Evaluating a Nutritional Supplement in Enhancing the Effectiveness of Selective Dry Cow Therapy

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To the Graduate Council:

I am submitting herewith a thesis written by Kody Hash entitled "Evaluating a Nutritional Supplement in Enhancing the Effectiveness of Selective Dry Cow Therapy." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Gina M. Pighetti, Major Professor

We have read this thesis and recommend its acceptance:

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
Evaluating a nutritional supplement in enhancing the effectiveness of selective dry cow therapy

A Thesis Presented for the

Master of Science

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Kody Max Hash

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ABSTRACT

The objective of this study was to assess the efficacy of a nutritional supplement (Omnigen-AF®; OG) fed during the dry period through early lactation on milk production, somatic cell count (SCC), and intramammary infections (IMI) of multiparous Holstein cows receiving selective dry cow therapy (SDCT). We hypothesized that feeding OG beginning in the dry period through 30 days in milk (DIM) would increase milk yield, reduce somatic cell count and IMI. To test our hypothesis, 113 multiparous pregnant Holstein cows were enrolled into a selective dry cow therapy (SDCT) program and alternately assigned at dry-off to either OG (n = 52) or control (CON, n = 61) treatments. Efforts were made to balance the number of cows and quarters receiving selective dry cow therapy across treatments. Quarter milk samples were taken 7 d before dry off to assess SCC and microbial status. Either positive growth on culture media or >150,000 SCC resulted in the quarter being treated with intramammary antibiotics. At dry off, quarters considered uninfected were rechecked for SCC and treated with antibiotics if >150,000. An internal teat sealant was used on every quarter regardless of SDCT. Both OG and CON (placebo) supplements were fed individually once per day at a rate of 60 g/d per head from dry off through 28 DIM by restraining in a headlock system. Consumption of grain mixture was observed and recorded daily. Milk samples and yield were taken approximately 3.7 ± 0.1 d after calving, and 7, 14, and 28 d into lactation to assess SCC and microbial status. SDCT resulted in 61.5% reduction in antibiotics over both treatments compared to using antibiotics in every quarter. Cows supplemented with OG had greater (2.4 ± 1.1 kg) milk production compared to CON, while SCC did not differ between treatments. Supplementing OG while selectively administering antibiotics indicated no increased risk of new IMI. In conclusion, supplementing OG, an immunomodulatory feed additive, to dry
and early lactation dairy cows participating in a SDCT program was associated with increased milk production and no effect on risk of new IMI over the dry period.
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CHAPTER I:

MASTITIS PREVENTION AND NON-ANTIBIOTIC ALTERNATIVES: A REVIEW
INTRODUCTION

Mastitis is an inflammation of the mammary gland that is the most common disease in the dairy industry. Dairy production systems use antibiotics mostly toward treating mastitis that counts for 85.4% of antibiotic usage on U.S. dairy farms (USDA, 2016). The occurrence of intramammary infections (IMI) in a herd depends upon effective application of standard mastitis control practices (Oliver and Mitchell, 1984). With these mastitis control practices being highly implemented, the shift in mastitis-causing pathogens from contagious to environmental has caused a change in the management of mastitis prevention (Klaas et al., 2018). During the transition period when the cow goes from a non-lactating to a lactating state, physiological changes compromise the cow’s immune system that increases her susceptibility to disease. The use of blanket dry cow antibiotic therapy (BDCT) is common practice in preventing and eliminating IMI during the dry period. Growing concern regarding antibiotic, or antimicrobial, use in dairy production systems on the rise of antimicrobial-resistant bacteria (Oliver et al., 2011). The World Health Organization in 2017 identified several antimicrobials that are of importance for human medicine, that are also used for mastitis treatment including cephalosporins (e.g. ceftiofur). With growing concern of the use of antibiotics in food animals, current strategies including blanket antibiotic dry cow therapy in the dairy industry are being heavily reviewed and has motivated research into alternative practices (Ekakoro et al., 2018). Finding alternatives for preventive antibiotics at dry-off is key in controlling mastitis and easing concerns of antibiotics usage.

Two current strategies to limit the use of antibiotics at dry off are internal teat sealants and selective dry cow therapy. Internal teat sealants (ITS) are a non-antimicrobial product that
acts similar to the cow’s natural teat canal defense system. Internal teat sealants are proven to prevent IMI during the dry period (McParland et al., 2019) and reduce new IMI after calving when used with or without antibiotics (Rabiee et al., 2013). Selectively treating only infected quarters at dry-off, otherwise known as selective dry cow therapy (SDCT), is another alternative available to lessen the reliance on antibiotic use. In a recent national survey, SDCT was only applied on 10% of US dairy operations (USDA, 2016). Limited adoption thus far likely has several potential reasons. One limitation of adoption of SDCT is the cost of labor and supplies needed to identify IMI for antibiotic use. Studies investigating implementation of SDCT have had mixed results and can be partly attributed to methods to identify mastitis, herd prevalence of IMI, and SCC (Berry and Hillerton, 2002b; Scherpenzeel et al., 2014). Even though SDCT programs have been known to reduce antibiotic use, the risk of possible IMI that are misclassified and not treated exist when compared to BDCT (Browning et al., 1994; Cameron et al., 2014; Rowe et al., 2020).

Although not traditionally used to reduce antibiotic use, several nutritional supplements have been implemented at dry-off and through the dry period to promote immunity and milk production in the next lactation. Well-known dietary supplements in the dairy industry are Vitamin E and selenium (Se) when fed daily promotes immune capabilities and reduced duration of clinical mastitis (Spears and Weiss, 1997; Weiss et al., 1997). Similarly, daily feeding of a commercially available nutritional supplement (Omnigen-AF, OG; Phibro Animal Health, Teaneck, NJ) that boosts the cow's immune functions during times of heightened physiological stress has the capability to reduce disease (Ryman et al., 2013; Wang et al., 2007). This non-antibiotic feed supplement consists of a mixture of yeast, B-complex vitamins and other
proprietary ingredients. When OG was fed to transition cows, Wu et al. (2017) found peripheral blood neutrophil function was maintained during the periparturient period. Mammary gland tissue transition from non-lactating to lactating was improved when fed OG during the prepartum period into lactation (Nace et al., 2014). Therefore, use of an immune booster such as a nutritional supplement combined to enhance a SDCT program to further protect against IMI, is unknown in dairy cattle. This chapter will provide an overview of different strategies to control and prevent mastitis in dairy cattle.

**Mastitis: Clinical and Subclinical**

Mastitis can be clinical, having visible signs, or subclinical, with no visible signs. Clinical mastitis (CM) is a noticeable infection that includes abnormal and/or discoloration in the milk, and swollen quarters with much higher somatic cell counts with or without presence of bacteria. Cows having >250,000 cells/ml are at a greater risk of CM infections (Beaudeau et al., 2001). Tomazi et al. (2018) calculated incidence of CM at the quarter level was 9.7 cases per 10,000 quarter days at risk. Clinical mastitis risk has also been correlated with increased parity, poor udder hygiene, and severe hyperkeratosis (Breen et al., 2009). Subclinical mastitis does not have a visible inflammatory response in the udder and milk but can be detected by an increase in somatic cell count. Somatic cells include 75% leucocytes such as neutrophils, lymphocytes, macrophages, and erythrocytes that have entered the mammary gland in response to injury or infection, and 25% milk-secreting epithelial cells that have shed from the mammary gland lining (Sharma et al., 2010). Measurement of cells in milk, otherwise known as somatic cell count (SCC) is used to assess milk quality and mastitis control. Cow and quarter level somatic cell
count of <200,000 cells/ml are considered uninfected quarters and associated with limited milk production loss (Harmon, 1994). Chronic subclinical mastitis infections having a somatic cell count >200,000 cells/ml that were also culture positive, reduced milk yield by 24.5% and total milk solids by 22.4% (Martins et al., 2020). Bulk tank somatic cell count (BTSCC) can give an estimation of infection prevalence at the herd level where with every increase of 100,000 cells/ml in BTSCC, quarters infected increase by 3.3% (Eberhart et al., 1982). High bulk tank somatic cell count impacts the quality of milk by reducing the amount of lactose and casein in the milk that are critical for various milk products (Wickstrom et al., 2009).

Common Organisms Isolated from Mastitis Cases

As herds have reduced the incidence of mastitis caused by contagious pathogens (i.e. Staphylococcus aureus, Streptococcus agalactiae) by the means of mastitis control programs, clinical and subclinical mastitis resulting from environmental pathogens (i.e. Streptococcus uberis, coliform bacteria) have increased (Hogan et al., 1989; Hillerton et al., 1995). Escherichia coli, Klebsiella spp., and Enterbacter spp. accounted for more than 40% of clinical coliform mastitis from 5 different farms (Schukken et al., 2011). The most common environmental pathogen causing clinical mastitis is E. coli with an incidence rate of 0.05 (Barkema et al., 1997). Culling or removal from the herd was highest in cows having clinical mastitis from gram-negative pathogens at 26.7% (Oliveira et al., 2013). Risk of death for cows with gram-negative clinical mastitis is 2.3 times more likely than a cow without clinical infection (Hertl et al., 2011). Streptococcus uberis is known as a common major pathogen causing mastitis that is classified as
the most difficult environmental pathogen to handle, as it can act in a contagious pattern as well (Wente et al., 2019).

More recently, Rowe et al., (2019) conducted a survey from 78 US dairy herds and found quarter-level prevalence of subclinical IMI before dry off at 21.1% with non-aureus Staphylococcus species (11.4%) such as Staphylococcus chromogenes being most commonly (6.9%) and gram-negative species least commonly (0.8%) isolated. As NAS species are considered minor pathogens, they are less likely to cause clinical mastitis but can cause increases in SCC (Green et al., 2002; Rowe et al., 2021). Taponen et al. (2007) found the most common organism that persisted throughout the entire lactation with mean quarter SCC >600,000 was Staph. chromogenes. Infection with NAS species can increase SCC and protect the quarter from mastitis caused by major pathogens like Staph. aureus (Schukken et al., 1989). A recent study by De Vliegher et al. (2004) also has shown evidence that certain NAS species can have protective effects in the teat canal against major mastitis-causing pathogens such as Staph. aureus.

Host Immunity

The early non-lactating period and periparturient period are the most vulnerable times for developing new IMI as both stages are rapidly changing in the mammary gland (Burton and Erskine, 2003; Nickerson et al., 2019). The first stage lasts approximately 3 weeks following the cessation of milking, the mammary gland undergoes involution by an increase in mammary pressure from milk buildup and disruption of secretory structures (Eberhart, 1986). During involution, milk fat and casein concentrations decline while immune cells and proteins that inhibit bacterial growth increase (Bradley and Green, 2004). Infection rate is highest during the first and last 25% of the dry period originating from environmental pathogens such as coliform
and streptococci (Smith et al., 1985). In the later part of the dry period, colostrogenesis occurs in preparation for calving and is a time of immune dysfunction for the cow. Colostrum production involves massive production of immunoglobulins necessary for building the calf’s immune system, and if antibody production is reduced, could lead to lowered immune responses during the periparturient period (Aleri et al., 2016). Proper management during the dry period is vital for setting up the next lactation for success.

As the cow’s environment can be the source of infection, managing the housing properly can lead to reduced infection rate. Dairy farms that had reduced SCC paid more attention to details in the cows’ environment than farms that had higher SCC that worked quickly rather than precisely (Barkema et al., 1999). When a designated maternity area that is isolated and clean for parturition was used, a lower level of incidence of clinical mastitis rate occurred (Bartlett et al., 1992). Wenz (2007) determined strong relationships associated with bulk tank SCC and the housing and bedding used in the cow’s housing. Bedding material and teat canal colonization of environmental coliform pathogens had the highest bacterial counts (2.4 CFU/ml) when compared to other mastitis-causing bacteria (Paduch et al., 2013). Exposure to mastitis-causing pathogens is directly affected by environmental conditions that the cow is exposed to along with the cow’s natural defense mechanisms.

A natural defense mechanism from invading bacteria called the keratin plug is built in the teat canal during involution as early as 16 days but more commonly occurs 30-40 days into the dry period (Williamson et al., 1995). Keratin is a waxy material that traps invading bacteria and contains antimicrobial fatty acids such as lauric, myristic, and palmitoleic acids that are bacteriostatic (Sordillo et al., 1997). Rate of teat canal closure differs among cows where 50% of
teats were open during the first month of dry off, which leaves some cows more vulnerable to new infections (Williamson et al. 1995). Cows with higher milk production have lower mass of keratin in the teat canal, which leaves more opportunity for mastitis-causing bacteria to invade the mammary gland (Capuco et al., 1990). Teat closure was 1.8 times less likely if milk production was >21 kg (Dingwell et al., 2004). In Holstein heifers as parturition got closer, teat canals rapidly began to open resulting in culture positive quarters that increased from 57% at 60-31 days before calving to 80% at 30 days to calving (Kromker and Friedrich, 2009). Teat canal keratin can be compromised during events such as teat damage that can increase the chances of IMI. Teat-end damage increased the odds of contracting new infections by 5% (Dingwell et al., 2004). Teat canal injury resulted in 2.4 times higher in having subclinical infections with SCC >400,000 cells/ml (Geishauser et al., 1999). Teat-ends with very rough hyperkeratosis associated with machine milking, had more than 70% culture positive teat canal colonization of coliform bacteria (Paduch et al., 2012).

Innate immunity represents the initial defense mechanisms during early stages of infection. This includes cellular defenses such as neutrophils, macrophages and polymorphonuclear neutrophilic lymphocytes (PMN). The promptness of these immune response cells is critically important to the severity and duration of mastitis infections. Macrophages represent the most common cell type in healthy milk and mammary tissue. These cells also have phagocytic capabilities and can consume bacteria and can signal the PMN to the site of infection for elimination of bacteria (Paape et al., 2000). Neutrophils are the most common cell type found in early stages of inflammation in mammary tissue and can account for >90% of total leukocytes in the mammary gland during an infection (Sordillo et al., 1989). After
being “called” to the infection site by cytokines and other chemotactic peptides, neutrophils engulf and kill the bacterial pathogens.

Adaptive immunity, or an acquired immune response, is the recognition of specific elements of a pathogen that enables selective elimination. Antibodies, macrophages and lymphocytes facilitate recognition of pathogen characteristics. Due to the “memory” of lymphocytes, acquired immune responses can be increased by multiple exposures to a specific pathogen. When antibody and cell-mediated immune responses were used as indicators for adaptive immunity, cows with average immune responses were 2.5 times more likely to have a case of severe mastitis than cows with a high immune response (Thompson-Crispi et al., 2012).

**MASTITIS CONTROL AND PREVENTION**

*Role of Vaccines in Reducing Severity of Mastitis*

Vaccination strategies against mastitis-causing bacterial pathogens also play an important role in infection control. Use of vaccines has been shown to reduce severity and decrease the occurrence of coliform infections (Bradley and Green et al., 2004). Use of a commercially available *Escherichia coli* vaccine reduced the occurrence of clinical coliform mastitis from 29 infections in unvaccinated cows to 6 infections in vaccinated cows (Gonzalez et al., 1989). Milk production of JVac® (Merial Ltd., Duluth, GA) immunized cows was 6-15 kg higher 21 d following coliform infection compared to unvaccinated cows (Wilson et al., 2008). Wenz (2007), when surveying dairy herds to evaluate associations between bulk tank SCC and management
practices, found use of a coliform mastitis vaccine was related to a lower bulk tank SCC ($P = 0.02$). Vaccination with an experimental *Klebsiella pneumoniae* vaccine reduced the risk of infection by 76.9% of Klebsiella and 47.5% of total coliform mastitis (Gorden et al., 2018).

Tomita et al. (2000) showed increased antibody titers ($P > 0.05$) from dry off to 45 DIM against *E. coli* in cows vaccinated with a commercially available Enviracor J-5 (Zoetis, Parsippany, New Jersey). A vaccine efficacy study using a commercially available *Streptococcus uberis* vaccine UBAC® (Laboratorios Hipra S.A., Amer, Spain) against *Streptococcus uberis* resulted in reduced mastitis clinical severity by 19% when compared to non-vaccinated cows (Collado et al., 2018).

In a vaccine trial against *Staph. aureus*, incidence of IMI decreased from 11.2% in the quarters of control cows to 6.2% in vaccinated cows (Calzolari et al., 1997). In a field trial using a commercially available *Staph. aureus* vaccine Startvac® (Hipra UK Ltd., Nottingham, UK) resulted in decreased odds of developing clinical mastitis (odds ratio: 0.58; CI: 0.35-0.98) (Bradley et al., 2015). In heifers, an experimental trial using a *Staph. aureus* vaccine resulted in vaccinated heifers showed no clinical signs when 6% of control heifers had clinical *Staph. aureus* (Nordhau et al., 1994). A review of efficacy of *Staph. aureus* vaccines concluded that most have not consistently prevented infection in the mammary gland due to the complexity of the organism and diversity of strains (Middleton et al., 2008). Due to the lack of efficacy in the *Staph. aureus* vaccine, controlling this contagious pathogen is better managed in the parlor. This was shown in a USDA report in 2014 where mastitis vaccination against *Staph aureus* was used in only 1.4% of dairy operations, while gram-negative bacteria was used the highest at 18.1%. 
Blanket Dry Cow Antibiotic Therapy

Blanket dry cow therapy (BDCT) cures current IMI at drying off and prevents new IMI in the early dry period. Intramammary blanket dry cow therapy has been part of the National Mastitis Council’s recommendation in controlling mastitis (NMC, 2016). Industry-level implementation of blanket dry cow therapy has played a substantial role in reducing contagious mastitis pathogens, reduction of IMI, and reduction in bulk tank somatic cell count (Hillerton et al., 1995; Ruegg, 2012; Rowe et al., 2020). In the U.S., approximately 80 percent of dairies use antimicrobials on all cows at dry off (USDA, 2016). Dry cow antibiotics achieve >70% cure rate of existing infections and remains >50% effective in preventing new infections, but new IMI still occur (Oliver et al., 1990; Dingwell et al., 2003; Rowe et al., 2020). Cows not given blanket antibiotic therapy had >30% higher incidence of clinical mastitis when compared to cows given blanket antibiotic therapy (14.7% vs. 10%, respectively) (Browning et al., 1990). When comparing six (6) different antimicrobial products, blanket dry cow therapy had an overall cure rate of 83.94% and quarter new infection rate during the dry period was 17.44% (Petzer et al., 2009). Quarters given antibiotics at dry off had lower incidence of clinical mastitis at calving (12 infections) compared to quarters not given antibiotics (33 infections) (Berry and Hillerton, 2002b).

Selective Dry Cow Therapy

Assessing the intramammary infection status before dry off can reduce unnecessary use of dry cow antibiotic therapy, or commonly known as selective dry cow therapy. Selectively treating quarters infected at dry off requires an accurate selection method and if done incorrectly
could result in financial losses (Berry et al., 2004). Selection criteria ranging from bacteriological culture, somatic cell count, California mastitis test and clinical mastitis history have been used with varying degrees of success. A Petrifilm-based on-farm culture system used with SCC <200,000 cells/ml for antimicrobial treatment decisions used with internal teat sealants in all quarters was not different in post-calving IMI between blanket (15.3%) and selective dry cow therapy cows (15.8%) (Cameron et al., 2014). The use of mastitis history, California mastitis test and somatic cell count resulted in up to 83% of infected cows treated with antibiotic therapy and approximately 70% of uninfected cows treated (Rindsig et al., 1979). Dry period IMI cure risk was similar in culture guided (87.5%) and algorithm guided SDCT using SCC and clinical mastitis history (88.1%) programs compared to BDCT (86.8%) (Rowe et al., 2020). Selectively treating with antibiotics resulted in 16 - 50% of untreated quarters infected at calving (Rindsig et al., 1979; Berry and Hillerton, 2002b; Cameron et al., 2014).

Selective dry cow therapy is not without limitations and can result in missing infections and/or treating quarters/cows with antibiotic therapy that are not infected. When SCC of 250,000 cells/ml was used as a threshold for treatment, 76% of cows were correctly determined while 2% of infected cows and 21% of uninfected cows were inaccurately diagnosed (Andrews et al., 1983). Even though selective quarter treatment used approximately 30% less antibiotics it was associated with a higher incidence of intramammary infections (2.24 relative risk) when compared to blanket dry cow therapy (1.77 relative risk) (Robert et al., 2006). New infection rate during the dry period was highest with selective quarter antibiotic therapy (6.4%) when compared to blanket dry cow therapy (2.6%) and selective dry cow therapy (3.9%) (Browning et al., 1994). Selective dry cow therapy has a higher mean incidence of quarters at risk of
contracting new IMI (9.9%) when compared to blanket dry cow therapy (6.5%) (Robert et al., 2006). Even after infected quarters have been identified, proper antibiotics used to combat the pathogen responsible for infection is key to elimination of the infection. Depending on the antibiotic used, quarters given antibiotics varied in incidence of IMI during the dry period from 6.6% to 8.0% (Robert et al., 2006). Therefore, since the vulnerability of infections increase with the use of SDCT programs, more protection is needed to prevent pathogen intrusion into the udder.

**Internal Teat Sealant**

The use of an internal teat sealant has shown to be an effective non-antimicrobial alternate to dry cow therapy in preventing new intramammary infections during the dry period but not eliminating current IMI (Berry and Hillerton, 2002a). In approximately 40 percent of U.S. dairies, internal teat sealants are used at dry off in adding to the physical barrier to prevent bacteria from entering the teat canal (USDA, 2016). Herds with proper mastitis control that have somatic cell count <200,000 cells/ml across lactation may use internal teat sealants with little effect on herd somatic cell count (McParland et al., 2019). In seasonally calving pasture-based herds, prevalence of cows with somatic cell count >150,000 cells/ml 60-80 days after parturition was reduced from 0.15 for the antibiotic only group to 0.09 in the antibiotic with internal teat sealant group (Bates et al., 2016). Internal teat sealants alone reduced new IMI by 73% compared to cows not given internal teat sealants (Rabiee et al., 2013). Quarters given internal teat sealants had 0.39 times less risk of new IMI than quarters not given teat sealants (Halasa et al., 2009). During an extended dry period of >10 wk, internal teat sealants reduced new quarter infections in
cows (3.7%) compared to cows given antibiotic treatment alone (11.4%) (Berry and Hillerton, 2007). The use of internal teat sealants with dry cow antibiotic therapy reduced IMI after calving by 25% when compared to antibiotics alone (Rabiee et al., 2013). When a commercially available internal teat sealant was used with intramammary antibiotics, there was a 30% lower risk of developing a new IMI between dry off and 1 – 3 days in milk when compared to intramammary antibiotics alone (Godden et al., 2003). Cows that received internal teat sealants had significantly less infections of major pathogens, including *Escherichia coli* and all *Enterobacteriaceae*, compared to cows not given internal teat sealants at dry off (103 vs 145 infections, respectively) (Huxley et al., 2002). Additionally, use of an internal teat sealant in heifers reduced the risk of new infection rate with *Streptococcus uberis* by 70% in quarters with an IMI before calving (Parker et al., 2008). Internal teat sealants showed a higher protection against new environmental IMI at time of calving in cows of parity > 4 than cows in parity 2 (1.05 vs. 3.82 relative risk) (Sandford et al., 2006). Heifers administered internal teat sealants approximately 30 d before calving compared to no internal teat sealant tended to decrease post-calving IMI with any bacterial species (13.7% vs. 9.6%, respectively) and reduce risk of clinical mastitis cases (6.8% vs. 4.2%, respectively) (Parker et al., 2007). Use of an internal teat sealant alone reduced clinical mastitis compared to untreated controls (24 cases versus 45, respectively) in herds averaging 250,000 SCC (Bhutto et al., 2011). Quarters given an internal teat sealant in combination with antibiotics had significantly less clinical mastitis (4/562) caused by *Streptococcus uberis* compared to when a teat sealant was not used (14/552) (Newton et al., 2008). Additionally, use of an internal teat sealant with antibiotics reduced cows acquiring clinical mastitis into 60 DIM by 2.79 times compared to antibiotics alone (Freu et al., 2020).
Clinical mastitis caused by coliforms was reduced when internal teat sealants were used when compared to using antibiotic alone (3.0% vs 5.4%, respectively) (Baillargeon and LeBlanc, 2010). Pasture based herds with longer dry periods also benefit in using internal teat sealants alone, where the use reduced the probability of clinical mastitis from 0.21 to 0.05 compared to no internal teat sealant or antibiotic used (Bates and Saldias, 2018). In contrast, use of an internal teat sealant with antibiotics on quarters that had SCC <200,000 cells/ml with no clinical mastitis history had less benefit in preventing new infections post-calving when compared to internal teat sealant alone (32.48% vs 37.08% total pathogen prevalence, respectively) (Bradley et al., 2010). Improper use of teat sealants can lead to mastitis by possibly inducing pathogens during insertion due to udder hygiene, and/or contamination of the teat sealant itself (Crispie et al., 2004). This suggests that proper use of internal teat sealant can be used as a replacement or in combination with dry cow therapy in occurrence of new IMI and clinical mastitis after calving (Woolford et al., 1998).

Role of Diet in Preventing Disease

The diet of the cow plays an important role on the immune system to defend against infection-causing bacteria. Inefficient quantities of antioxidant vitamins and minerals has negative effects on mammary gland immunity (Yang and Li, 2015). During the periparturient period the dairy cows’ immune system was boosted by enhancing neutrophil function resulting from addition of higher concentrations of vitamin E and selenium (Spears and Weiss, 1997). Supplemental vitamin E and selenium in the diet reduced clinical mastitis by 62% and had 41% shorter duration of clinical symptoms (Smith et al., 1984). Cows receiving a high vitamin E
supplement had 11.8% reduction in the percentage of quarters with infections at calving when compared to cows fed a low vitamin E concentration that resulted in 32% IMI at calving (Weiss et al., 1997). Clinical mastitis during the first 7 d of lactation was also reduced by 22.4% in high vitamin E diets compared to low vitamin diets (Weiss et al., 1997).

A proper dry cow diet will affect how well the cow will perform in the following lactation, as high producing dairy cows experience massive metabolic changes during the transition period that can predispose the cow to disease. Nutrition during the far-off period, defined as the first 25 days after dry off, plays a substantial role in periparturient metabolism (Dann et al., 2006). There are many interrelationships between nutrition and disease during the periparturient period that are affected by various management factors. Cows fed a low energy diet when compared to a high energy diet had higher dry matter intake after calving (19.7 kg/d vs 18.8 kg/d respectively) (Huang et al., 2014). Decreased dry matter intake (DMI) before and after calving, insufficient amounts of vitamins, trace minerals and/or antioxidants, imbalanced dietary cation-anion difference (DCAD) diets, and insufficient dietary effective fiber can all create metabolic disorders that could predispose the cow to further disorders and diseases (Goff 2006).

**Nutritional Supplement**

Another strategy as an alternative to antibiotics is a commercially available nutritional supplement that can boost the cow's immune system, improve milk quality, and improve cow’s health overall. One of the most studied products thus far, OmniGen-AF (OG) consisting of yeast, B-complex vitamins and other proprietary ingredients, is a non-antibiotic feed supplement with immunomodulatory benefits including enhanced leukocyte function, surface L-selectin
concentrations, and phagocytosis (Ryman et al., 2013). Wang et al. (2007) also observed in sheep fed OG increased concentrations of neutrophils and lymphocytes in circulation, and greater abundance of neutrophil L-selectin proteins. L-selectin receptors provide an initial tether that allows neutrophils to adhere to capillaries for migration into the infected tissue (Kimura et al., 1999; Ozawa et al. 2012). Dairy heifers fed OG resulted in enhanced neutrophil binding and increased engulfing of bacteria such as *Escherichia coli* (Ryman et al., 2013). Dairy heifers fed OG also had an enhanced response to a bovine respiratory disease vaccine by increasing IL-4 concentrations and generating a stronger proliferative response to BVDV types 1 and 2 when compared to controls in vitro (Hurley et al., 2019). Lymphocytes and white blood cells were increased when cows were fed OG during the periparturient period when compared to controls cows not fed OG (Wang et al., 2009). OmniGen-AF fed 60 day before dry off, through the dry period and 30 DIM decreased IMI after calving by 5%, reduced SCC after calving by 281,000 cells/ml, and increased milk production by 4.56 kg (Nickerson et al., 2019). OG fed to cows during the dry period under heat stressed, immune compromising conditions had higher milk production (41.3 and 40.5 kg/d) compared to heat-stressed cows not fed OG (35.9 kg/d) (Fabris et al., 2017). When OG was fed 35 days before calving through 46 DIM, milk yield was higher in OG fed cows (30.3 kg/d) compared to controls (27.1 kg/d) and SCC was lower in OG fed cows (326,000 cells/ml) compared to controls (450,000 cells/ml) (Brandão et al., 2016).

The OG feed supplement also has shown to improve overall postpartum health of dairy cows. Cows fed OG during the dry period through 150 DIM, had fewer retained placentas (OG fed = 5.4%, controls = 7.6%) and fewer displaced abomasums (OG fed = 1.9%, controls 3.2%) (Casarotto et al., 2020). Culling rate of dairy cows was reduced when fed OG (1%) during the
dry period into lactation when compared to cows not fed OG (7.4%) (Mammi et al., 2018).

Under heat-stressed conditions, OG supplementation reduced respiration rates by 7.5 breaths/min for cows in the morning hours (Fabris et al., 2017). Pasture-based cows under a high temperature-humidity index supplemented with OG had higher pasture dry matter intake (7.68 kg/d) when compared to controls (6.99 kg/d) (Gandra et al., 2019). Also observed during heat stress conditions, early post-partum dairy cows not supplemented with OG had higher vaginal temperatures by 0.38 to 0.52% compared to supplemented cows (Leiva et al., 2017). Heifers fed OG 60 days prepartum through calving had lower incidents of mammary edema when compared to control heifers (36% vs. 75%) (Nace et al., 2014). With OG improving post-partum disorders, increased reproductive success would also be apparent. Cows fed OG during the dry period into lactation spent on average half a day less in the hospital pen than control cows (6.02 d vs. 6.56 d), and were confirmed pregnant 10 d earlier than control cows (139 d vs. 149 d) (Casarotto et al., 2020).

Thus feeding OG benefits not only milk yield, but also the immune system and the cow’s overall health. The use of OG with other non-antibiotic alternatives to improve the health and productivity of dairy cows should be investigated further.

**CONCLUSION**

With the use of preventive antibiotics being increasingly scrutinized, other strategies have gained attention to reduce the amount of antibiotics used on commercial dairy farms. Selectively treating intramammary infections at dry-off offers a solution to reduce antibiotic use, but can miss infections that would otherwise be treated by the blanket use of intramammary antibiotics. Combining alternative strategies, such as a nutritional supplement to enhance the effectiveness of
SDCT program is unknown and needs to be investigated further. Our study evaluates the use of a commercially available nutritional supplement in enhancing the effectiveness of a SDCT program that includes an internal teat sealant. Our objective was to compare milk production, SCC, IMI risk, and microbiological status between cows fed OG and cows not fed OG during the dry period through early lactation in combination with a SDCT program. Our hypothesis was by feeding OG beginning at dry off and continuing through 28 DIM would enhance a SDCT program to increase milk production and decrease SCC and IMI after calving in multiparous Holstein cows.
CHAPTER II

POTENTIAL OF A NUTRITIONAL SUPPLEMENT TO ENHANCE
SELECTIVE DRY COW THERAPY EFFECTIVENESS
ABSTRACT

The objective of this study was to assess the efficacy of a nutritional supplement (Omnigen-AF®; OG) fed during the dry period through early lactation on milk production, somatic cell count (SCC), and intramammary infections (IMI) of multiparous Holstein cows receiving selective dry cow therapy (SDCT). We hypothesized that feeding OG beginning in the dry period through 30 days in milk (DIM) would increase milk yield, reduce somatic cell count and IMI. To test our hypothesis, 113 multiparous pregnant Holstein cows were enrolled into a selective dry cow therapy (SDCT) program and alternately assigned at dry-off to either OG (n = 52) or control (CON, n = 61) treatments. Efforts were made to balance the number of cows and quarters receiving selective dry cow therapy across treatments. Quarter milk samples were taken 7 d before dry off to assess SCC and microbial status. Either positive growth on culture media or >150,000 SCC resulted in the quarter being treated with intramammary antibiotics. At dry off, quarters considered uninfected were rechecked for SCC and treated with antibiotics if >150,000. An internal teat sealant was used on every quarter regardless of SDCT. Both OG and CON (placebo) supplements were fed individually once per day at a rate of 60 g/d per head from dry off through 28 DIM by restraining in a headlock system. Consumption of grain mixture was observed and recorded daily. Milk samples and yield were taken approximately 3.7 ± 0.1 d after calving, and 7, 14, and 28 d into lactation to assess SCC and microbial status. SDCT resulted in 61.5% reduction in antibiotics over both treatments compared to using antibiotics in every quarter. Cows supplemented with OG had greater (2.4 ± 1 kg) milk production compared to CON, while SCC did not differ between treatments. Supplementing OG while selectively administering antibiotics indicated no increased risk of new IMI. In conclusion, supplementing
OG, an immunomodulatory feed additive, to dry and early lactation dairy cows participating in a SDCT program was associated with increased milk production and no effect on risk of new IMI over the dry period.

INTRODUCTION

The early non-lactating (dry) period and the periparturient period are the most vulnerable times for developing new intramammary infections (IMI) as both stages are rapidly changing the environment of the mammary gland (Burton and Erskine, 2003; Nickerson et al., 2019). After dry off, teat canal colonization of bacteria increases by cessation of milking procedures including teat cleaning, teat dipping with a germicidal agent, and milk removal that limit bacterial penetration (Nickerson 1985). Treatment of all quarters with antibiotic therapy at dry off reduced contagious mastitis pathogens, IMI, and bulk tank somatic cell count (SCC) by curing current infections and reducing new infections during the dry and periparturient periods (Hillerton et al., 1995; Ruegg, 2018; Rowe et al., 2020). Treatment of all quarters at dry off, or blanket dry cow therapy has been widely adopted in the dairy industry. In 2014, most dairy cows (93%) in the US were given antimicrobials at dry off (USDA, 2016).

Recently, concern from consumers and dairy product processors regarding antibiotic use has motivated research into alternative practices that lower the need for antibiotics (Lipsitch et al., 2002). Development of alternative approaches for sustaining mammary health during times of physiological stress could positively influence the cow’s ability to defend against IMI and reduce the need for antibiotics. Selective dry cow therapy (SDCT) offers an alternative by administering antibiotics based on infection status at dry off. Effective SDCT requires accurately identifying infections at dry off. Bacteriological culture (Cameron et al., 2014; Rowe et al.,
2020), SCC and clinical mastitis history (Torres et al., 2008) have been used with varying degrees of success. Depending on the criteria used, SDCT reduced antibiotic use at dry off by 55% (Rowe et al. in 2020), 21% (Cameron et al., 2014), 48% (McParland et al., 2019), and 58% (Kabera et al., 2019) when compared to blanket antibiotic treatment.

Although SDCT has been shown to reduce antibiotic use, a higher level of new IMI and missing infections can occur. Blanket dry cow therapy offered a 10% infection rate of cows given antibiotics in one or multiple quarters after parturition (Berry et al., 2004). However, selectively treating with antibiotics resulted in 16 to 50% of untreated quarters infected at calving (Rindsig et al., 1979; Berry and Hillerton, 2002b; Cameron et al., 2014). For further protection against new IMI during the dry period, addition of an ITS to a SDCT culture-guided program has resulted in decreased IMI during the dry period (Cameron et al., 2014; Kabera et al., 2019).

With the highest IMI risk before and after calving, internal teat sealants can offer protection against new IMI to cows that are immunosuppressed (Mehrzad et al., 2001). Whereas dry cow antibiotics only benefit the early dry period, internal teat sealants (ITS) that function like a keratin plug can prevent IMI throughout the entire dry period. During involution, the teat canal builds a keratin plug that blocks invading bacteria but rate of canal closure differs among cows (Capuco et al., 1990; Sordillo et al., 1997). A review by Rabiee et al. (2013), concluded that ITS reduced risk of new IMI by 25% when used with antibiotics compared to use of antibiotics alone and a 75% reduced risk in new IMI when compared to cows not given ITS or antibiotics.

Optimizing dairy cattle nutrition could improve immune function and reduce disease incidence. Daily vitamin E and selenium supplementation to dairy cows enhanced immune
function and reduced mastitis incidence by 37% after parturition (Smith et al., 1984; Spears and Weiss, 1997). The OmniGen-AF (OG) feed supplement containing yeast and B-complex vitamins enhanced key immune functions (Ryman et al., 2013; Brandão et al., 2016; Nickerson et al., 2019). OmniGen-AF boosted leukocyte phagocytosis and increased cell surface trafficking proteins (Ryman et al., 2013). Fed daily to dairy cows during the dry period and into lactation, OG decreased SCC and new IMI, and increased milk production during the next lactation when used in combination with a blanket dry cow therapy program (Brandão et al., 2016; Fabris et al., 2017; Nickerson et al., 2019). As SDCT becomes more frequent, understanding the effectiveness of alternative strategies to aid in curing missed IMI and limit new infections will be needed.

The aim of this study was to evaluate the use of OG in enhancing the effectiveness of a SDCT program that includes an internal teat sealant. Our objective was to compare milk production, SCC, IMI risk, and microbiological status between cows fed and not fed OG during the dry period through 28 DIM in combination with a SDCT program. Our hypothesis was feeding OG from dry off through 28 DIM would increase milk production and decrease SCC and IMI after calving in multiparous Holstein cows enrolled in a SDCT program.

**MATERIALS AND METHODS**

**Sample Size Calculation**

Sample size calculations were based on published IMI prevalence of 5% for cows receiving OG and 10% in cows not receiving OG (Woolford et al., 1998). With a significance level of 95% (2-sided) and a power of 80%, it was determined that approximately 434 quarters (approx. 54 cows in each group) would be required to detect a minimum change in IMI from 5 to 10%. To account for potential losses in animals, samples, etc. we included 15% extra quarters
that totaled 500 quarters or 125 cows per study group. Due to the reduction in cow numbers in the herd during the course of this project and the need for other research projects at this facility, the cow numbers needed to achieve sufficient power for the level of significance was not reached (143 cows enrolled).

**Experimental animals**

Lactating Holstein dairy cows confirmed pregnant in at least their first lactation were enrolled into one of two treatments, 73 control cows not fed OG and 70 treatment cows fed OG. Animals were housed at the East Tennessee Research and Education Center - Little River Animal and Environmental Unit (ETREC – LAEU) dairy herd in Walland, Tennessee, USA. During July 2018 to July 2019, cows were enrolled 7 d before dry off with a dry period length of 62.6 ± 1.60 d and exited the study 28 ± 3 d after calving. Cows were enrolled by alternating treatment assignment at the day of dry off. If more than two cows were dried off on a given day, the number of quarters requiring intramammary antibiotics, *Staphylococcus aureus* infection status, milk production, and parity at calving were reviewed to maintain similar demographic profiles across treatments. Quarters were assessed for SCC and microbial status to determine the need for dry cow antibiotic therapy and is outlined more fully below. Milk yield was taken daily as cows entered the parlor. All procedures used for this project were approved by the Institutional Animal Care and Use Committee (IACUC #2622-0618) at the University of Tennessee, Knoxville.
**Farm Management Protocols**

At dry off each cow received 5cc Enviracor-J5 (Zoetis, Parsippany, New Jersey) against *E. coli*; 2cc Salmonella-SRP (Zoetis, Parsippany, New Jersey), a Salmonella siderophore receptor and porin (SRP) proteins vaccine; and 2cc Vision-7 Somnus with SPUR (Merck, Whitehouse Station, New Jersey) against Clostridium chauvoei (Blackleg), Clostridium septicum (malignant edema), Clostridium novyi (acute toxemia or black disease), Clostridium sordellii (wound infection), Clostridium perfringens types c & d (enterotoxemia), and Haemophilus somnus (Pneumonia). All total mixed rations (TMR) rations were formulated using the National Research Council (NRC, 2001) requirements for dairy cattle. From dry off to approximately 28 days before calving (a far-off TMR was fed once daily and consisted of a grain mixture, orchard grass hay, corn silage and rye-grass silage (Table 1). Treatment groups were assigned to separate but similar pastures that consisted of separate water troughs and feed bunks with 20 headlocks in each pasture. The headlocks and feed bunks were located on the perimeter of the pasture on a concrete pad. A hay ring was also located on the concrete pad for feeding hay, and both feeding areas were uncovered and exposed to environmental conditions.

Cows were moved 30 ± 4 days before calving to a pack barn bedded with straw that was re-bedded weekly. Each cow received 5cc Enviracor-J5, 2cc Salmonella-SRP, and 3cc Inforce-3 (Zoetis, Parsippany, New Jersey), an intranasal vaccine against bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis (IBR) and Parainfluenza virus type 3 (PI3) infection. The calving facility consisted of a covered barn that was divided into two sections, one section for each treatment, with each section consisting of 25 headlocks, and a 4-hole water trough that
was shared between the two sides. Each side had access to separate pastures in equal size. A close-up TMR was fed once daily consisting of prepartum grain mix, Animate (Phibro Animal Health Corporation Teaneck, New Jersey), rye-grass hay, rye-grass silage and corn silage (Table 1).

After calving, cows were transported to a freestall barn consisting of 12 stalls and a 2-hole water trough. They were examined for postpartum metabolic diseases daily for 2 weeks while restrained in headlocks. Examinations included: rumination, urine ketone level, rectal temperature, and uterine infection status. Immediately after calving and 24 h later cows were given one Bovikal bolus orally (Boehringer Ingelheim, Duluth, Georgia) to provide a quick and sustained release of calcium for milk fever (hypocalcemia) prevention. Stalls were bedded with recycled sand and replenished once a week. Cows were fed a lactation TMR twice daily consisting of corn silage, rye grass silage, rye grass hay, grain mix, and water (Table 1) and balanced for 32 kg of milk using NRC guidelines (2001). Before feed rations were distributed to cows, refused feed was removed from the feed bunk.

**Feeding Procedures**

Omnigen-AF® was donated by Phibro Animal Health Corporation (Teaneck, New Jersey, USA). Control cows were fed 228 grams of a grain mixture formulated for far-off dry cows (Table 1). Treatment cows were fed 60 g of OG per cow daily that was mixed with the same far-off dry cow grain mixture to total 228 g. The amount of OG chosen was slightly above the amount provided by other studies of 56 g/cow daily (Brandão et al., 2016; Leiva et al., 2017; Wu et al., 2019). The OG mixture was premixed in batches by a feed mixer located at the East Tennessee Research and Education Center - Johnson Animal Research and Teaching Unit.
(ETREC-JARTU, Knoxville, Tennessee, USA). Each batch consisted of 29.5 kg of Omnigen-AF® and 83.5 kg of grain mixed for 2.5 minutes to adequately disperse the OG into the grain. The final mixture was stored in 45-gallon plastic drums with lids until needed for feeding.

All materials were labeled either “T” for treatment for OG fed cows, or “C” for control that did not receive OG. Treatment materials were red in color and controls were blue to further keep materials separate. The OG and control grain were weighed for each cow daily as needed to be fed the following day and were gently mixed in the batch containers before weighing to decrease settling. A total of 0.23 kg were weighed for each individual bag that was then fed to the cows.

Cows were fed after restrained by headlocks. After the TMR was fed, the grain mixture was fed individually by pushing away the TMR and pouring in front of the cow to consume. Visual assessment of supplement consumption was recorded daily for each animal and measures were taken to ensure cows did not eat other cow’s grain mixture by attempting to leave 1-2 empty headlocks between each animal. On occasion when cows declined to eat the grain mixture, it was lightly mixed with the TMR for improved consumption. After consumption of the mixture, the TMR was pushed back up to the cow and this procedure was completed for the far-off, close-up and lactation groups. After all animals consumed the grain mixture, they were released from the headlocks. Cows were left locked in headlocks for 5 to 15 min depending on how aggressive individual cows were eating and consumption of the grain mixture.

Feeding of the grain mixture occurred between 6:30 to 9:30 ET daily. Daily feeding logs and observation sheets were maintained. If a cow did not lock herself in the headlock or did not eat the grain mixture, this was recorded with the cow’s number and a description of the
occurrence. Occurrence of cows not consuming the mixture resulted in 43 cows in control group (1 to 19 occurrences per cow) and 44 cows in treatment group (1 to 27 occurrences per cow). The feeding was completed by undergraduate students that were trained on the procedures of feeding the grain mixture.

**Selective Dry Cow Therapy**

Seven days before scheduled dry off, quarter microbiological and somatic cell samples were taken to determine infection status. Study technicians wearing clean disposable gloves, scrubbed teat ends with 70% isopropyl alcohol-soaked gauze swabs to collect aseptic samples (ie. free of microorganisms outside the quarter) from foremilk. One set of microbiological samples were taken for culturing in the Tennessee Quality Milk Lab (TQML, Brehm Animal Science, Knoxville, TN) for species identification. Another set of microbiological samples were taken for on-farm culturing in the lab at Little River Animal and Environmental Unit, Walland, TN. Quarter and composite SCC samples were taken and then processed at the Tennessee DHIA lab, Knoxville, TN.

On-farm culturing was performed using the Minnesota Easy Culture System (University of Minnesota Laboratory for Udder Health, St. Paul). The Tri-plates consisted of MacConkey Media selective for gram-negative bacteria growth, Focus Media selective for *Streptococcus* and *Streptococcus*-like growth, and Factor Media selective for gram-positive growth. Each quarter sample was plated on individual Tri-plates according to Minnesota Easy Culture System guidelines (Royster et al., 2014). An individual cotton swab dipped in milk was used to streak.
the sample on the Tri-plate on a lab bench in a controlled environment. Tri-plates were labeled with tube number and placed inverted in an incubator at 37 degrees Celsius. The plates were checked for growth 24 h, 48 h and 72 h and the number of colonies (0 to >10) recorded.

From the samples taken 7 d before dry off, a combination of quarter SCC and culture results determined whether the quarter was treated with dry cow intramammary antibiotic (Spectramast DC; Zoetis, Parsippany, NJ). Quarters were treated when the quarter SCC was >150,000 and growth occurred on culture media from samples processed on-farm or the TQML laboratory. On farm culture media growth patterns indicative of contamination (numerous independent isolates) were categorized as growth. At dry off, SCC of quarters not scheduled for dry cow antibiotic therapy were re-tested with a portable SCC reader (DeLaval cell counter DCC, DeLaval Tumba, Sweden) to assess if subclinical mastitis had developed during the preceding week. All quarters received an internal teat sealant (Lockout, Boehringer Ingelheim, Ingelheim am Rhein, Germany) regardless of SDCT.

Post-calving Sampling Timeline and Record Keeping

Aseptic collection of quarter foremilk samples was taken at 3.7 ± 0.1 d after calving, and 7, 14, and 28 DIM (+/- 1 d) for SCC and microbiological analysis. Sampling days were strategically moved to Mondays, Wednesdays and Fridays for ease of labor and farm sampling preferences. If any sign of infection was visible, the affected quarter was palpated, and milk was evaluated and assigned a clinical score ranging from 0 to 3 (Almeida et al., 2015). Daily milk yield was recorded by SmartDairy management system (Boumatic, Madison, WI).
**Laboratory Bacteriological Culture**

Quarter aseptic samples were chilled on ice and taken to Tennessee Quality Milk Laboratory (TQML) for identification of bacterial species using National Mastitis Council’s guidelines starting with 10 µl of milk sample (NMC, 1999). Individual staphylococci, streptococci, and coliforms were identified using biochemical profiles generated by API strips (BioMetrieux Inc., Durham, NC). Laboratory technicians were blinded to the treatment groups from the results obtained from culture and SCC. Samples with 3 or more different colonies were considered contaminated and any growth of *Bacillus* spp. was considered non-significant growth.

For the purpose of data analysis, gram-negative bacteria were grouped together and encompassed *Escherichia coli*, *Klebsiella* spp., *Serratia* spp., and other non-differentiated gram-negative bacteria. Gram-positive bacteria were grouped together and encompassed *Enterococcus* spp., *Corynebacterium* spp., *Aerococcus* spp., *Lactococcus* spp., *Streptococcus* spp., *Streptomyces*, and other non-differentiated gram-positive bacteria. Non-aureus *Staphylococcus* spp. were not differentiated beyond the group level, and growth of just 1 colony did not meet the definition for IMI and was removed from statistical analysis.

**Definitions**

*Dry off and Post-calving IMI and Subclinical Mastitis*

Definition of IMI was defined by interpretation of results from bacteriological culture and SCC for samples collected prior to dry off and post-calving. Dry off samples included those collected 7 days before and at dry off. When both samples were missing or contaminated, the associated quarter was omitted from analysis. If one of the dry off samples were missing or
contaminated, the quarter infection status was determined exclusively by the results of the remaining sample. Post-calving samples included those collected at 3.7 ± 0.1 d after calving, and 7, 14, and 28 DIM (± 1 d). If all post-calving samples were contaminated or missing, the associated quarter was removed from analysis. When one post-calving sample was missing or contaminated, the quarter infection status was determined by the remaining samples. To define IMI, species and number of colonies as reported by TQML were used (NMC, 1999). A quarter was considered infected if ≥100 cfu/mL of milk of any organism, except for NAS. For NAS, a definition of ≥200 cf/mL was used. These IMI definitions are based on findings from single-sample bacteriological testing (Dohoo et al., 2011; Cameron et al., 2014). Subclinical mastitis was defined as >200,000 SCC.

**Dry Period Cure**

Dry period cure was determined separately by organism and SCC at the quarter level. An organism isolated in either dry off sample was considered cured if it was not isolated at any post-calving sample or absent in 3 of the 4 post-calving samples. The exception was *Staph. aureus* where only 1 post-calving sample was needed to be considered a non-cure. Analyzed separately, a composite SCC >200 in either dry off sample was considered cured over if all post-calving samples were ≤ 200 SCC.

**New IMI**

A quarter was defined as having a new IMI if an organism was cultured in ≥ 2 post-calving samples and was not present in either dry off sample. An exception was made for *Staph. aureus* where new IMI was defined when only 1 post-calving sample was identified. Both infected and uninfected quarters were at risk of new IMI during the dry period. Analyzed
separately, a composite SCC ≤ 200 in both dry off samples was considered a new IMI if any post-calving sample was >200 SCC.

**Chronic IMI**

A quarter was considered as having a chronic IMI when an organism was isolated in either dry off samples and cultured in ≥ 2 post-calving samples. An exception was made for *Staph. aureus* where a chronic IMI was defined when present in either dry off sample and at least 1 post-calving sample. Analyzed separately, a composite SCC >200 in either dry off sample was classified as a chronic IMI if the composite SCC was >200 at any post-calving sample.

**Statistical Analysis**

Statistical analysis was performed using SAS Version 9.4 (SAS Inc., Cary, SC). To generate population demographics, either PROC MEANS or PROC FREQ procedures were used to determine the means or proportions for parity, dry cow period length, cow and quarters receiving antibiotics, and number of quarters per cow receiving antibiotics.

Cows with only 3 functional quarters (23 cows: 8 in control, 15 in OG) were removed from analysis. Previous literature by Valckenier et al. (2019) suggests hind quarters gave 0.93 kg/d more milk than front quarters and risk of an IMI in the hind quarter tended to be 1.76 higher than the front quarter. Furthermore, Cameron et al. (2014) also showed higher chance of IMI in hind quarters compared to front quarters in a SDCT program. For these reasons, cows with only 3 functional quarters were removed from the sample population to minimize the potential of drawing a wrong conclusion.

For milk production analysis, an ANOVA mixed model (PROC GLIMMIX, MMAOV) was used with treatment group as the main fixed predictor. Other fixed effects included the
number of quarters treated with antibiotics per cow, time and two- and three-way interactions of all fixed effects. Composite SCC was log10 transformed after adding 1 to all values and used as a covariate. Other covariates included parity at calving and days at first sample from calving date. For analysis of composite SCC, an ANOVA mixed model (PROC GLIMMIX, MMAOV) was used with treatment group as the main fixed predictor. Other fixed effects of number of quarters treated with antibiotics per cow, time and, the two- and three-way interactions of all fixed effects. Cow milk yield and parity were used as covariates. A random effect of cow nested within treatment was also included in all models. An autoregressive covariance structure was used to account for within animal variance over time in all models.

A multivariable or univariable GLIMMIX model with a binary distribution was used to determine the effect of treatment and dry cow therapy use for the following outcomes: quarter-level prevalence of cultured organism groups and SCC>200,000, risk for cure over the dry period, risk for new IMI during the dry period, and risk of chronic IMI over the dry period. The same model was used for quarter SCC in determining cure over the dry period, new IMI, and chronic IMI over the dry period. Determination of the association between cow-level cure and new IMI based on composite SCC>200,000 was not possible because it would not fit a univariate GLIMMIX model due to the low sample population (n = 113) and prevalence. For all analyses, significance was determined at a $P < 0.05$.

RESULTS

Final Sample Population Characteristics

Descriptive statistics of cows included in the final sample population are located in in Table 2. Post-enrollment exclusions resulted in the loss of 7 cows (4 control, 3 treatment) for the
following reasons: no post-calving samples were collected due to missed sampling (n = 2: 1 control, 1 treatment), death (n = 4: 2 control, 2 treatment), and miss-diagnosed pregnant (n = 1, control). A total of 23 cows had 1 non-functional quarter with 8 in control and 15 in treatment that were removed from the final sample population due to potential confounding effects related to milk yield, SCC, and IMI. In total, 61 control cows (244 quarters) and 52 treatment cows (208 quarters) were included in the final sample population. The lactation completed at dry off was similar between treatment groups with mean 305ME milk yields of 13,405.9 ± 273.7 kg for control and 13,611.9 ± 320.2 kg in treatment (P = 0.71). Mean somatic cell score (SCS) for this same period also was similar between treatments with 2.58 ± 0.2 in control cows and 2.44 ± 0.2 in treatment cows (P = 0.55). Overall, 32.7% (37 total cows: 18 in OG, 19 in CON) cows were not treated in any quarter with intramammary antibiotics (Table 2). Quarters categorized as uninfected and not receiving intramammary antibiotic at dry off were 59.0% (144 out of 244) in the control group and 64.4% (134 out of 208) in the treatment group.

A total of 2,712 quarter samples were collected from 113 cows. Over six sampling periods, there were 452 functional quarters (n = 61 cows with 244 quarters in the control group; n = 52 cows with 208 quarters in the treatment group). Total number of samples for analysis were 2,660 samples. Of these, 126 samples were excluded due to contamination with 66 in control and 60 in treatment. Level of contamination was 4.7% overall. The majority of samples (n = 2,045; 76.9% of total samples) resulted in no growth with 1,117 (42.0% of total samples) in control and 928 (34.9% of total samples) in treatment. Excluding contamination, the remaining 380 samples (14.3% of total samples) displayed growth on culture media with 201 (7.6% of total samples) in control and 179 (6.7% of total samples) in treatment.
**Milk Production and SCC**

Milk production increased over time where OG fed cows produced 2.4 ± 1.1 kg more milk than control cows across all sampling time points ($P = 0.03$; Table 3). Milk weight did not differ relative to the number of quarters per cow administered antibiotic therapy ($P = 0.75$). Several other factors were included in the final model due to their association with milk weight. The number of quarters per cow administered antibiotics per treatment group was trending to differ in milk production ($P = 0.07$), where 3 quarters administered antibiotics differed between treatments (OG: 38.6 ± 2.6; CON: 30.8 ± 1.9). Parity had an effect on milk yield where for every increase in parity there was 1.35 ± 1.5 kg increase in milk production ($P = 0.05$). Composite SCC had an effect on milk yield where for every log10 increase of SCC there was 2.6 ± 0.5 kg loss in milk production ($P < .0001$). Because samples were collected Monday, Wednesday, Friday for convenience and resulted in slightly different DIM on sample collection days, we assessed days from calving to first sample collection in the model and it was not associated with milk production ($P = 0.83$). Post-treatment lactation yields also were analyzed using 305 d mature-equivalent milk and were similar with least square means of 13,334 ± 303.5 kg and 13,736 ± 345.6 kg in control and treatment cows, respectively ($P = 0.54$).

Composite SCC decreased across time but did not differ between treatments for the first 28 sampling days (Table 3). Number of quarters per cow administered antibiotics did not effect composite SCC per treatment group ($P = 0.29$). Number of quarters treated with antibiotics per cow, which ranged from no antibiotics (0) to all quarters given antibiotics (4), did have an effect on composite SCC where none (1.6 ± 0.09), 1 (1.9 ± 0.10) and 3 (2.8 ± 0.16) antibiotics used per
cow were the main differences \((P = 0.0006)\). The OG treatment over time did have an effect on composite SCC at the sampling time points of 7 and 28 \((P = 0.0005)\). Milk production had an effect on composite SCC, where for every 0.45 kg of milk production there was 0.01 log10 SCC decrease \((P <.0001)\). Parity had an effect on composite SCC where for every increase in parity there was \(0.31 \pm 0.7\) log10 SCC increase in composite SCC \((P = 0.05)\). Days from calving to first sample collection did not have an effect on composite SCC \((P = 0.90)\). Beyond the initial 28 DIM of the study, the lactation mean SCS were analyzed and were similar between treatment groups \((\text{CON} = 2.9 \pm 0.2\ \text{SCS}, \ \text{OG} = 3.1 \pm 0.3\ \text{SCS}; P = 0.49)\). The prevalence of subclinical mastitis, as defined by a composite SCC >200,000 also was assessed and occurred in 28.5\% and 32.1\% of the OG and CON sample populations, respectively (Table 3).

**Risk of Subclinical Mastitis at Dry off and Post-Calving**

Quarter-level SCC > 200,000 prevalence at dry off and post-calving samples are shown in Table 4. In Table 4, data also are provided that reflect the breakdown in quarters with SCC >200,000 for quarters receiving ITS only and ITS+DCT within a treatment group. The proportion of quarters with a quarter SCC >200,000 at dry off was higher in the CON group than the OG group \([\text{CON}: 16.9\% (95\%\ CI: 9.7, 25.1\%) \text{ vs } \text{OG}: 6.5\% (95\%\ CI: 3.4, 12.2\%); P = 0.01; \text{Table 5}]\). Following calving, the prevalence of SCC>200,000 was not different between study groups, \([\text{CON}: 16.9\% (95\%\ CI: 12.3, 22.6\%) \text{ vs } \text{OG}: 15.7\% (95\%\ CI: 11.0, 21.8\%); P = 0.76]\). As expected, the proportion of quarters with a quarter SCC >200,000 at dry off and post-calving samples were higher in quarters given DCT compared to quarters not given DCT (Table 5).
Microbiological Culture Results

The microbiological culture results from quarter-level milk samples collected before dry off and post-calving are shown in Tables 6 and 7, respectively. A more detailed breakdown also is provided that reflects species isolated from quarters receiving ITS vs ITS+DCT within the OG and CON treatments. Total microbiological prevalence on lab-based culture media for all samples was 14.3% (n = 380 of 2,660 total samples). *Staphylococcus aureus* growth was observed in 66 samples (17.4% of growth) with 52 in control and 14 in treatment. *Staphylococcus* species, excluding *Staph. aureus* (non-aureus *Staphylococcus*; NAS) were grouped resulting in 142 samples (37.4% of growth) with 62 in control and 80 in treatment. The most prominent NAS was *Staph. chromogenes* with 54 samples (38.0% of NAS species) with 24 in control and 30 in treatment, and *Staph. hyicus* with 33 samples (23.2% of NAS species) with 11 in control and 22 in treatment. The frequency of *Staph. hyicus* should be interpreted with caution, as identifications were made using an API Staph detection system that can misclassify *Staph. agnetis* as *Staph. hyicus* (Adkins, et al. 2017). Others included in the NAS group: *Staph. epidermidis, Staph. hominis, Staph. caprae, Staph. cohnii, Staph. lugdunensis, Staph. simulans, Staph. warneri, Staph. sciuri, Staph. xylosus*. Other gram-positive bacteria included: *Aerococcus spp., Enterococcus spp., Streptococcus ssp., Streptomyces spp.*, and undifferentiated gram-positive bacteria. Other gram-negative bacteria included: *Klebsiella spp., Serratia spp.*, and undifferentiated gram-negative bacteria.
Risk of IMI at Dry off and Post-calving

At dry off, the probability of quarters with an IMI, regardless of organism, was not different between treatment groups [CON: 11.5% (95% CI: 7.4, 17.4%) vs OG: 13.1% (95% CI: 8.4, 19.7%); \( P = 0.59 \); Table 8]. As expected, the proportion of quarter IMI was higher in quarter’s given DCT when compared to quarters not given DCT (Table 8). In post-calving samples, the prevalence of quarter IMI or bacterial species was not different between study groups [CON: 13.7% (95% CI: 9.9, 18.6%) vs OG: 16.6% (95% CI: 12.1, 22.2%); \( P = 0.34 \); Table 9]. Post-calving proportion of quarter IMI continued to be greater in quarters given DCT compared to quarters not given DCT (Table 9).

SCC > 200,000 and IMI Cure Risk Over the Dry Period

Quarter-level cure risk over the dry period was measured by quarter SCC and bacteriological culture results. Quarter SCC >200,000 cure risk over the dry period was not different between study groups [CON: 7.7% (95% CI: 4.4, 13.1%) vs OG: 6.9% (95% CI: 3.7, 12.5%); \( P = 0.78 \); Table 10]. Quarters that received DCT had a higher cure risk over the dry period when compared to quarters not given DCT according to quarter SCC >200,000 (Table 10). Quarter bacteriological cure risk over the dry period was not different between study groups [CON: 15.2% (95% CI: 10.3, 21.9%) vs OG: 21.6% (95% CI: 15.0, 30.2%); \( P = 0.18 \); Table 11]. While, quarters that received DCT had a higher cure risk over the dry period when compared to quarters not given DCT (Table 11).
**New IMI Risk Over the Dry Period**

New quarter SCC >200,000 risk over the dry period was not different between study groups [CON: 16.3% (95% CI: 11.0, 23.4%) vs OG: 20.2% (95% CI: 13.8, 28.6%); \( P = 0.40 \); Table 10]. Quarters not given DCT had a higher risk of new SCC>200,000 over the dry period compared to quarters given DCT (Table 10). Quarter new IMI risk over the dry period was not different between study groups [CON: 10.9% (95% CI: 7.2, 16.0%) vs OG: 11.8% (95% CI: 7.7, 17.6%); \( P = 0.78 \); Table 11]. Quarters given DCT had a higher risk of new IMI over the dry period when compared to quarters not given DCT (Table 11).

**IMI Chronic Over the Dry Period**

Chronic quarter SCC>200,000 risk over the dry period was significant between study groups [CON: 6.5% (95% CI: 2.3, 17.1%) vs OG: 2.6% (95% CI: 0.8, 8.3%); \( P = 0.05 \)]. Quarters given DCT had higher chronic SCC>200,000 when compared to quarters not given DCT (Table 10). The risk of quarter bacteriological chronic IMI was not different between study groups [CON: 2.0% (95% CI: 0.8, 5.2%) vs OG: 2.6% (95% CI: 1.1, 6.7%); \( P = 0.61 \); Table 11]. Similarly, quarters given DCT had higher chronic IMI than quarters not given DCT (Table 11).

**DISCUSSION**

The primary goal of this study was to assess the ability of OG to effect production outcomes when used in combination with SDCT. As a first step, we needed to assess the degree to which SDCT was used in this sample population. The current study demonstrated that quarter-
level antibiotic use at dry off was considerably reduced using SCC and culture guided SDCT. The observed decrease of 61.5% in antibiotic use when compared to administering every quarter with antibiotics at dry-off is similar to other studies. Kabera et al. (2019) showed at 58% reduction in antibiotic usage at dry off at the quarter level using on-farm culture. Rowe et al. (2020) also demonstrated a 55% reduction in antibiotic usage at the quarter level based on treating quarters with a probable infection determined by culture and an algorithm-guided process. When antibiotics were applied at the cow level, e.g. all four quarters were treated if determined to have a probable IMI by culture growth, Cameron et al. (2014) had a 21% reduction in use of dry cow antibiotics. Interdependence of quarters within a cow for acquiring a new IMI during the dry period has been shown to support administering antibiotics at the cow level (Berry et al., 2004). Robert et al. (2006) reported the effective use of applied prevention methods, such as ITS, reduced interdependence of quarters within a cow regarding acquiring new IMI during dry off. In the current study, the relatively low prevalence of IMI after calving suggests the employed SDCT program that includes an ITS was effective.

In the current study, feeding OG from dry off until 28 DIM was associated with a 2.4 ± 1.1 kg more milk yield than control cows across all sampling time points (P = 0.03). Our results agree with Brandão et al. (2016) who also observed an increase in milk yield when cows received OG during the dry period and continued 46 days into lactation. Similarly, Casarotto et al., (2020) observed higher milk yield at 150 DIM when OG was fed during the dry period and extending into lactation. In contrast, Nickerson et al. (2019) fed cows OG 60 d before dry off through the dry period and into 30 DIM but did not show a difference in milk production (P > 0.34), with one possible explanation that cows in that study were experiencing health issues that
could have affected milk yield. Each of these mentioned studies used blanket dry cow therapy, whereas the current study used a SDCT program and continued to observe increased milk production with OG supplementation. Literature supporting the effect of milk production in selective dry cow therapy programs is limited. A study by ØSterås and Sandvik (1996) demonstrated that SDCT increased milk yield by 186 kg per lactation compared to cows not given any antibiotic therapy at dry off. The basis for greater milk yield with supplementation of OG most likely is linked to its ingredients such as yeast and B-complex vitamins that can alter the microbiome in the rumen to improve the efficiency of mobilizing nutrients from the feed (McKay et al., 2019). These findings suggest that OG fed to cows during the prepartum and postpartum periods will increase milk production while selectively administering intramammary antibiotics at dry off.

In the current study, composite SCC was higher in OG fed cows relative to CON cows at 7 and 28 DIM sampling time points, but did not differ between treatment groups overall ($P = 0.20$). Casarotto et al. (2020) observed higher SCC in OG fed cows from dry off through 150 DIM, whereas Hall et al. (2018) reported SCC was similar in OG fed cows relative to control cows in a controlled setting. In contrast, Leiva et al. (2017) reported 97% greater SCC in control cows compared to OG supplemented cows in heat stress conditions, which should be interpreted with caution as SCC was not examined in the cows before treatment started and OG supplement was given during mid to late lactation. In the current study, one possible explanation for the higher SCC in the OG fed group is from increased surface L-selectin mRNA expression in leukocytes from the OG supplement (Wang et al., 2007). L-selectin is an adhesion molecule on neutrophil cell surfaces that allows migration from capillaries into the mammary tissue.
Additionally, OG supplementation to dairy heifers resulted in greater concentrations of IL-8 receptors expressed on neutrophils that bind IL-8 cytokines in inflammatory regions (Ryman et al., 2013). Therefore, increased L-selectin and IL-8 receptor concentrations allows neutrophils to respond more effectively to inflammatory signals and can potentially increase neutrophil migration from circulation into the mammary gland, increasing SCC.

Quarter level IMI prevalence in our current study was 13.7% in CON and 14.5% in OG with use of culture and SCC criteria. Cameron et al. (2014) resulted in a similar quarter IMI prevalence within 18 DIM after calving of 15.3% in BDCT and 15.8% in SDCT when antibiotics were applied at the cow level using culture-guided treatment decisions in a SDCT program. At the quarter level, antibiotic use determined by Rowe et al. (2020) used culture-guided results similar to this study but did not include SCC, and resulted in 23.9% quarter level prevalence of IMI at 1 to 13 DIM. Inclusion of SCC in the current study as an additional antibiotic treatment criterion potentially reduced our IMI prevalence after calving.

The risk of new IMI over the dry period was low with similarity between CON and OG study groups. The use of an ITS in the current study that was given to all quarters regardless of antibiotic use, has been proven to prevent IMI during the dry period (Berry and Hillerton, 2002a; Godden et al., 2003). When ITS was used alone in the absence of antibiotics, it was shown to be as effective as DCT in reduction of new IMI (Rabiee et al., 2013). This tool should be used with caution as improper use can lead to introduction of pathogens during insertion (Crispie et al., 2004). Proper use was demonstrated in the current study as bacteriological culture new IMI risk was not higher than quarters that received DCT and ITS compared to ITS alone.
Several potential limitations should be noted. In the current study, the final sample size used in the analysis was smaller than what was needed to maintain a power of 0.80 and is of concern to detect differences. When assessing quarter level IMI status in terms of cure, chronic, new and none, we used major categories of *Staph. aureus* and NAS as definitions, not individual species. Further investigation at the species level could reveal different results in cure, chronic, and new IMI, but would need more animal numbers and/or higher prevalence to investigate effectiveness of OG supplementation. More animals would have given us the opportunity to address IMI at the cow level and its potential relationship to OG treatment with milk production. Feeding the cows OG individually has its drawbacks as we observed a small subset of cows were not willing to consistently consume the mixture. Adding the supplement to the TMR is more practical and replicable in modern day dairy operations. For this study, feeding individually was key to not confound other research projects that was also being conducted at this dairy. Lastly, because blanket dry cow therapy is still used on most dairy operations, inclusion of a blanket dry cow therapy treatment group would have enabled a more effective comparison to prior research. This additional treatment group would have required more animals, but would have given use the opportunity to compare OG feeding in both blanket and selective dry cow therapy programs.

**CONCLUSIONS**

Selective dry cow therapy, based on quarter microbiological culture coupled with SCC, reduced antibiotic dry cow therapy by 61.5% from total therapy. In this SDCT program, supplementing OG through the dry period and into lactation increased milk production by 2.4 ±
1.1 kg with no change in SCC or IMI. Additional research should be conducted to strengthen the relationship between enhancing a SDCT program with immunomodulatory nutritional supplements to continue to increase the well-being and productivity of dairy cows.

ACKNOWLEDGMENTS

We appreciate the participation of East Tennessee Research and Education Center, ETREC staff, and undergraduate and graduate team members. This work was partly supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number USDA-NIFA-AFRI (2013-68004-20424). Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture. We also greatly appreciate the donation of OmniGen by Phibro to conduct this study.

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Table 1. Ingredient composition of far off, prepartum and postpartum diets fed to cows receiving 60 g/d of OmniGen-AF (n = 61) or control (n = 52) from dry off through 28 DIM

<table>
<thead>
<tr>
<th>Item</th>
<th>Far-off diet</th>
<th>Prepartum diet</th>
<th>Postpartum diet</th>
<th>Grain mixture¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients, % of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn Silage</td>
<td>21.7</td>
<td>42.5</td>
<td>30.4</td>
<td>-</td>
</tr>
<tr>
<td>Grass Hay</td>
<td>48.3</td>
<td>-</td>
<td>4.4</td>
<td>-</td>
</tr>
<tr>
<td>Wheat Straw</td>
<td>-</td>
<td>10.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ryegrass Silage</td>
<td>-</td>
<td>-</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>Grain – postpartum</td>
<td>-</td>
<td>-</td>
<td>57.1</td>
<td>-</td>
</tr>
<tr>
<td>Grain – far-off</td>
<td>28.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Grain – prepartum¹</td>
<td>-</td>
<td>45.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Animate²</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, %</td>
<td>44.8</td>
<td>41.6</td>
<td>41.4</td>
<td>47.5</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>10.9</td>
<td>16.0</td>
<td>18.1</td>
<td>17.2</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>53.0</td>
<td>39.4</td>
<td>29.5</td>
<td>45.2</td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>31.7</td>
<td>25.8</td>
<td>19.4</td>
<td>21.8</td>
</tr>
<tr>
<td>EE, % of DM</td>
<td>3.7</td>
<td>3.7</td>
<td>5.9</td>
<td>-</td>
</tr>
<tr>
<td>Calcium, % of DM</td>
<td>0.8</td>
<td>1.8</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Phosphorus, % of DM</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

¹Prepartum diet was fed starting at ~30 days before expected calving
²Phibro Animal Health Corporation Teaneck, New Jersey
³Ingredients: gluten feed, distiller grains, soybean mill, cracked corn, cottonseed hull, liquid molasses, limestone, salt, selenium, vitamin ADE, monensin
Table 2. Descriptive statistics of cows in the final study population enrolled in a selective dry cow therapy program and provided one of two Omnigen-AF (OG) treatments during the dry period through 28 days into lactation.1

<table>
<thead>
<tr>
<th>Item</th>
<th>CON (n = 61)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>OG (n = 52)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry period length (d)</td>
<td>60.5</td>
<td>1.6</td>
<td>60.8</td>
<td>2.8</td>
<td>60.8</td>
<td>2.8</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Parity at calving</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td></td>
<td>22</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>3</td>
<td>21</td>
<td></td>
<td>15</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 or above</td>
<td>16</td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows receiving IMAM2 antibiotics, %</td>
<td>68.9</td>
<td></td>
<td>65.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Quarters receiving IMAM2 antibodies, %</td>
<td>41.0</td>
<td></td>
<td>35.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quarters per cow receiving IMAM2 antibiotics</td>
<td>1.6</td>
<td>0.2</td>
<td>1.4</td>
<td>0.2</td>
<td>1.4</td>
<td>0.2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

1Quarters were selectively treated with dry cow antibiotic and ITS, or ITS alone based on quarter bacteriological culture and SCC results.
2Intramammary
3Cows not fed Omnigen-AF
Table 3. Mean values and standard deviations for milk weight and composite SCC for cows in the final study population to evaluate the effectiveness of OG in a selective dry cow therapy program

<table>
<thead>
<tr>
<th>Item</th>
<th>Sampling period relative to expected calving and calving</th>
<th>TRT</th>
<th>-60</th>
<th>3</th>
<th>7</th>
<th>14</th>
<th>28</th>
<th>Overall</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Weight (kg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OG</td>
<td></td>
<td>18.7 ± 1.2</td>
<td>32.3 ± 1.2</td>
<td>39.6 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.5 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.2 ± 1.3</td>
<td>35.9 ± 1.2</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>CON</td>
<td></td>
<td>19.9 ± 1.0</td>
<td>29.5 ± 1.0</td>
<td>35.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.4 ± 1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.0 ± 1.0</td>
<td>33.5 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite SCC (log10)</td>
<td></td>
<td>1.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9 ± 0.1</td>
<td>2.1 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9 ± 0.1</td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>OG</td>
<td></td>
<td>1.9 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td></td>
<td>1.9 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite SCC [no. (%)]</td>
<td></td>
<td>9 (8.0)</td>
<td>24 (21.1)</td>
<td>15 (13.3)</td>
<td>13 (11.5)</td>
<td>13 (11.5)</td>
<td>74 (28.5)</td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>OG</td>
<td></td>
<td>23 (20.4)</td>
<td>33 (29.2)</td>
<td>17 (15.0)</td>
<td>15 (13.3)</td>
<td>10 (8.9)</td>
<td>98 (32.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CON</td>
<td></td>
<td>23 (20.4)</td>
<td>33 (29.2)</td>
<td>17 (15.0)</td>
<td>15 (13.3)</td>
<td>10 (8.9)</td>
<td>98 (32.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Cows were selectively treated with intramammary antibiotic based on quarter bacteriological culture and SCC.

<sup>a,b,c,d</sup>Values with different letters within a column were significantly different (P < .0001).
Table 4. Quarter SCC >200,000\(^1\) prevalence for dry off and post-calving samples for cows in the final study population in a trial to evaluate the effectiveness of OG in a selective dry cow therapy program\(^2\)

<table>
<thead>
<tr>
<th>Item</th>
<th>CON Overall</th>
<th>CON ITS(^3)</th>
<th>CON DCT(^3)+ITS</th>
<th>OG Overall</th>
<th>OG ITS</th>
<th>OG DCT+ITS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dry off Samples (no.)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 200,000 SCC [no. (%)]</td>
<td>346 (70.9)</td>
<td>282 (98.3)</td>
<td>66 (32.5)</td>
<td>344 (82.7)</td>
<td>262 (98.8)</td>
<td>82 (54.3)</td>
</tr>
<tr>
<td>&gt; 200,000 SCC [no. (%)]</td>
<td>142 (29.1)</td>
<td>5 (1.7)</td>
<td>137 (67.5)</td>
<td>72 (17.3)</td>
<td>3 (1.1)</td>
<td>69 (45.7)</td>
</tr>
<tr>
<td><strong>Post-calving Samples (no.)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 200,000 SCC [no. (%)]</td>
<td>771 (79.0)</td>
<td>497 (86.3)</td>
<td>278 (68.8)</td>
<td>671 (80.7)</td>
<td>448 (84.2)</td>
<td>223 (74.3)</td>
</tr>
<tr>
<td>&gt; 200,000 SCC [no. (%)]</td>
<td>205 (21.0)</td>
<td>79 (13.7)</td>
<td>126 (31.2)</td>
<td>161 (19.4)</td>
<td>84 (15.8)</td>
<td>77 (25.7)</td>
</tr>
</tbody>
</table>

\(^1\)SCC>200,000 considered to be infected (Dohoo et al., 1991).

\(^2\)Cows were selectively treated based on quarter bacteriological culture and SCC results.

\(^3\)ITS = Internal teat sealant; DCT = Dry cow therapy
Table 5. Probability of quarter SCC >200,000 at dry off and post-calving samples for cows enrolled in a trial to evaluate the effectiveness of OG in a selective dry cow therapy program

<table>
<thead>
<tr>
<th>Item, %</th>
<th>Prob²</th>
<th>95% CI</th>
<th>Prob²</th>
<th>95% CI</th>
<th>P value</th>
<th>Prob²</th>
<th>95% CI</th>
<th>Prob²</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry off</td>
<td>16.9</td>
<td>9.7</td>
<td>25.1</td>
<td>6.5</td>
<td>3.4</td>
<td>12.2</td>
<td>.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post calving</td>
<td>16.9</td>
<td>12.3</td>
<td>22.6</td>
<td>15.7</td>
<td>11.0</td>
<td>21.8</td>
<td>.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹P-values with multivariable analysis: Treatment and Dry Cow Therapy
²Probability
### Table 6. Prevalence of quarter IMI and bacterial species isolated 7 d before dry off and at dry off of control (CON) cows and cows fed Omnigen-AF (OG) during the dry period through 28 DIM in a selective dry cow therapy program using dry cow antibiotic (dry cow therapy, DCT) and internal teat sealant (ITS), or ITS alone

<table>
<thead>
<tr>
<th>Item</th>
<th>CON Overall (n = 488)</th>
<th>CON ITS only (n = 285)</th>
<th>CON DCT + ITS (n = 203)</th>
<th>OG Overall (n = 416)</th>
<th>OG ITS only (n = 265)</th>
<th>OG DCT + ITS (n = 151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total quarter IMI [no. (%)]</td>
<td>71 (14.5)</td>
<td>14 (4.9)</td>
<td>57 (28.1)</td>
<td>62 (14.9)</td>
<td>13 (4.9)</td>
<td>49 (32.5)</td>
</tr>
<tr>
<td>Bacterial species culture [no. (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>22 (31.0)</td>
<td>1 (7.1)</td>
<td>21 (36.8)</td>
<td>5 (8.1)</td>
<td>0 (0)</td>
<td>5 (10.2)</td>
</tr>
<tr>
<td>NAS²</td>
<td>19 (26.8)</td>
<td>2 (14.3)</td>
<td>17 (29.8)</td>
<td>35 (56.5)</td>
<td>4 (30.8)</td>
<td>31 (63.3)</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>8 (11.3)</td>
<td>5 (35.7)</td>
<td>3 (5.3)</td>
<td>12 (19.4)</td>
<td>6 (46.2)</td>
<td>6 (12.2)</td>
</tr>
<tr>
<td><em>Streptococcus</em> uberis</td>
<td>3 (4.2)</td>
<td>0 (0)</td>
<td>3 (5.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Streptococcus</em> dysgalactiae</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other gram-positive bacteria³</td>
<td>17 (23.9)</td>
<td>6 (42.9)</td>
<td>11 (19.3)</td>
<td>6 (9.7)</td>
<td>2 (15.4)</td>
<td>4 (8.2)</td>
</tr>
<tr>
<td>Total gram-positive bacteria</td>
<td>47 (66.2)</td>
<td>13 (92.9)</td>
<td>34 (59.6)</td>
<td>53 (85.5)</td>
<td>12 (92.3)</td>
<td>41 (83.7)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>1 (7.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other gram-negative bacteria⁴</td>
<td>2 (2.8)</td>
<td>0 (0)</td>
<td>2 (3.5)</td>
<td>3 (4.8)</td>
<td>0 (0)</td>
<td>3 (6.1)</td>
</tr>
<tr>
<td>Total gram-negative bacteria</td>
<td>2 (2.8)</td>
<td>0 (0)</td>
<td>2 (3.5)</td>
<td>4 (6.5)</td>
<td>1 (7.7)</td>
<td>3 (6.1)</td>
</tr>
<tr>
<td>Yeast [no. (%)]</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

¹Definition of IMI: all pathogens except NAS ≥100 cfu per 1.0 ml of milk; NAS ≥200 cfu per 1.0 ml of milk (Dohoo et al., 2011).
²Cows were selectively treated based on quarter bacteriological culture and SCC results.
⁴Other gram-positive bacteria: *Aerococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Streptomyces* spp., undifferentiated gram-positive bacteria

⁵Other gram-negative bacteria: *Klebsiella* spp., *Serratia* spp., undifferentiated gram-negative bacteria
### Table 7. Prevalence of quarter IMI and bacterial species isolated within 28 d after calving of control (CON) cows and cows fed Omnigen-AF (OG) during the dry period through 28 DIM in a selective dry cow therapy program using dry cow antibiotic (dry cow therapy, DCT) and internal teat sealant (ITS), or ITS alone

<table>
<thead>
<tr>
<th>Item</th>
<th>CON Overall (n = 948)</th>
<th>ITS (n = 561)</th>
<th>DCT+ITS (n = 387)</th>
<th>OG Overall (n = 808)</th>
<th>ITS (n = 510)</th>
<th>DCT+ITS (n = 298)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total quarter IMI [no. (%)]</strong></td>
<td>130 (13.7)</td>
<td>46 (8.2)</td>
<td>84 (21.5)</td>
<td>117 (14.5)</td>
<td>54 (10.6)</td>
<td>63 (21.1)</td>
</tr>
<tr>
<td><strong>Bacterial species culture [no. (%)]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30 (23.1)</td>
<td>7 (15.2)</td>
<td>23 (27.4)</td>
<td>9 (7.7)</td>
<td>2 (3.7)</td>
<td>7 (11.1)</td>
</tr>
<tr>
<td><em>NAS</em>[^2^]</td>
<td>43 (33.1)</td>
<td>10 (21.7)</td>
<td>33 (39.3)</td>
<td>45 (38.5)</td>
<td>24 (44.4)</td>
<td>21 (33.3)</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>18 (13.8)</td>
<td>10 (21.7)</td>
<td>8 (9.5)</td>
<td>22 (18.8)</td>
<td>9 (16.7)</td>
<td>13 (20.6)</td>
</tr>
<tr>
<td><em>Streptococcus uberis</em></td>
<td>7 (5.4)</td>
<td>4 (8.7)</td>
<td>3 (3.6)</td>
<td>12 (10.3)</td>
<td>4 (7.4)</td>
<td>8 (12.7)</td>
</tr>
<tr>
<td><em>Streptococcus dysgalactiae</em></td>
<td>3 (2.3)</td>
<td>0 (0)</td>
<td>3 (3.6)</td>
<td>2 (1.7)</td>
<td>1 (1.9)</td>
<td>1 (1.6)</td>
</tr>
<tr>
<td>Other gram-positive bacteria[^3^]</td>
<td>17 (13.1)</td>
<td>9 (19.6)</td>
<td>4 (4.8)</td>
<td>21 (17.9)</td>
<td>11 (20.4)</td>
<td>10 (15.9)</td>
</tr>
<tr>
<td><strong>Total gram-positive bacteria</strong></td>
<td>88 (67.7)</td>
<td>33 (71.7)</td>
<td>51 (60.7)</td>
<td>102 (87.2)</td>
<td>49 (90.7)</td>
<td>53 (84.1)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (1.7)</td>
<td>1 (1.9)</td>
<td>1 (1.6)</td>
</tr>
<tr>
<td>Other gram-negative bacteria[^4^]</td>
<td>7 (5.4)</td>
<td>3 (6.5)</td>
<td>4 (4.8)</td>
<td>2 (1.7)</td>
<td>0 (0)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td><strong>Total gram-negative bacteria</strong></td>
<td>7 (5.4)</td>
<td>3 (6.5)</td>
<td>4 (4.8)</td>
<td>4 (3.4)</td>
<td>1 (1.9)</td>
<td>3 (4.8)</td>
</tr>
<tr>
<td>Yeast [no. (%)]</td>
<td>5 (3.8)</td>
<td>3 (6.5)</td>
<td>2 (2.4)</td>
<td>2 (1.7)</td>
<td>2 (3.7)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

[^1^]Definition of IMI: all pathogens except NAS ≥100 cfu per 1.0 ml of milk; NAS ≥200 cfu per 1.0 ml of milk (Dohoo et al., 2011).

[^2^]Cows were selectively treated based on quarter bacteriological culture and SCC results.


Table 8. Probability of quarter IMI and bacterial species isolated 7 d before dry off and at dry off in control (CON) cows and cows fed Omnigen-AF (OG), and dry cow therapy use\(^1\)

<table>
<thead>
<tr>
<th>Item, %</th>
<th>CON Prob(^2)</th>
<th>95% CI</th>
<th>Prob(^2)</th>
<th>95% CI</th>
<th>P value</th>
<th>OG Prob(^2)</th>
<th>95% CI</th>
<th>Prob(^2)</th>
<th>95% CI</th>
<th>P value</th>
<th>NO Prob(^2)</th>
<th>95% CI</th>
<th>Prob(^2)</th>
<th>95% CI</th>
<th>P value</th>
<th>YES Prob(^2)</th>
<th>95% CI</th>
<th>Prob(^2)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>11.5</td>
<td>7.41</td>
<td>17.4</td>
<td>13.1</td>
<td>8.4</td>
<td>19.7</td>
<td>0.59</td>
<td>5.5</td>
<td>2.6</td>
<td>11.1</td>
<td>25.1</td>
<td>20.2</td>
<td>30.8</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>4.6</td>
<td>2.2</td>
<td>9.3</td>
<td>1.2</td>
<td>1.2</td>
<td>0.3</td>
<td>0.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>NAS</td>
<td>1.1</td>
<td>0.3</td>
<td>4.0</td>
<td>2.7</td>
<td>0.8</td>
<td>8.9</td>
<td>0.01</td>
<td>0.3</td>
<td>0.0</td>
<td>3.5</td>
<td>8.9</td>
<td>6.4</td>
<td>12.3</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram +</td>
<td>5.6</td>
<td>3.3</td>
<td>9.4</td>
<td>4.5</td>
<td>2.4</td>
<td>8.3</td>
<td>0.57</td>
<td>4.1</td>
<td>1.9</td>
<td>8.6</td>
<td>6.1</td>
<td>3.9</td>
<td>9.3</td>
<td>0.37</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)P-values with multivariable analysis: Treatment and Dry Cow Therapy

\(^2\)Probability
Table 9. Probability of quarter IMI and bacterial species isolated within 28 d after calving in control (CON) cows and cows fed Omnigen-AF (OG), and dry cow therapy use

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CON</th>
<th>OG</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item, %</td>
<td>Prob</td>
<td>95% CI</td>
<td>Prob</td>
<td>95% CI</td>
</tr>
<tr>
<td>All</td>
<td>13.7</td>
<td>9.9</td>
<td>18.6</td>
<td>16.6</td>
</tr>
<tr>
<td>SA</td>
<td>2.1</td>
<td>0.8</td>
<td>5.7</td>
<td>0.8</td>
</tr>
<tr>
<td>NAS</td>
<td>4.1</td>
<td>2.3</td>
<td>7.1</td>
<td>5.5</td>
</tr>
<tr>
<td>Gram +</td>
<td>4.4</td>
<td>2.7</td>
<td>7.0</td>
<td>6.8</td>
</tr>
</tbody>
</table>

1P-values with multivariable analysis: Treatment and Dry Cow Therapy
2Probability
Table 10. Quarter SCC >200,000 probability over the dry period for cows enrolled in a trial to evaluate the effectiveness of a nutritional supplement in a selective dry cow therapy program

<table>
<thead>
<tr>
<th>Item, %</th>
<th>Treatment</th>
<th>Selective Dry Cow Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>OG</td>
</tr>
<tr>
<td></td>
<td>Prob^2</td>
<td>95% CI</td>
</tr>
<tr>
<td>Cure (n = 49)</td>
<td>7.7</td>
<td>4.4</td>
</tr>
<tr>
<td>Chronic (n = 80)</td>
<td>6.5</td>
<td>2.3</td>
</tr>
<tr>
<td>New (n = 108)</td>
<td>16.3</td>
<td>11.0</td>
</tr>
</tbody>
</table>

^1P-values with multivariable analysis: Treatment and Dry Cow Therapy

^2Probability
Table 11. Probability of quarter bacterial species IMI over the dry period for cows enrolled in a trial to evaluate the effectiveness of OG in a selective dry cow therapy program.

<table>
<thead>
<tr>
<th>Item, %</th>
<th>CON</th>
<th>95% CI</th>
<th>Probability</th>
<th>95% CI</th>
<th>P-value</th>
<th>Selective Dry Cow Therapy</th>
<th>95% CI</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cure (n = 88)</td>
<td>15.2</td>
<td>10.3</td>
<td>21.9</td>
<td>21.6</td>
<td>0.18</td>
<td>7.8</td>
<td>5.0</td>
<td>12.0</td>
<td>0.0017</td>
</tr>
<tr>
<td>Chronic (n = 16)</td>
<td>2.0</td>
<td>0.8</td>
<td>5.2</td>
<td>2.6</td>
<td>0.61</td>
<td>0.7</td>
<td>0.2</td>
<td>2.8</td>
<td>0.2</td>
</tr>
<tr>
<td>New (n = 51)</td>
<td>10.9</td>
<td>7.2</td>
<td>16.0</td>
<td>11.8</td>
<td>0.78</td>
<td>8.1</td>
<td>5.3</td>
<td>12.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^1\)P-values with multivariable analysis: Treatment and Dry Cow Therapy

\(^2\)Probability
VITA

Kody Hash was born on April 22, 1991 in Wytheville, Virginia. Kody graduated from Grayson County High School in 2009 and continued his education at Wytheville Community College from 2009 to 2010. Kody transferred to Virginia Tech, Blacksburg, Virginia and gained his Bachelor’s degree in Dairy Science in 2013. Kody began full-time employment at Little River Animal and Environmental Unit, then began his Master’s degree in Fall 2014. Kody also managed up to 15 undergraduates during his thesis for data collection on farm while working a full-time job. After completing the degree, Kody plans to continue working within the dairy industry and furthering the research mission.