96	46.91	0.7473	0.0821

Footnote: Bd-Before dry-off, Ad-After dry-off, S-Sealant, NS-No sealant, SE-Standard error

Table 15. Number of quarters with somatic cell count (DHIA) >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml in sealant-treated and non-sealant treated cows before and after dry-off.

SCC/ml (x1000)	No Sealant					Sealant					% Overall difference
	Number of cows Before Dry-off	% Before Dry-off	Number of cows After Dry-off	% after dry off	% Difference before and after dry-off	Number of cows Before Dry-off	% before dry off	Number of cows After Dry-off	% after dry off	% Difference before and after dry-off	
>165	11	42.30	5	22.72	46.28	25	54.34	23	50.00	7.98	-82.75
>200	8	30.76	2	9.09	70.44	20	43.47	20	43.47	0	-100.00
>400	4	15.38	2	9.09	40.89	10	21.73	7	15.21	30.00	-27.22
>750	2	7.69	1	4.54	40.96	9	19.56	5	10.86	44.47	7.89

Foot note: 26 total cows before dry-off in non-sealant cow group, 22 total cows after dry-off in non-sealant, 46 total cows before and after dry-off in sealant cow group

Table 16. Least square means and standard errors for DHIA somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml for the non-sealant and sealant-treatment cow groups before and after dry-off.

SCC/ml (x1000)	No Sealant				Sealant				Difference				P value Pr > t	
	Estimate		SE		Estimate		SE		Estimate		SE			
	Bd	Ad	Bd	Ad	Bd	Ad	Bd	Ad	NS	S	NS	S	NS	S
>165	0.4348	0.2222	0.1029	0.1164	0.5556	0.5610	0.0735	0.0770	0.2126	-0.005	0.1553	0.1066	0.1737	0.9595
>200	0.3043	0.0555	0.0980	0.1108	0.4444	0.4878	0.0700	0.0734	0.2488	0.0433	0.1479	0.1015	0.0951	0.6699
>400	0.1304	0.0555	0.0778	0.0879	0.2222	0.1707	0.0556	0.0582	0.0748	0.0514	0.1175	0.0805	0.5250	0.5240
>750	0.0869	0.0555	0.0712	0.0805	0.2000	0.1220	0.0509	0.0533	0.0314	0.0708	0.1075	0.0737	0.7708	0.2921

Footnote: Bd-Before dry-off, Ad-After dry-off, S-Sealant, NS-No sealant, SE-Standard error

Table 17. Least square means and standard errors for the number of times quarters infected with microbes for the non-sealant and sealant-treatment cows before and after dry-off.

Qt	No Sealant (NS)				Sealant (S)				Difference				P value Pr > t	
	Estimate		SE		Estimate		SE		Estimate		SE			
	Bd	Ad	Bd	Ad	Bd	Ad	Bd	Ad	NS	S	NS	S	NS	S
LF	0.4091	0.0500	0.0834	0.0875	0.2558	0.1190	0.0597	0.0604	0.3591	0.1368	0.1210	0.0849	0.0036	0.1100
LR	0.3182	0.2000	0.0938	0.0984	0.3478	0.1778	0.0648	0.0656	0.1182	0.1700	0.1360	0.0922	0.3864	0.0676
RF	0.3636	0.2000	0.0898	0.0942	0.3333	0.1111	0.0628	0.0628	0.1636	0.2222	0.0888	0.1302	0.0136	0.2111
RR	0.4762	0.2632	0.0967	0.1017	0.3182	0.1591	0.0668	0.0688	0.2130	0.1591	0.1403	0.0944	0.1315	0.0947
T	1.5455	0.7000	0.2612	0.2739	1.2791	0.5714	0.1868	0.1890	0.8455	0.7076	0.3785	0.2658	0.0273	0.0088

Footnote: Bd-Before dry-off, Ad-After dry-off, S-Sealant, NS-No sealant, SE-Standard error, Qt-Quarters LF: Left Front, LR: Left Rear, RF: Right Front, RR: Right Rear, T:Total.

Table 18. Number of quarters infected with *Staphylococcus, Streptococcus,* coliforms and other bacteria in sealant-treated and non- sealant treated cows before and after dry-off .

Microbes	No Sealant					Sealant					% Overall difference
	Number of quarters Before Dry-off	% Before Dry-off	Number of quarters After Dry-off	% After Dry-off	% Difference before and after dry-off	Number of quarters Before Dry-off	% Before Dry-off	Number of quarters After Dry-off	% After Dry-off	% Difference before and after dry-off	
Staph	10	9.70	1	1.14	88.24	40	22.47	30	24.61	24.61	-72.11
Strep	18	17.47	13	14.94	14.48	12	6.74	9	24.62	24.62	41.18
Coli	3	2.91	2	2.29	21.30	1	0.56	0	100.00	100.00	78.7
O B	9	8.73	2	2.29	73.76	6	3.37	1	83.38	83.38	11.53

Foot note: 103 total quarters before dry-off in non-sealant cow group, 87 total quarters after dry-off in non-sealant cow group. 178 total quarters before dry-off in sealant cow group, 177 total quarters after dry-off in sealant cow group.

Staph stands for *Staphylococcus*, *Strep* stands for *Streptococcus*, Coli stands for coliforms and OB stands for Other Bacteria.

Table 19. Least square means and standard errors for number of times quarters infected with *Staphylococcus, Streptococcus,* coliforms and other bacteria for the non-sealant and sealant-treated cows before and after dry-off.

Microbe	No Sealant (NS)				Sealant (S)				Difference				P value Pr > t	
	Estimate		SE		Estimate		SE		Estimate		SE			
	Bd	Ad	Bd	Ad	Bd	Ad	Bd	Ad	NS	S	NS	S	NS	S
Staph	0.4545	0.0500	0.1825	0.1914	0.9070	0.2619	0.1306	0.1321	0.4045	0.6451	0.2645	0.1857	0.1287	0.0007
Strep	0.6818	0.4500	0.1749	0.1834	0.2826	0.2222	0.1210	0.1223	0.2318	0.0603	0.1720	0.2535	0.3621	0.7261
Coli	0.0909	0.1000	0.0549	0.0576	0.0222	0.0444	0.0384	0.0384	0.0090	0.0222	0.0796	0.0543	0.9093	0.6833
OB	0.3333	0.1053	0.0931	0.0979	0.1364	0.0227	0.0643	0.0643	0.2281	0.1136	0.1352	0.0910	0.0942	0.2144

Footnote: Bd-Before dry-off, Ad-After dry-off, S-Sealant, NS-No sealant, SE-Standard error, *Staph-Staphylococcus, Strep- Streptococcus*, Coli-Coliforms, OB-Other Bacteria.

Table 20. Somatic cell counts as they relate to estimated milk losses.

Lactation Average Liner SCC Score[#]	CMT (Score)	WMT (mm)	Somatic Cell Count (cells/ml)	Milk Loss (%)	Estimated Milk Production Loss Per Cow/Year* (lb)
2	Negative	--	50,000	---	---
3	Negative	2	100,000	3	-400
4		5	200,000	6	-800
	Trace	8	300,000	7	-1,000
5		10	400,000	8	-1,200
		12	500,000	9	-1,300
	1	14	600,000	10	-1,400
		16	700,000		-1,500
		18	800,000	11	-1,600
		20	900,000		-1,650
		21	1,000,000	12	-1,700
	<2	24	1,200,000	>12	-1,700
7		29	1,600,000		-2,000

*Based on 14,000-15,000 lb average/cow/year, lasted in >=2.
Linear score calculation from SCC. Example: SCC = 2000,000/ml.
CMT Interpretation:
Negative - Mixture remains liquid with no evidence of the formation of a precipitate.
Trace – A slight precipitate or small flakes form and then disappear.
1 (weak positive) - A distinct precipitate forms.
2 (distinct positive) – The mixture thickens immediately with some gel formation.
Source: J.W. Schroeder, 1997

Table 21. Economic benefit of using hydrated lime on dairy cow bedding*

Cost of Lime
50# bag at $5
Application rate 50 grams per stall
1gram=.0353 oz
50 grams = 1.765 oz (50 x .0353) per stall
50# x 16 ozs = 800 ozs per $5 bag of lime
$5/800oz = $.00625 per oz
1.765 oz lime per stall x $.00625 per oz = $.0110312 per stall per day
$.0110312 x 365 days = $4.026388 cost of lime per stall per year

Milk loss due to SCC
Loss due to SCC 1000 lbs milk / 300,000 scc = .0033333 (Schroeder, 1997)
Loss due to SCC 80 lbs milk / 200,000 scc = .0040000 (Schroeder, 1997)
Ave. SCC in Control group 329,715
Ave. SCC in Treatment group 203,308
329,715 x .0033333 = 1,099.039 total lbs milk lost per cow per year in control group
203,308 x .0040000 = 813.232 total lbs milk lost per cow per year in treatment group
1,099.039 - 813.232 = 285.807 total lbs lost per cow per year in the control group over the treatment group.

$ Loss in Control Group Over Treatment Group
285.807 x .1628 (avg. milk price per lb) = $46.529379 ($ savings per cow per year in treatment group)

Summary

Savings per cow per year in treatment group	$46.53
Cost of Lime per cow per year	- 4.03
Total Savings per cow per year with lime	$42.50

* Cost analysis was prepared by Boyd Brady, DHIA coordinator, Auburn University.

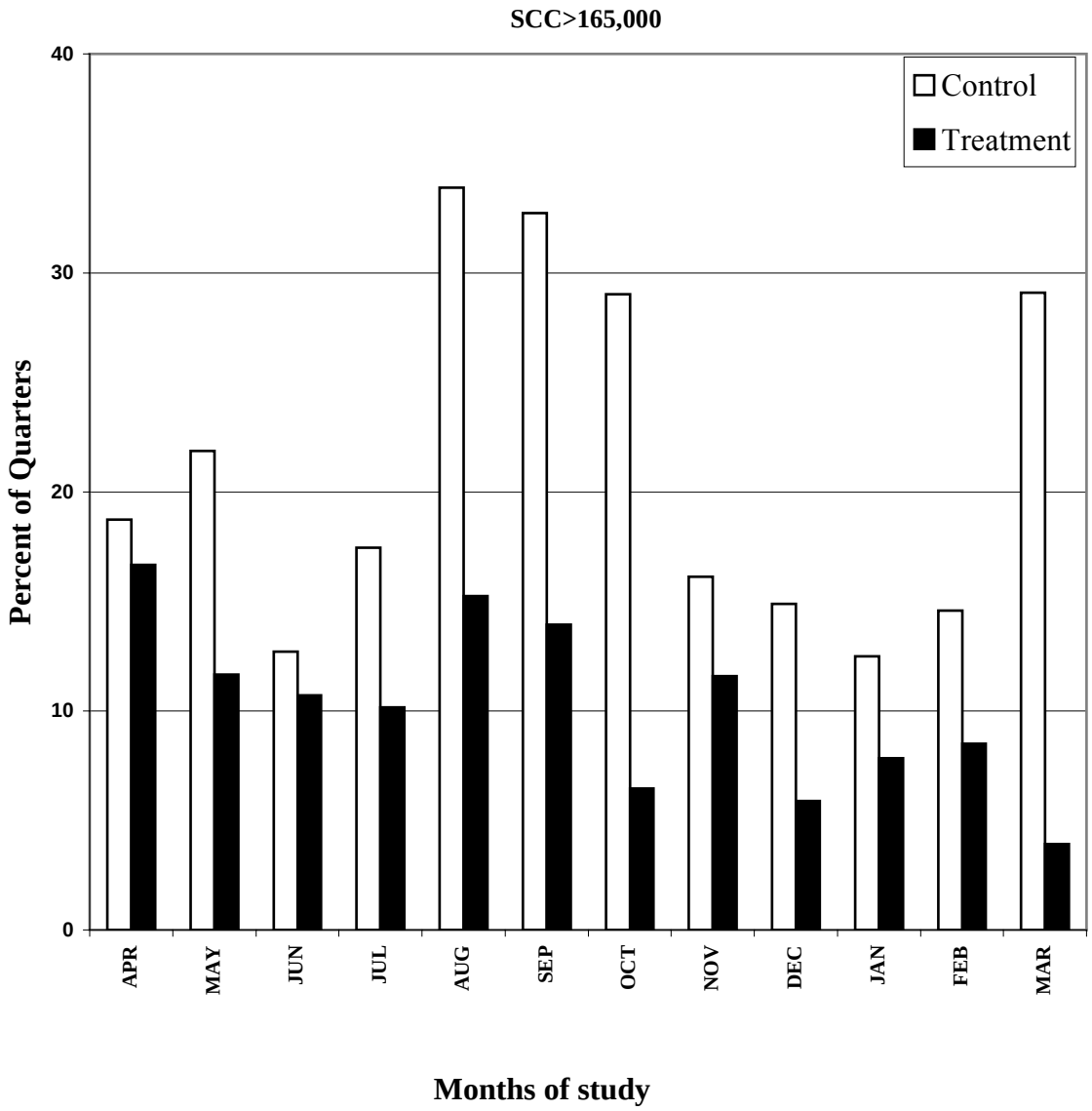

Figure 1. Percent of quarters with somatic cell count >165,000/ml for control and lime-treatment cows during the 12-month study period for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

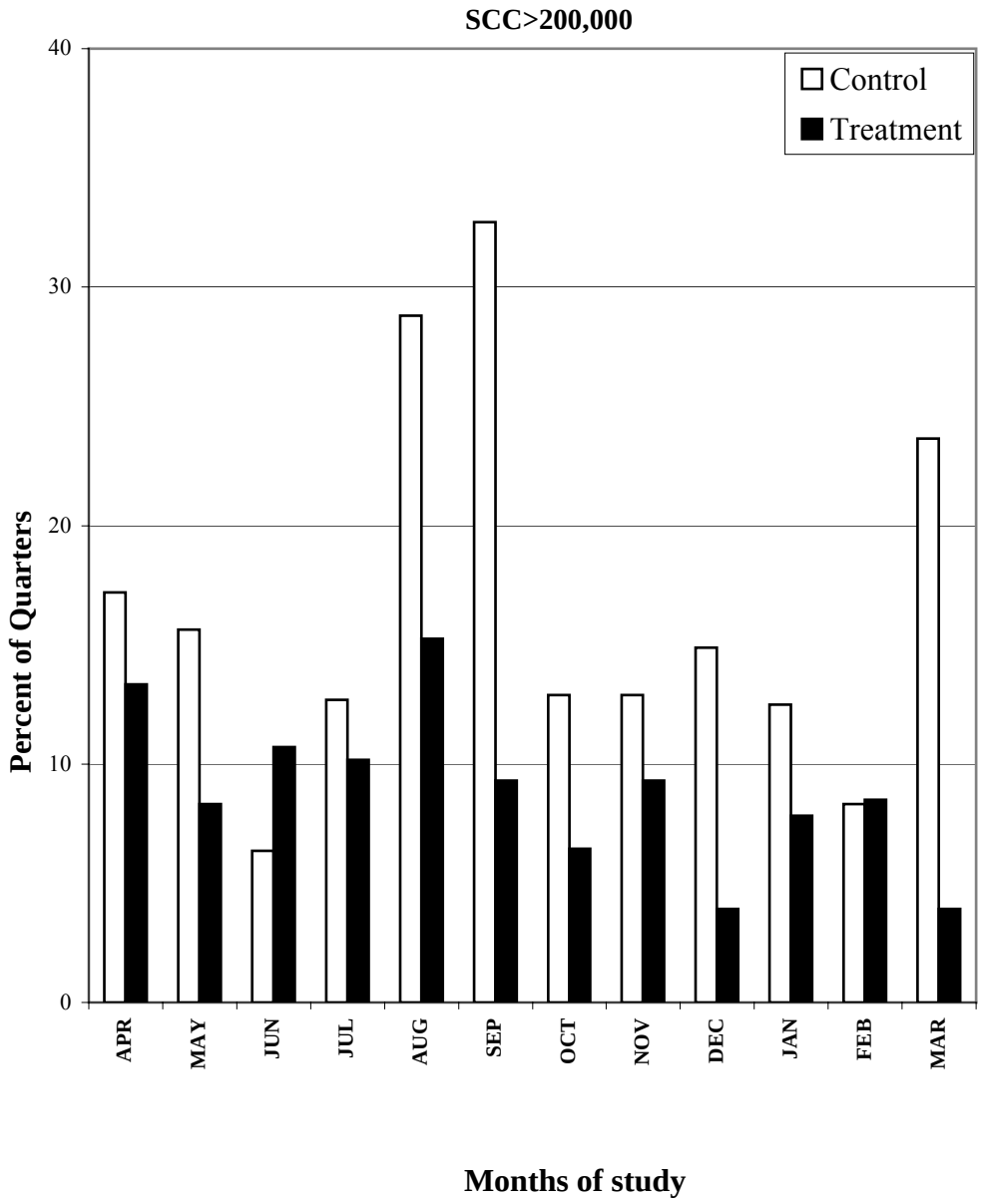

Figure 2. Percent of quarters with somatic cell count >200,000/ml for control and lime-treatment cows during the 12-month study period for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

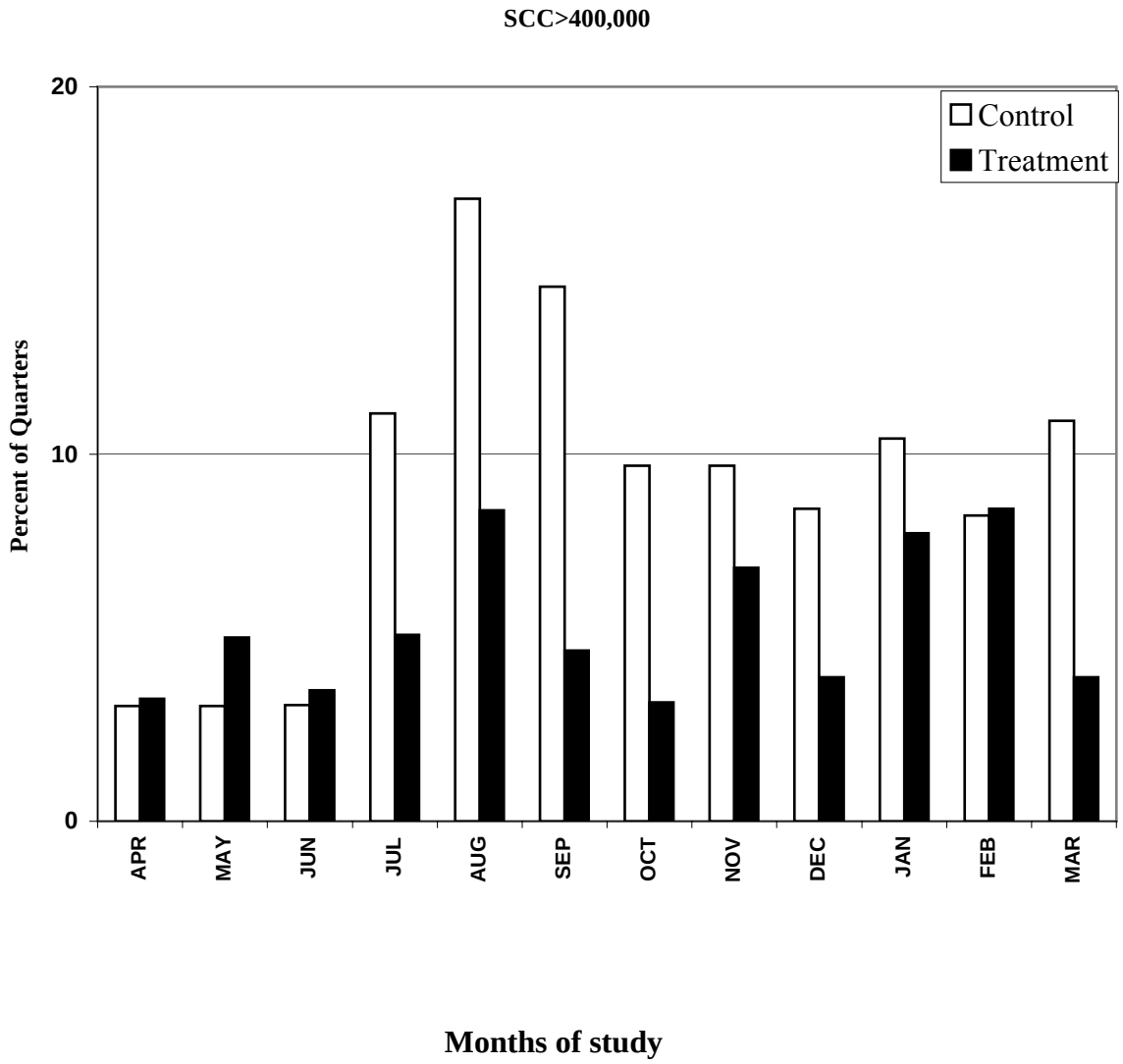

Figure 3. Percent of quarters with somatic cell count >400,000/ml for control and lime-treatment cows during the 12-month study period for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

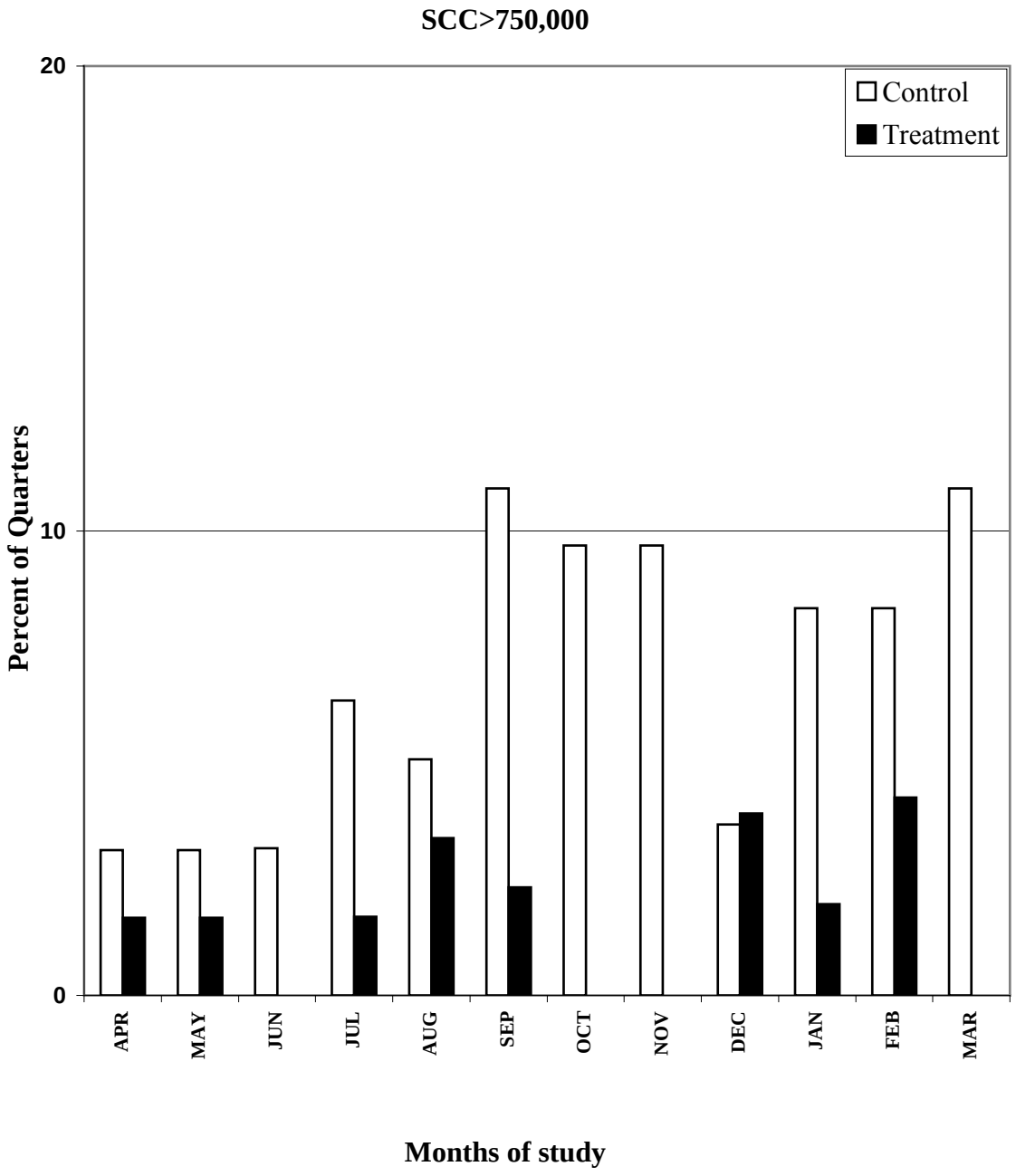

Figure 4. Percent of quarters with somatic cell count >750,000/ml for lime and non-lime treatment cows during the 12-month study period for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

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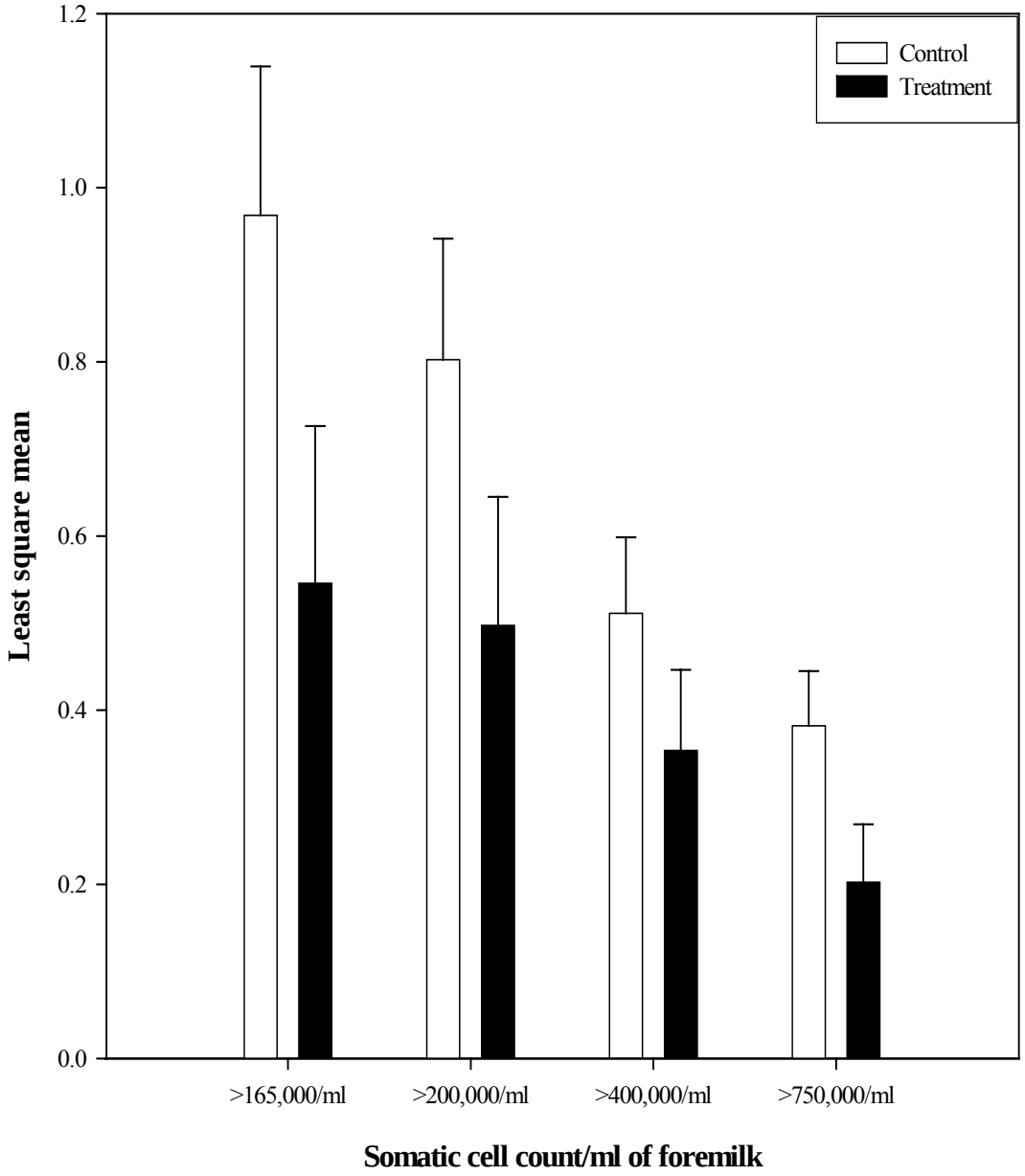

Figure 5. Least square means and standard errors of quarters with somatic cell count >165,000, >200,000, >400,000, and >750,000/ml in control and lime-treatment cow groups for the 12-month study period (data plotted from Table 6).

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

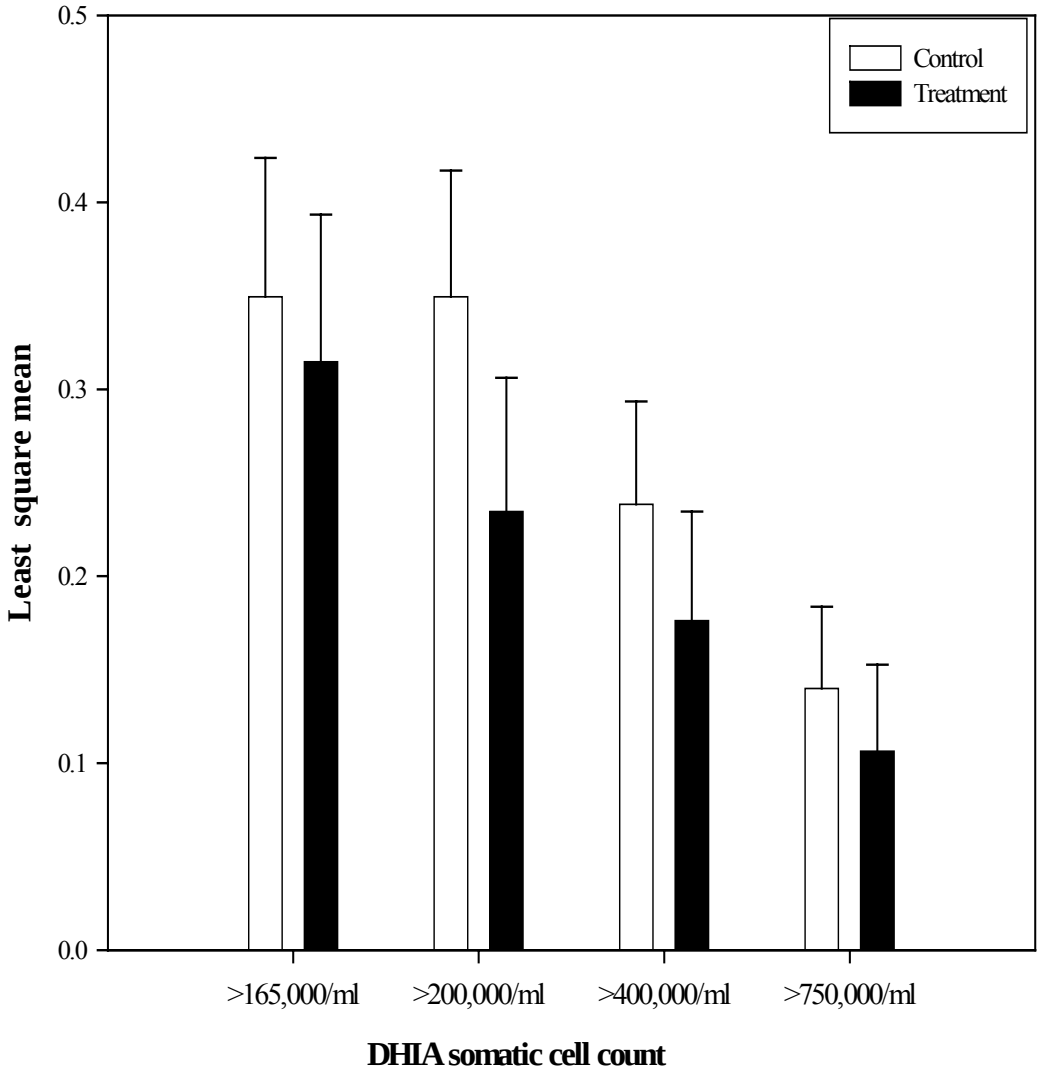

Figure 6. Least square means and standard errors of quarters with DHIA somatic cell count >165,000, >200,000, >400,000, and >750,000/ml in control and lime-treatment cow groups for the 12-month study period (data plotted from Table 7).

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

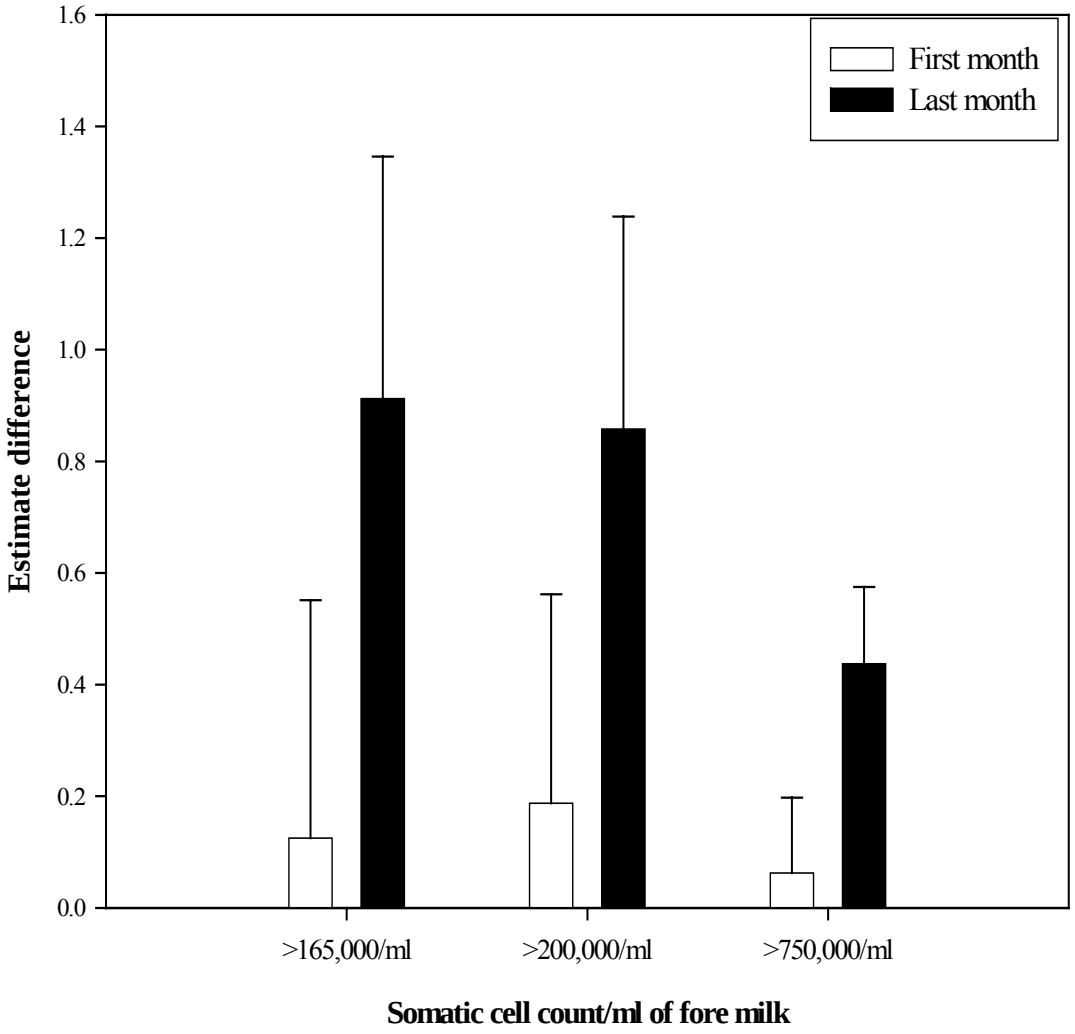

Figure 7. Least square means and standard errors for quarters with somatic cell count >165,000/ml, >200,000/ml and >750,000/ml for the control and lime-treatment cow groups in the first and last month of study (data plotted from Table 8).

Foot note: Differences in least square means reductions between first and last month quarter milk somatic cell counts for control and lime-treatment cow groups.

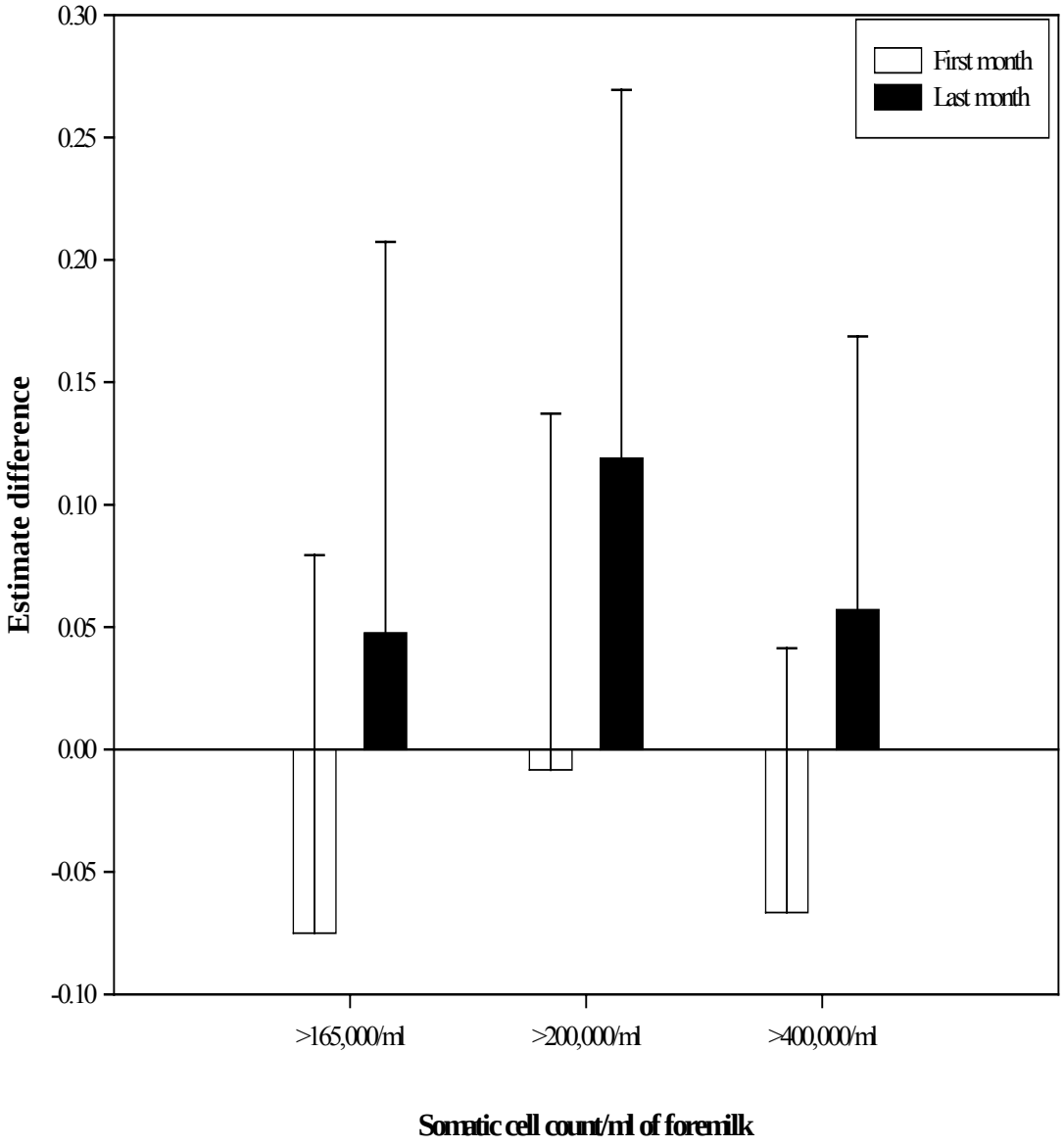

Figure 8. Least square means and standard errors for quarters with DHIA somatic cell count >165,000/ml, >200,000/ml and >750,000/ml for the control and lime-treatment cow groups in the first and last month of study (data plotted from Table 9).

Foot note: A negative estimate difference indicates that the control cow group had fewer cows with SCC>165,000, >200,000, and >400,000 compared to the lime-treatment cow group.

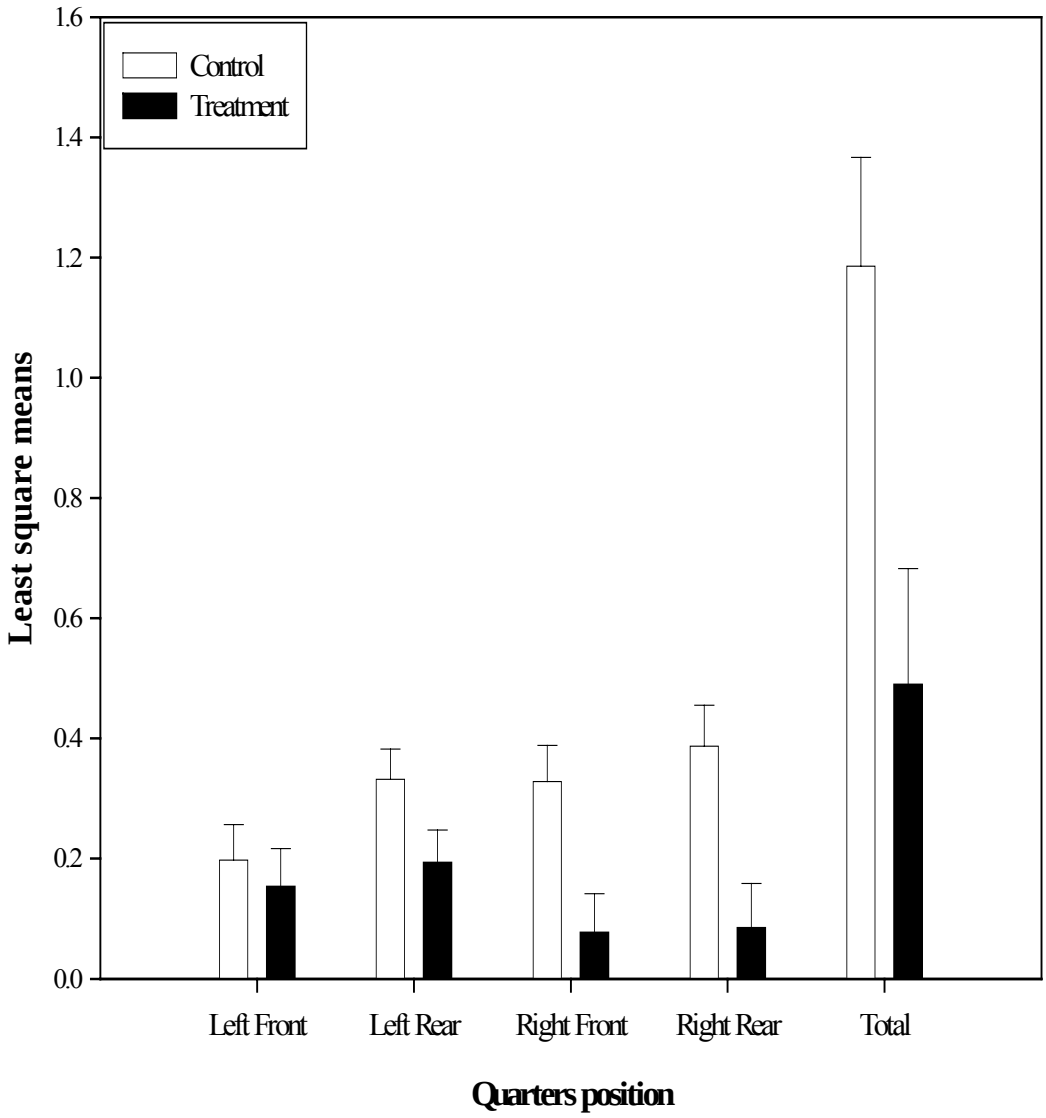

Figure 9. Least square means and standard errors for number of quarters colonized with microbes for control and lime-treatment cow groups during the 12-month study (data plotted from Table 10).

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

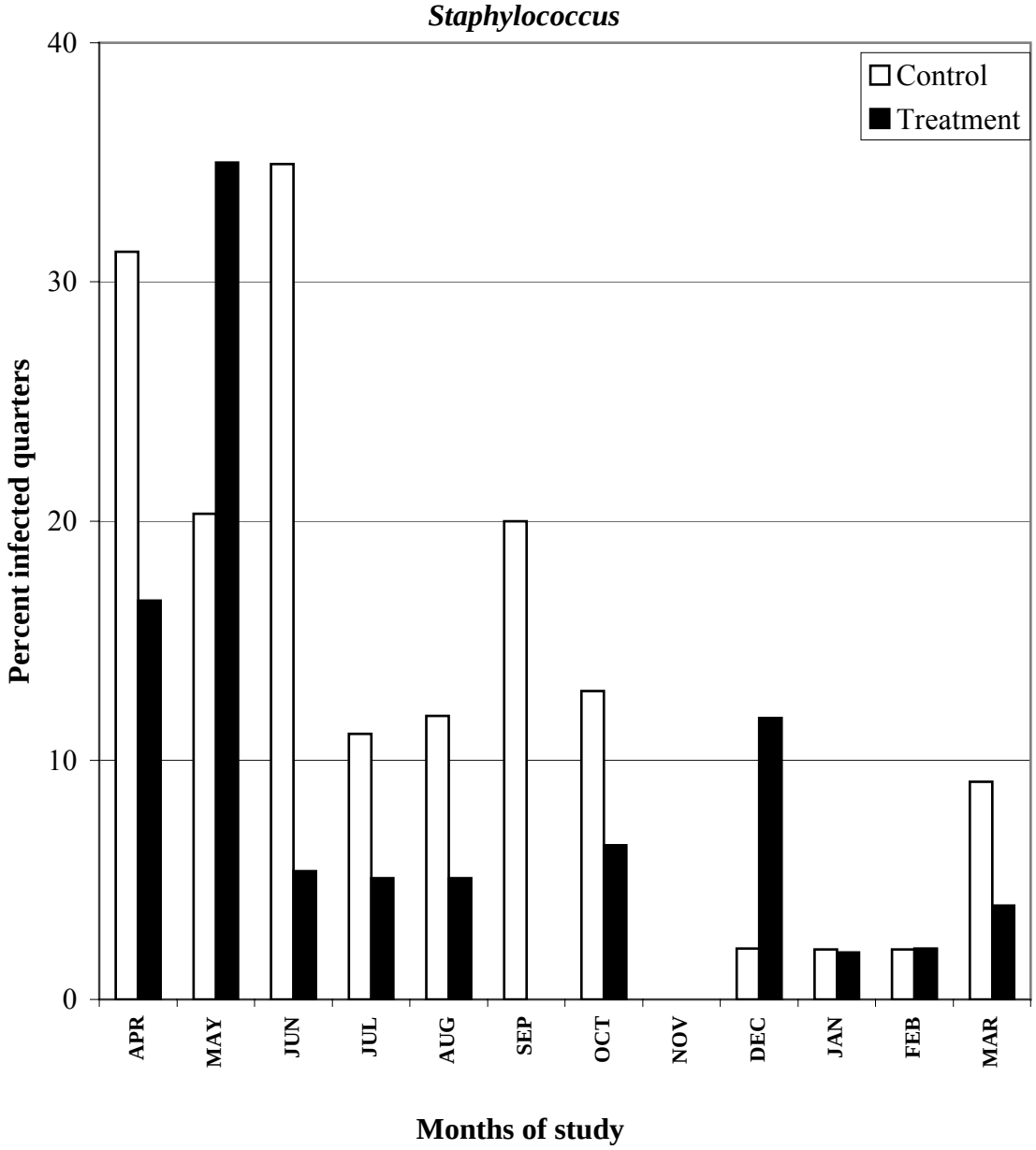

Figure 10. Percent of quarters infected with *Staphylococcus* in control and lime-treatment cow groups for each month of study for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

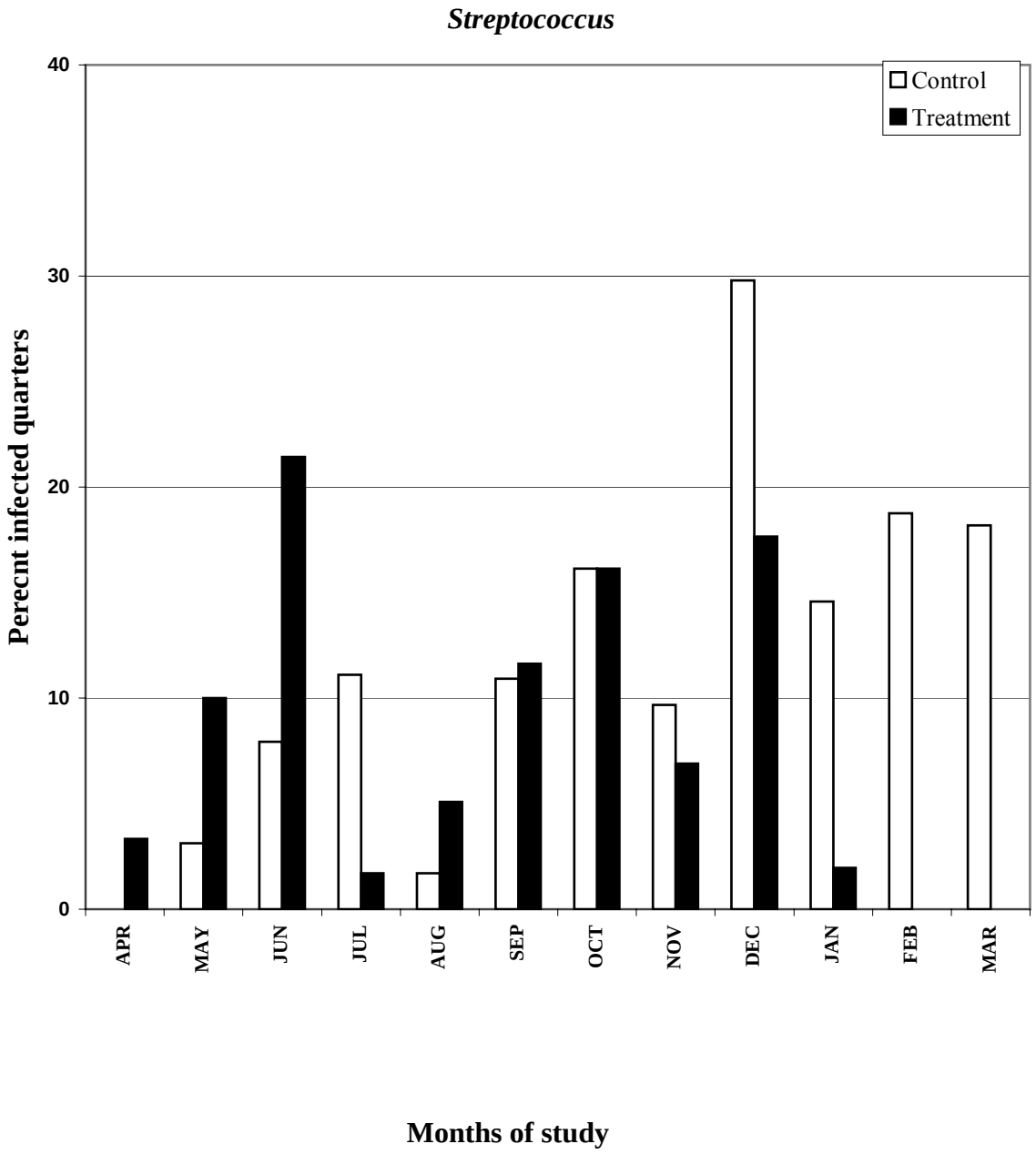

Figure 11. Percent of quarters infected with *Streptococcus* in control and lime-treatment cow groups for each month of study for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

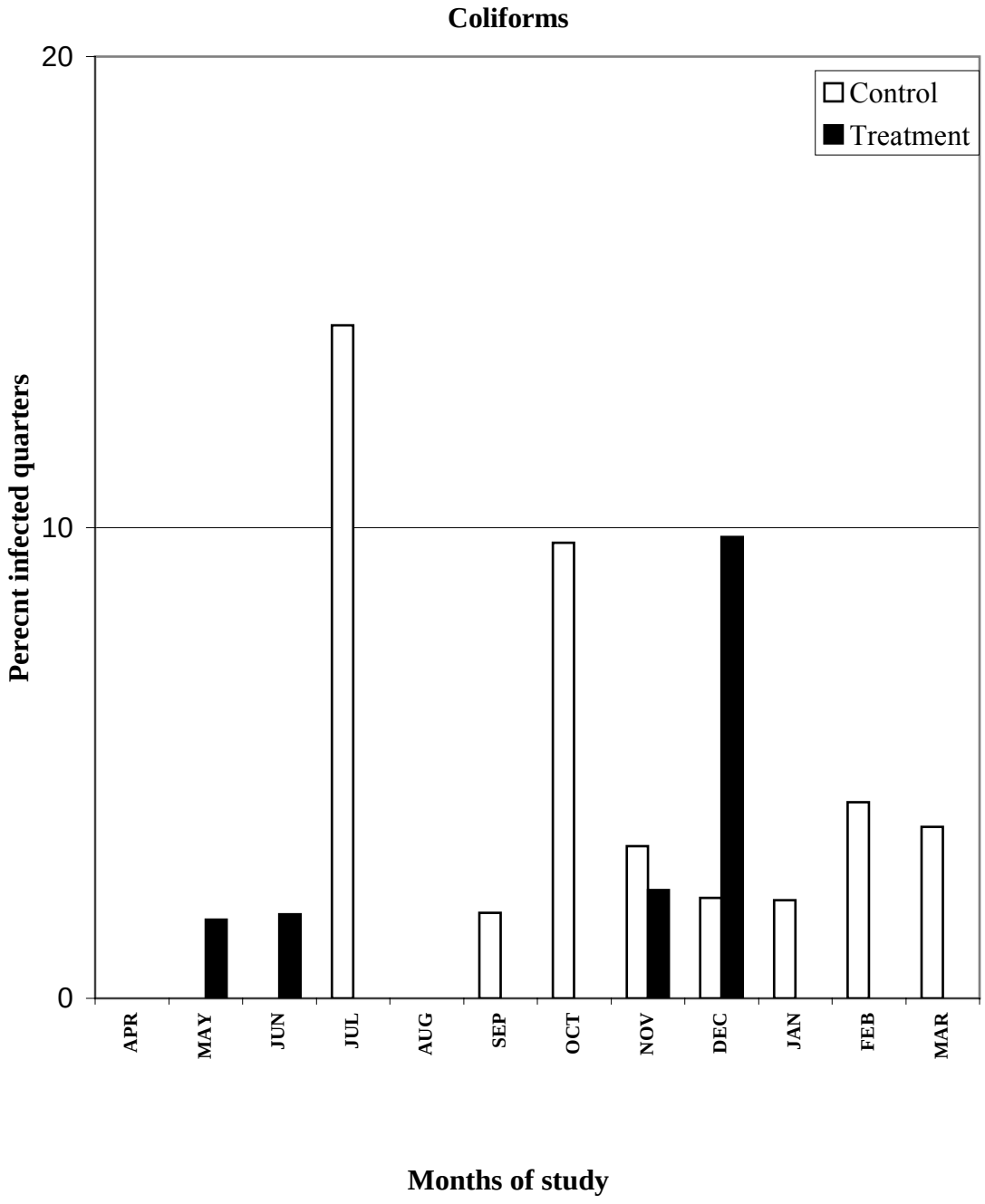

Figure 12. Percent of quarters infected with coliforms in control and lime-treatment cow groups for each month of study for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

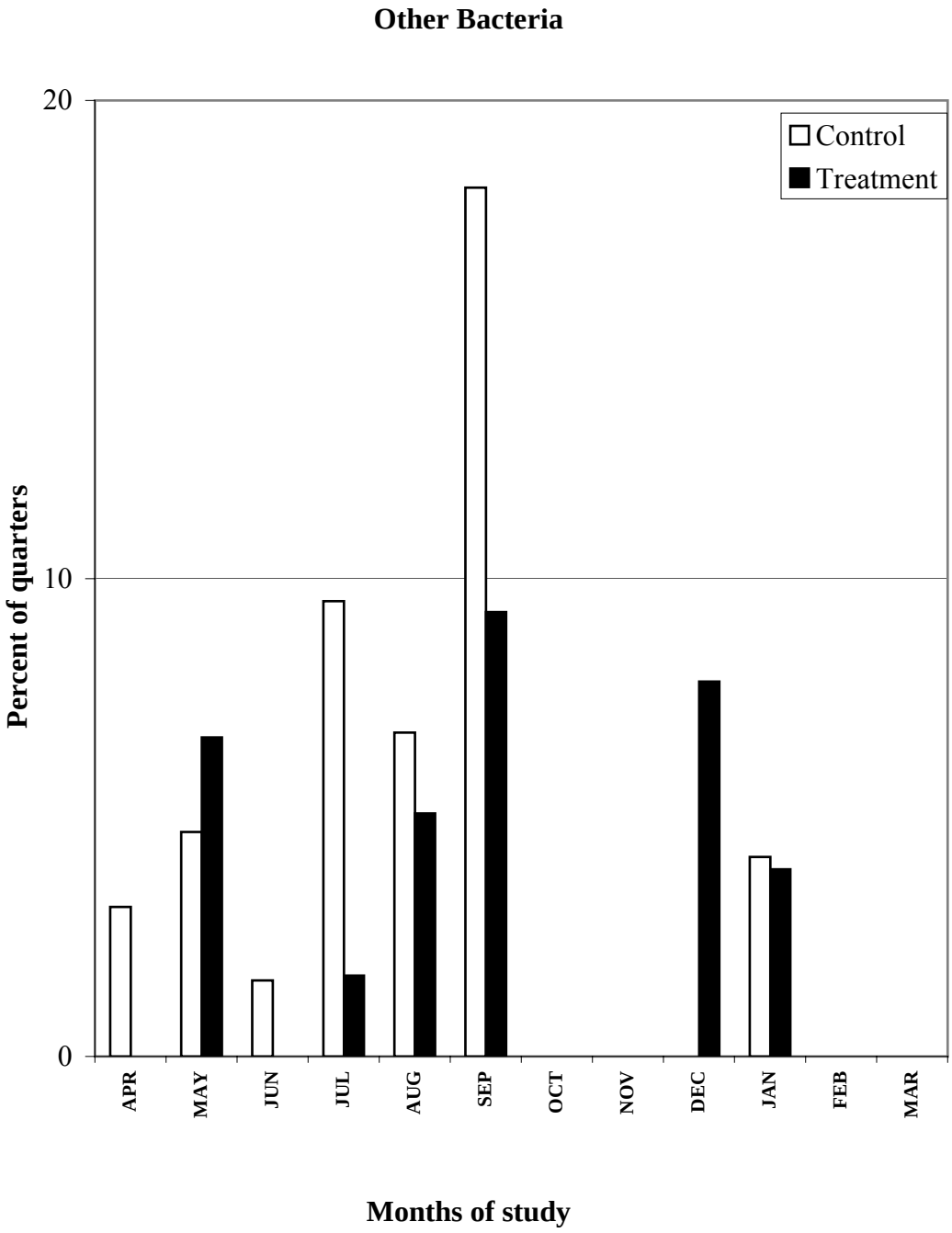

Figure 13. Percent of quarters infected with other bacteria in control and lime-treatment cow groups for each month of study for year 2004-2005.

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

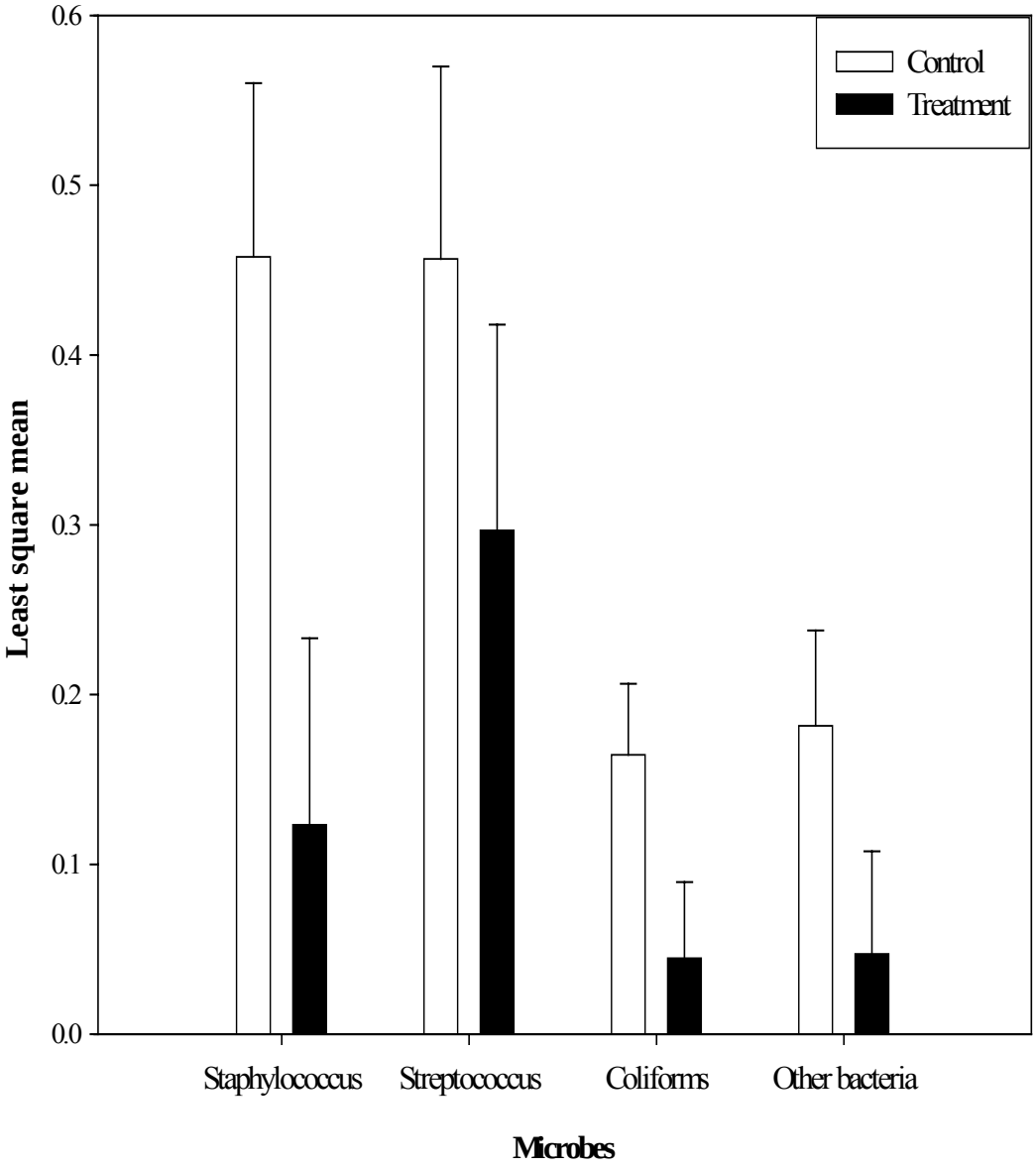

Figure 14. Least square means and standard errors for number of times quarters infected with *Staphylococcus, Streptococcus*, coliforms and other bacteria for control and lime-treatment cow groups during the 12-month study (data plotted from Table 11).

Foot note: Control stands for non-lime treatment cow group and Treatment stands for lime-treatment cow group.

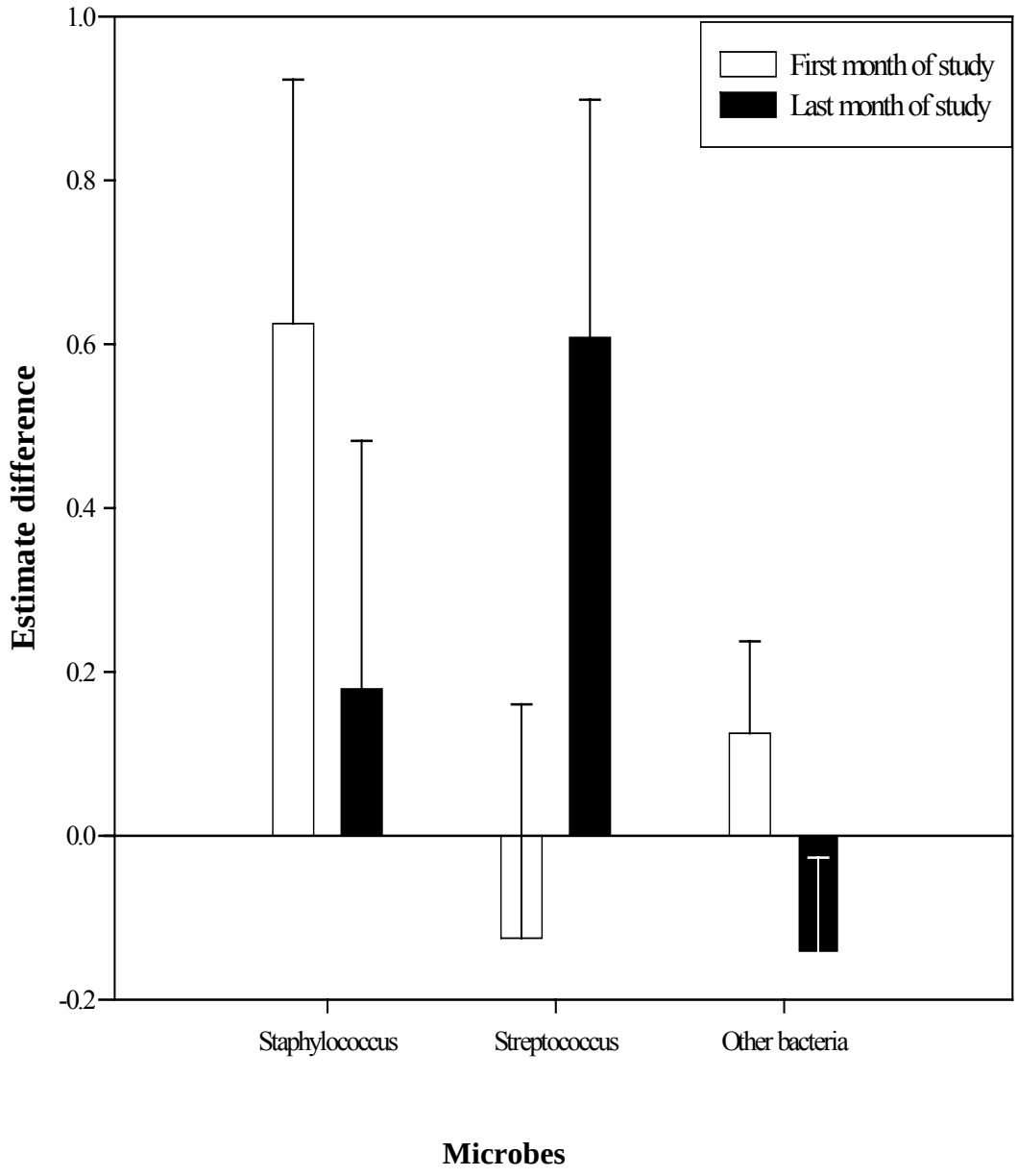

Figure 15. Least square means and standard errors for number of times quarters infected with *Staphylococcus, Streptococcus,* and other bacteria for control and lime-treatment cow groups during the 12-month study (data plotted from Table12).

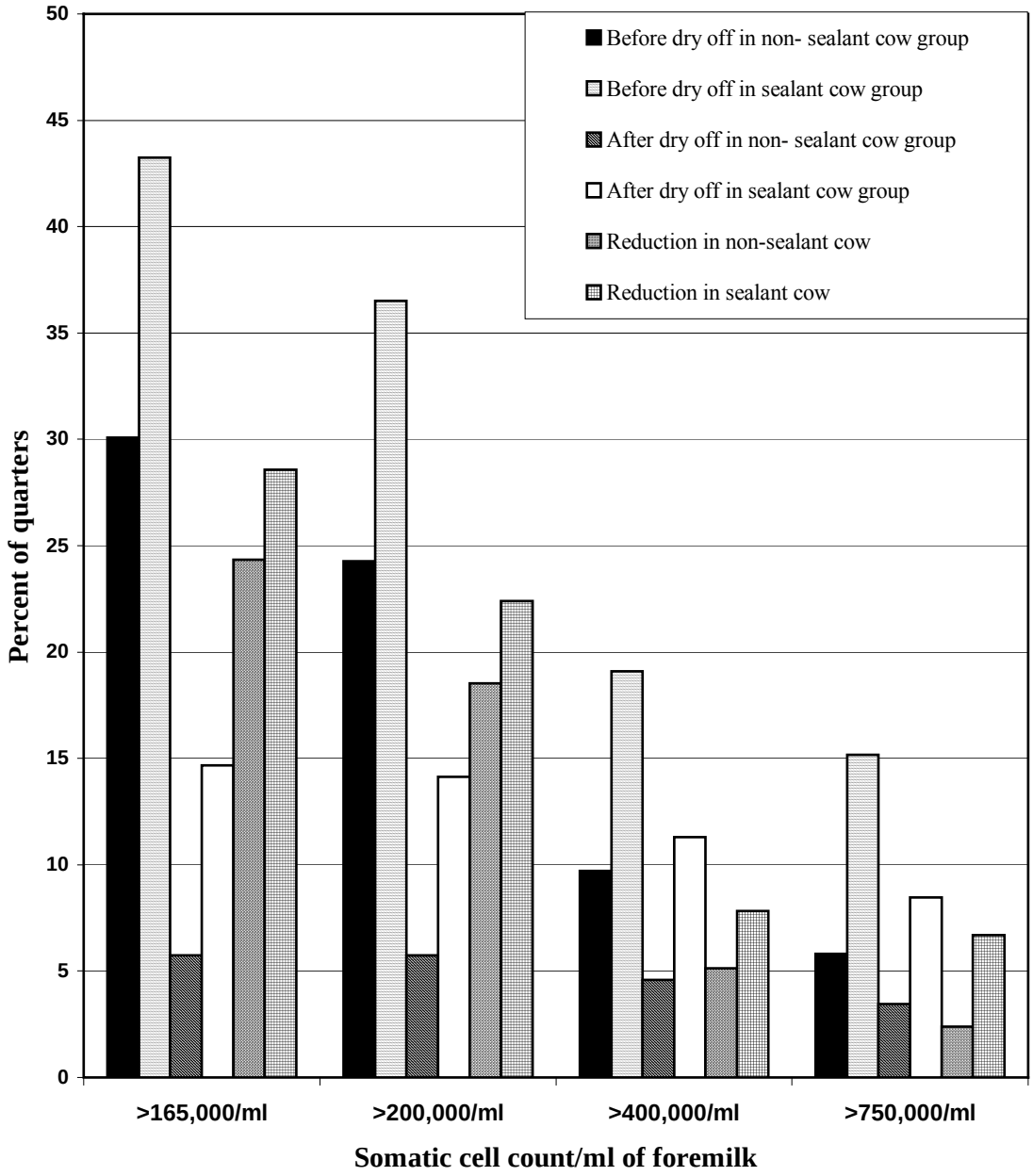

Figure 16. Percent of number of quarters with somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml in sealant-treated and non-sealant treated cows before and after dry-off (data plotted from Table 13).

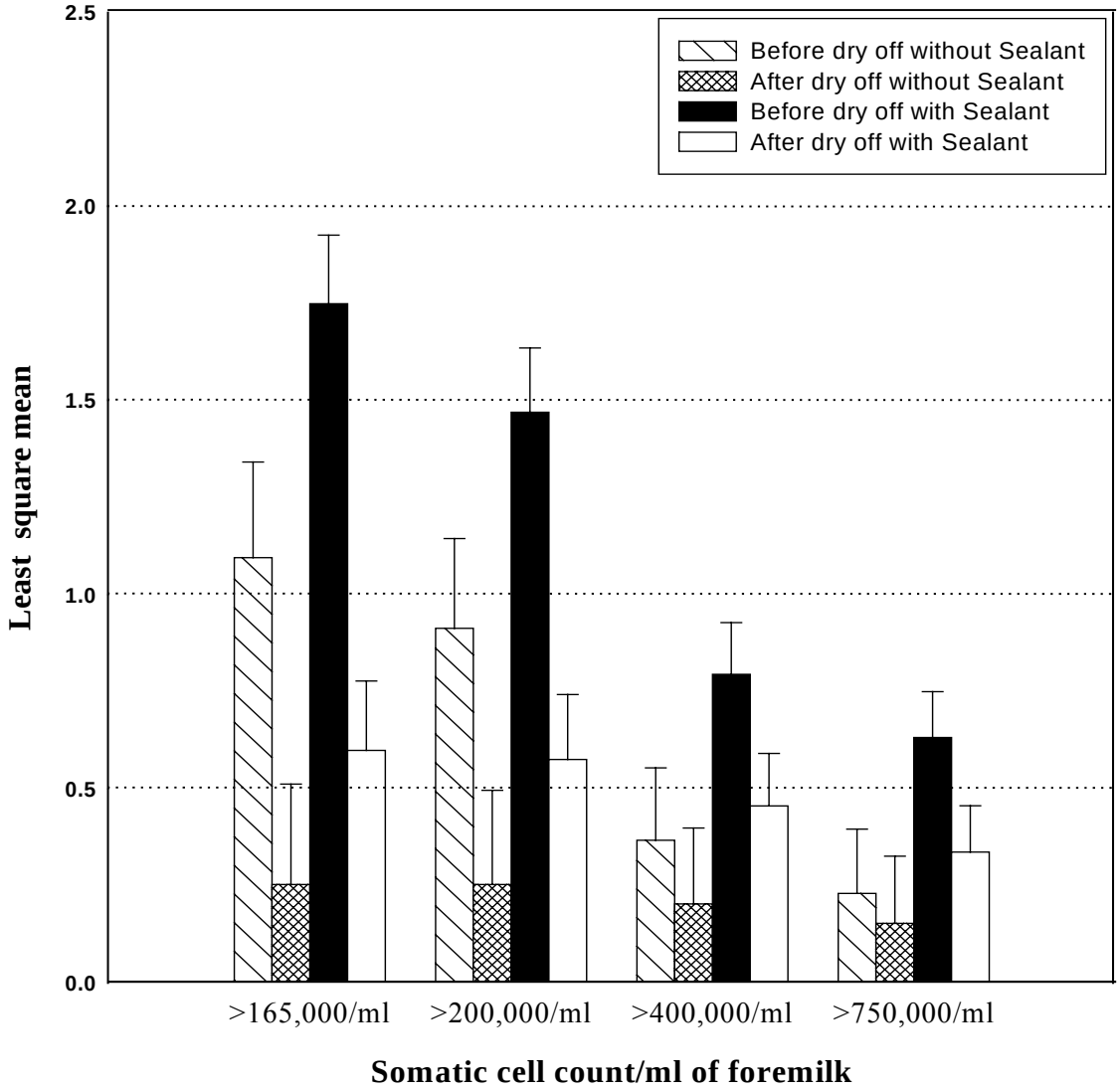

Figure 17. Least square means and standard errors for quarters with somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml in non-sealant and sealant-treated cow groups (data plotted from Table 14).

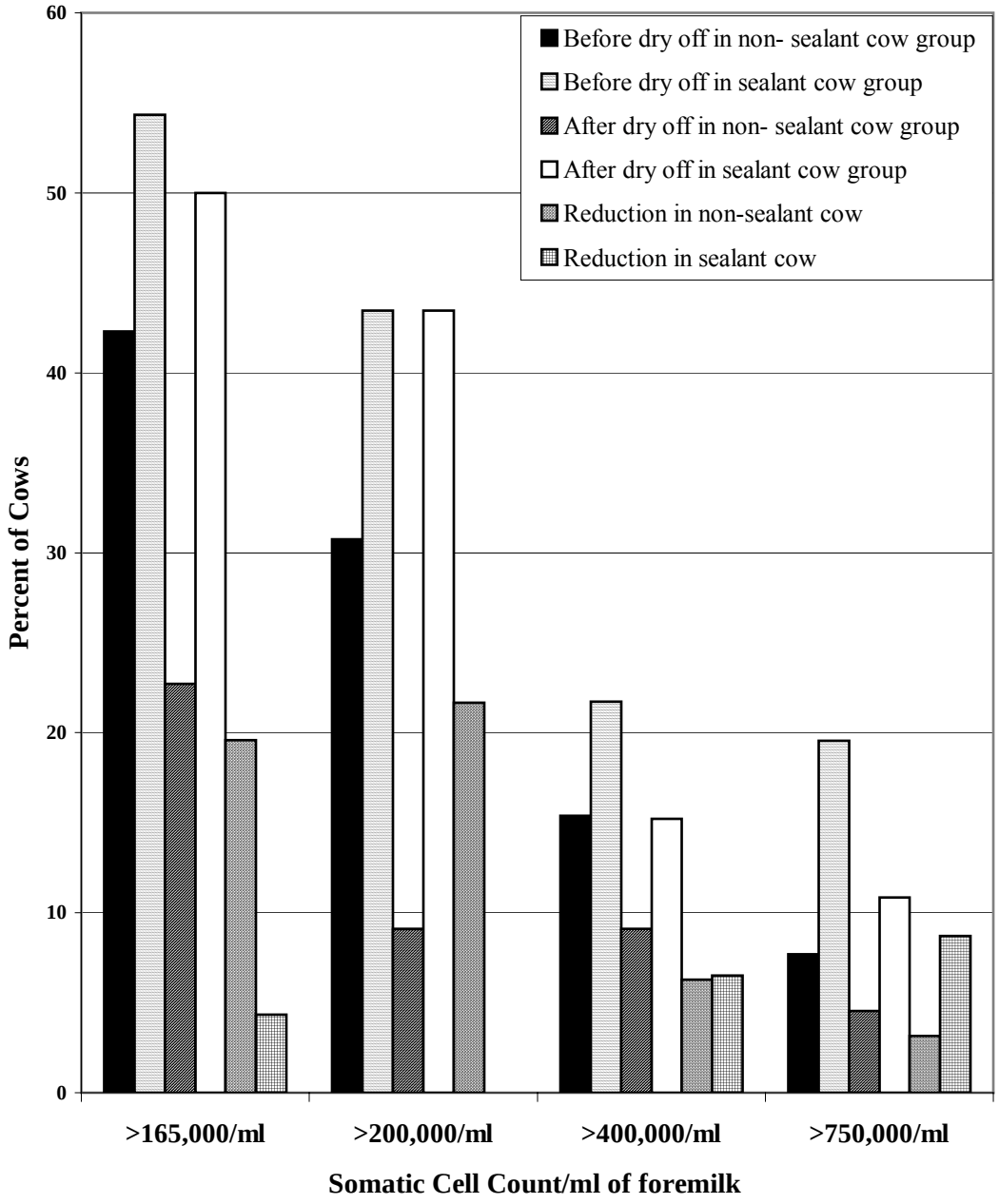

Figure 18. Percent of number of cows with somatic cell count (DHIA) >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml in sealant-treated and non-sealant treated cows before and after dry-off (data plotted from Table 15).

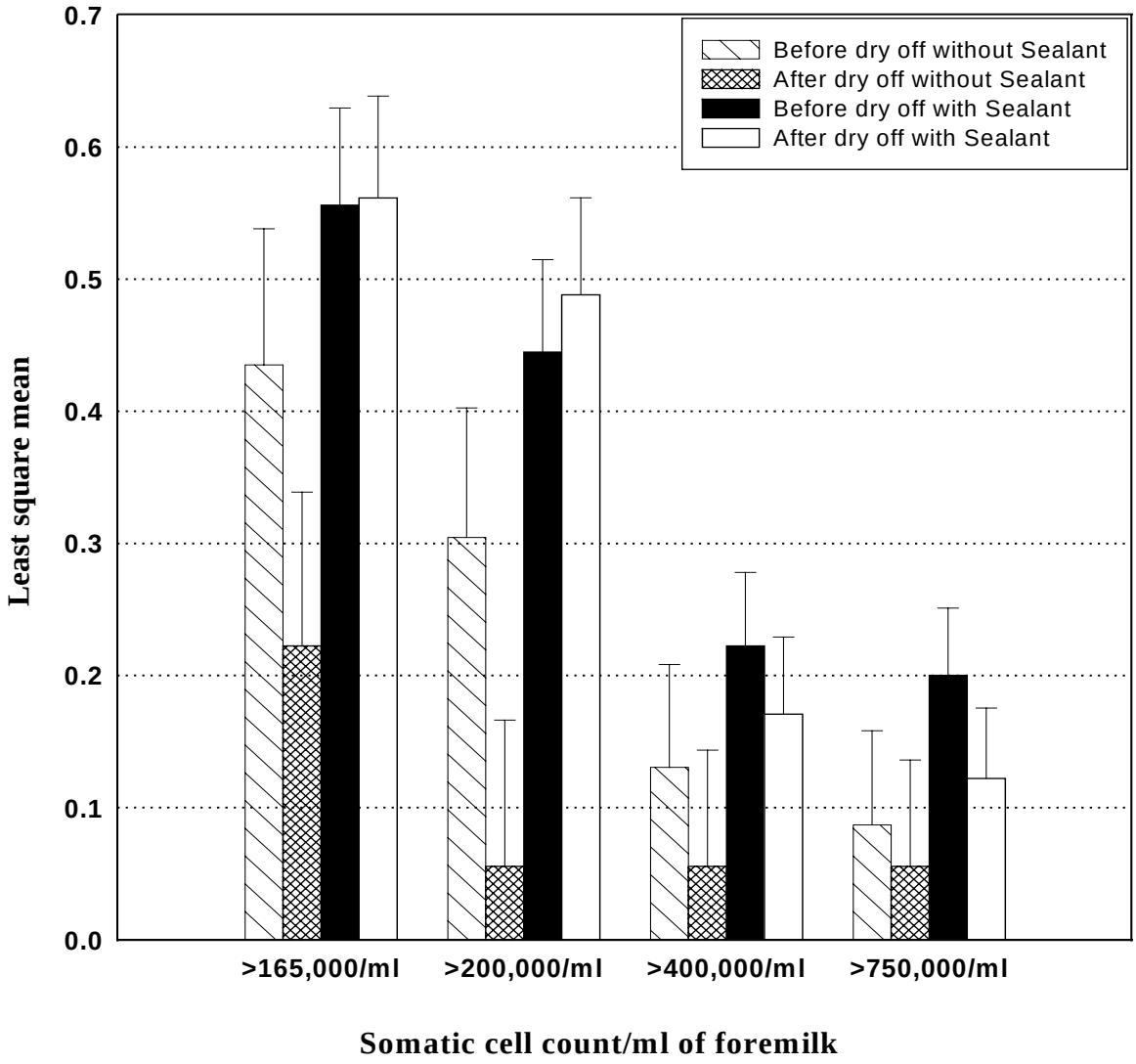

Figure 19. Least square means and standard errors for DHIA somatic cell count of milk for non-sealant and sealant-treatment cow groups for the 12-month study (data plotted from Table 16)

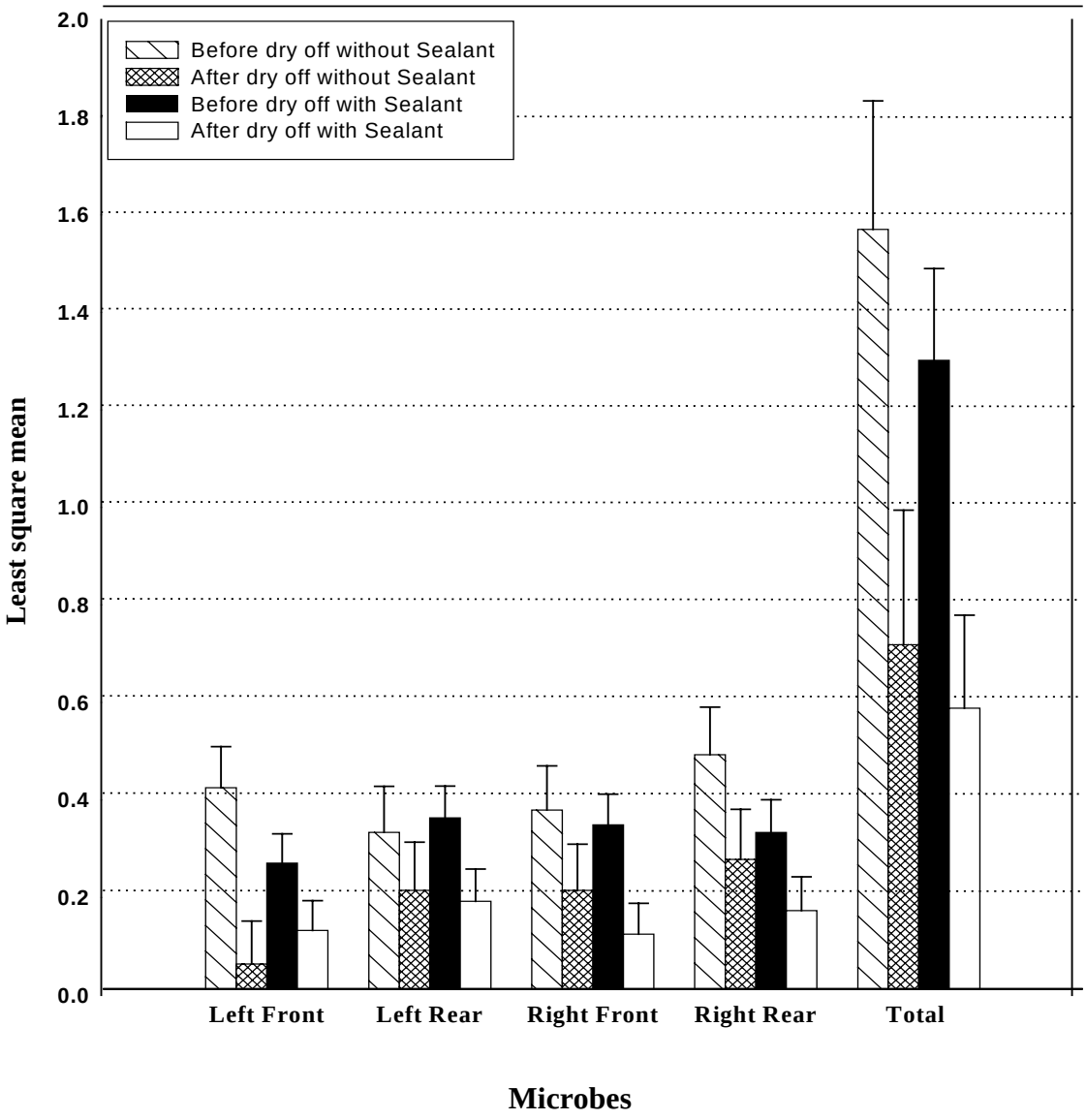

Figure 20. Least square means and standard errors for number of quarters infected with microbes for non-sealant and sealant-treatment cows during the 12-month of study (data plotted from Table 17).

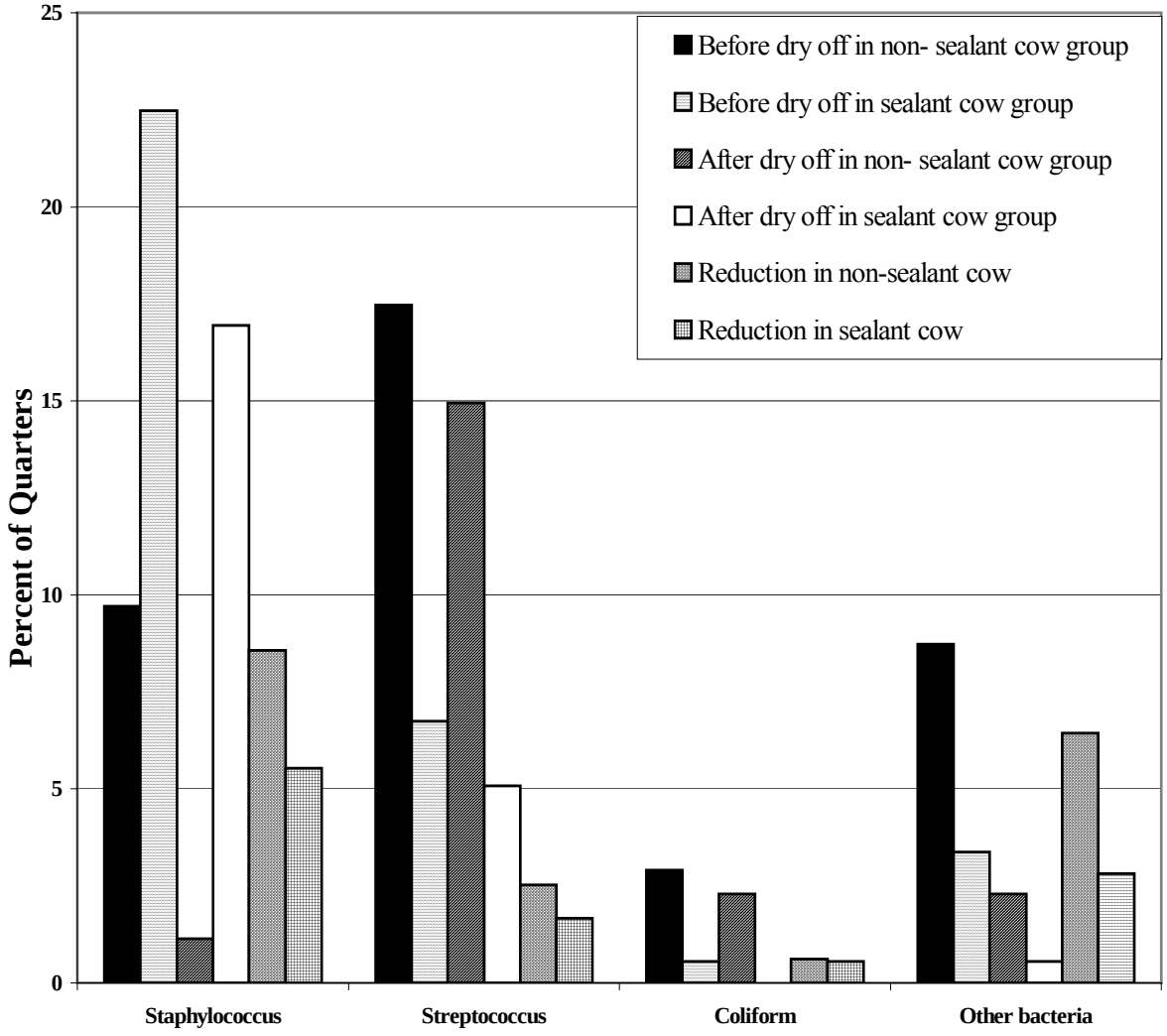

Figure 21. Percent of number of quarters infected with *Staphylococcus, Streptococcus,* coliforms and other bacteria in sealant-treated and non-sealant treated cows before and after dry-off (data plotted from Table 18).

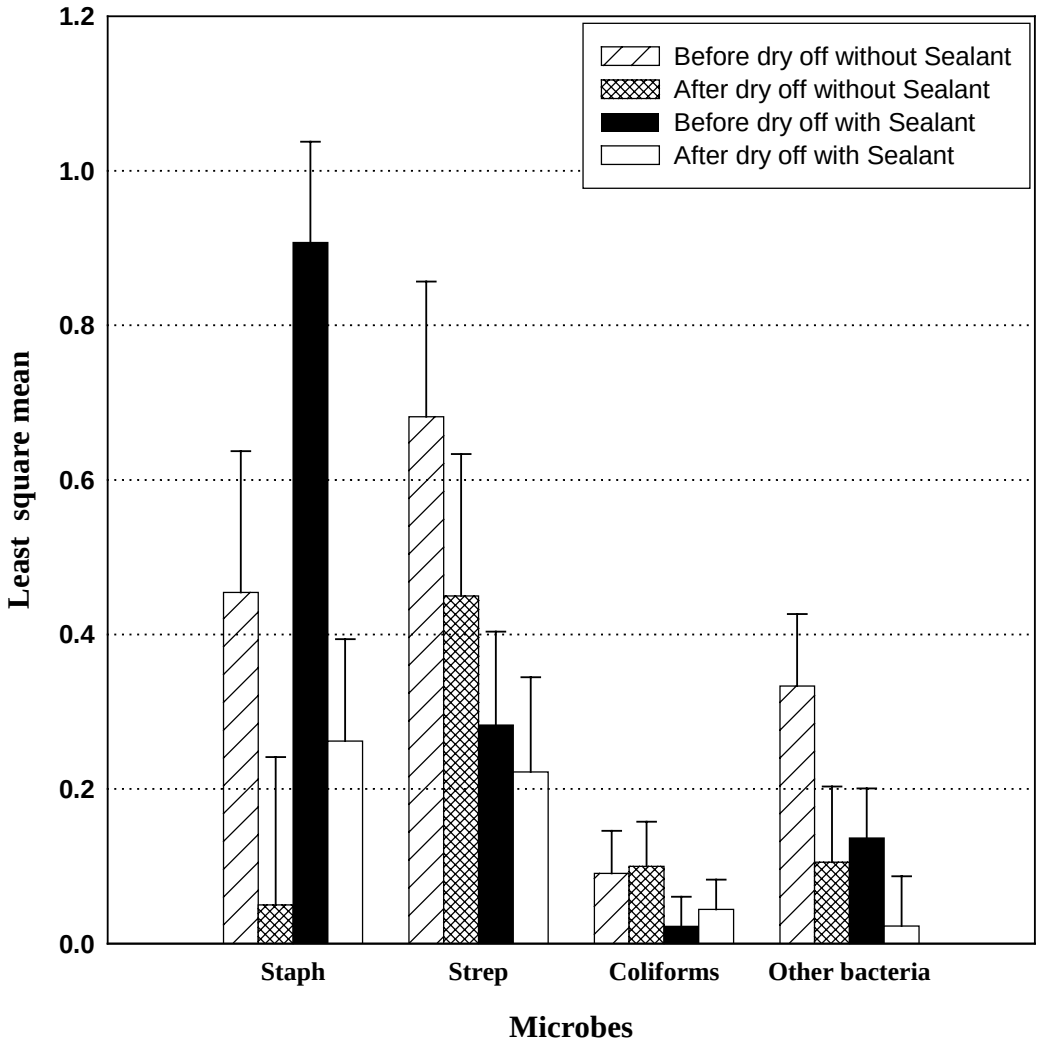

Figure 22. Least square means and standard errors for number of times quarters infected with *Staphylococcus*, *Streptococcus*, coliforms and other bacteria for non-sealant and sealant-treatment cows during the 12-month of study (data plotted from Table 19).

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VIII. APPENDIX

Appendix Table 1. Number of quarters with somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and 750,000/ml for the entire 12-month study for the control and lime-treatment cow groups.

SCC/ml (x1000)	Control Cows		Lime Bedding Cows		% decrease in quarters exceeding the four SCC levels
	Number of quarters	% quarters of 631 total	Number of quarters	% quarters of 611 total	
>165	133	21.07	62	10.14	51.87
>200	106	16.79	52	8.51	49.32
>400	57	9.03	33	5.40	40.20
>750	42	6.65	13	2.12	68.12

Appendix Table 2. Number of cows with DHIA somatic cell count >165,000/ml, >200,000/ml, >400,000/ml and 750,000/ml for the entire 12-month study for the control and lime-treatment cow groups.

SCC/ml (x1000)	Control Cows		Lime Bedding Cows		% decrease in cows exceeding the four SCC levels
	Number of cows	% cows of 163 total	Number of cows	% cows of 155 total	
>165	54	33.12	42	27.09	18.21
>200	45	27.60	27	17.41	36.92
>400	27	16.56	17	10.96	33.82
>750	15	9.20	11	7.09	22.93

Appendix Table 3. Number of quarters harboring *Staphylococcus, Streptococcus*, coliforms and other bacteria for the entire 12-month study for the control and lime-treatment cow groups.

Microbes	Control Cows		Lime Bedding Cows		% decrease in percentage of infected quarters
	Number of quarters	% infected of 631 total quarters	Number of quarters	% infected of 611 total quarters	
Staphylococcus	92	14.58	48	7.85	46.16
Streptococcus	69	10.93	42	6.87	37.15
Coliforms	20	3.16	2	0.32	89.87
Other Bacteria	28	4.43	14	2.29	48.31

Appendix Table 4. Least square means and standard errors for quarters with somatic cell count (SCC) >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml for control and lime-treatment cow groups.

SCC/ml (x1000)	First month						Last month					
	Control			Treatment			Control			Treatment		
	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t
>165	0.7500	0.3015	0.0157	0.6250	0.3015	0.0157	1.3125	0.3015	0.0157	0.4000	0.3113	0.2039
>200	0.6875	0.2647	0.0118	0.5000	0.2647	0.0638	1.125	0.2647	0.0638	0.2667	0.2733	0.3332
>750	0.1250	0.0955	0.1960	0.0625	0.0955	0.5157	0.4375	0.0955	0.0001	-326E-19	0.9872	1.000

Appendix Table 5. Least square means and standard errors for quarters with somatic cell count (DHIA) >165,000/ml, >200,000/ml, >400,000/ml and >750,000/ml for control and lime-treatment cow groups.

	First month						Last month					
	Control			Treatment			Control			Treatment		
DHIA SCC/ml (x1000)	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t
>165	0.1250	0.1074	0.2495	0.2000	0.1110	0.0040	0.3333	0.1110	0.0040	0.2857	0.1148	0.0159
>200	0.1250	0.1012	0.2219	0.1333	0.1045	0.2073	0.3333	0.1045	0.0023	0.2143	0.1082	0.0525
>400	0	0.0750	1.000	0.0666	0.07752	0.3934	0.2000	0.0775	0.0125	0.1429	0.0802	0.0804

Appendix Table 6. Least square means and standard errors for number of times quarters infected with *Staphylococcus*, *Streptococcus*, and other bacteria for control and treatment cows between the first and last month of the study.

	First month						Last month					
	Control			Treatment			Control			Treatment		
Microbes	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t	Estimate	± SE	P value Pr > t
Staph	1.250	0.2107	0.0001	0.625	0.2107	0.0044	0.3125	0.2107	0.1434	0.1333	0.2176	0.5425
Strep	0	0.2018	1.000	0.125	0.2018	0.538	0.875	0.2018	0.0001	0.2667	0.2084	0.2057
O B	0.125	0.078	0.1151	0	0.0806	1.000	0	0.0806	1.000	0.1429	0.0834	0.0926

Foot note: *Staph* stands for *Staphylococcus*, *Strep* stands for *Streptococcus*, Coli stands for coliforms OB stands for Other Bacteria.