The Effects of a Powdered Post-milking Teat Dip on Mastitis and Teat Condition in Dairy Cattle in the Winter Months

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Abstract

Mastitis is disease that is very prevalent in dairy herds around the world. It has many effects on both the cow and the farm. The effects of a powdered post dip (Derma Soft n' Dry) on teat end health and somatic cell count (SCC) were observed over a 21-day period. Holstein cows (n = 8 per treatment) were randomly assigned to the control group (regular iodine-based post-milking teat dip) or the treatment group (powdered post-milking teat dip). Daily milk yield and activity levels were measured throughout the study using the Afimilk® parlor system. Milk samples were collected weekly to test for SCC and teat end scores were assigned prior to collection of the milk samples. Samples with a SCC of >250,000 cells/mL were cultured using a tri-plate agar to determine which bacterial species were present. There were no significant differences in daily milk yield, conductivity, SCC, activity time, or rest time between the control and treatment groups ($P > 0.05$). Teat end sores in relation to the treatment were not significantly different ($P = 0.9794$). Furthermore, the control and treatment group showed similar bacterial growth indicating that there was no significant difference by treatment.

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Somatic cells: white blood cells produced to fight off infections.

Somatic cell count (SCC): The level of somatic cell in a sample of milk.

Post dip: a dip, usually a liquid, that is applied to the teat end of a cow after the milking process in order to protect the teat end from further infection.

Teat canal: the opening at the end of the teat.

Teat end scoring: a process in which the skin and shape of the teat end is assessed and recorded.

AFI milk: the computer system used by the MTSU dairy to collect data pertaining to milk production and activity levels of each lactating cow.

Intramammary infection (IMI): the presence of an infectious organisms in the mammary gland.

Delaval Cell Counter: a portable device designed for on farm testing of SCC in cattle.

trip-plate agar: an agar plate that is evenly divided into three sections.

Delaval Cell Counter Cassette: a cartridge designed to collect a small milk sample and is inserted into the Delaval Cell Counter.

Introduction

One of the most prevalent issues that dairy herds face is a disease called mastitis. Mastitis is an intramammary gland infection caused by bacteria and microorganisms entering the teat canal and moving upwards into the udder, infecting the secretory glands. This causes the cow's body to produce an excess of white blood cells, which will in turn contaminate the milk, making it unsafe for consumption. There are many different factors that can cause mastitis; therefore, it can be very difficult to keep at bay. Some common factors that can lead to mastitis include poor hygiene, heat stress, poor teat end condition, under milking, and dirty bedding. Udder health can be monitored with a somatic cell count (SCC) measurement. Somatic cell count is a measurement of the volume of white blood cells within a sample of milk. Somatic cells will increase as the cow's body attempts to fight off unwanted bacteria and microorganisms that have entered the teat canal. The SCC cannot exceed 750,000 cells/ml; otherwise, it is not safe for human consumption and therefore must be disposed. When the milk is picked up from the bulk tank at the dairy farm, a sample is taken. Once the milk arrives at the processing plant, the milk samples are tested to determine SCC.

Clinical mastitis vs. Subclinical mastitis

To ensure that a herd is not being overrun with mastitis cases, it is important to understand what mastitis is and that it has different forms. Mastitis is caused when microorganisms enter the teat canal and travel upwards into the secretory glands affecting the milk producing glands thus causing an intramammary infection. The cow's body then begins to attempt to fight off the infection by secreting an excess of white blood cells.

Clinical mastitis can be seen with the naked eye. When the teat is striped before the milking process one can see physical changes in the milk. This includes clumpy or flaky milk. One might also see blood in the milk. It is important to point out that the clumps, flakes, and blood that are commonly associated with clinical mastitis are not necessarily evidence of mastitis; this is the result of damage done to the udder via the bacteria and/or microorganisms (Bewley et al.). Clinical mastitis can also cause changes in the udder and teat structures. Extremely swollen, shriveled, or discolored udders or teats could denote that a cow has a case of clinical mastitis. Subclinical mastitis, however, is harder to catch as it does not have any visible signs to suggest that a cow has been infected. Affected quarters will appear normal rather than being swollen or discolored (Yutzy, 2018). If not detected, subclinical mastitis can lead to an infection that is unresponsive to treatment (Yutzy, 2018). It is imperative that dairy farms implement a strict milk testing schedule as that is one of the only ways that subclinical mastitis can be caught. An ongoing case, if not properly contained, can lead to lower overall milk production and result in the producer losing money (Yutzy, 2018). It can also be inferred that if a cow that usually has a high milk production suddenly begins to produce less milk, she may have a case of subclinical mastitis and should therefore be tested.

Prevention measures: Pre- and Postmilking Dip

While cows are likely to be exposed to mastitis while being outside or lying in soiled bedding, they can also be exposed during the milking process. Cows are at a higher risk of infection once they enter the parlor. Before the cow is milked, the teats are first cleaned with a pre-dip and stripped. Stripping the teat allows the worker to look for signs of clinical mastitis.

However, this opens the streak canal thus making it easier for microorganisms to enter inside the teat and cause mastitis. Therefore, having a strict milking procedure is important. The standard procedure that is used includes: pre-dip, strip the teat, wipe the teat, attach milkers, and lastly, post-milking dip. The pre-dip is used to clean the existing dirt off the teats. The teats must be completely dried before the milkers are attached as moisture is an optimal atmosphere for bacteria to grow. Finally, the post-milking dip is used to keep the teat ends clean while the teat end is still open. Post dipping is used to disinfect the teat from any pathogens that the cow may have been exposed to during the milking process, as well as helping to protect the teat end from environmental pathogens after milking (Morrill et al., 2019).

The milking procedures that are set in place in the parlor are the most effective preventive measures against mastitis. With that being said, it is very important to do research and choose the best possible teat dips. Consistently using an effective teat dip can prevent mastitis cases (Reneau, 2011). There are, however, certain aspects to keep in mind when choosing what products are going to be implemented into the milking procedure. One of which is weather. During the colder months, udders and teats are more susceptible to mastitis for several different reasons. In the South, the cold comes with excess amounts of moisture. Moisture is a breeding ground for bacteria and henceforth is problematic for dairy cows. The issue that this brings is that udders and teats will take longer to dry off after leaving the parlor. This allows bacteria to grow and enter the teat canal, causing mastitis.

Another problem that occurs during the winter is chapping. Before the milkers are attached the worker will dry the teats off. After the milkers come off, a liquid post dip is

used. The liquid post dip is left on the teats and is not dried off. This can contribute to chapping and frostbite. Skin that is washed repeatedly may lose some natural oils. Teats that are left wet are more vulnerable to mastitis pathogens than teats that are dry. (Reneau, 2011). Some farmers opt not to use a post dip for this reason:

> It could be reasoned that during severe cold, bacterial growth on teats and in the environment is much less; therefore, teat disinfection is not quite as crucial during those periods. However, in some regions of the northern United States the complete cessation of teat dipping during the cold winter months has allowed the spread of contagious mastitis pathogens. In addition, it should be pointed out that omitting teat dipping does not assure that teats are dry. (Reneau, 2011)

While cold temperatures slow down the growth of bacteria it does not stop the growth completely; therefore, a cow could still contract mastitis. Powder post-milking dips could be used in place of liquid teat dips to minimize the amount of moisture that is left on the teat after milking. A powder substitute could potentially be a better alternative than discontinuing the use of a post-milking dip altogether. In an attempt to mitigate the effects of severe cold weather conditions and to maintain teat health, management procedures on some farms involve switching from the normal liquid postmilking dip to a powdered dip. Using a powdered substitute has been reported to eliminate the occurrence of post dip freezing and becoming diluted (Knutson et al., 2011).

The health of the teat ends is imperative to the prevention of mastitis. If teat ends become compromised, they are not going to be able to adequately protect the streak canal from the invasion of microorganisms. Observing teat end conditions and scoring them can help to gage how healthy the teat end is and to determine whether the teat skin is in

need of ointment. It is important to use a constant teat end scoring method when making observations.

Literature Review

Economic Effects of Mastitis

Mastitis and high levels of SCC can have serious economic repercussions in the dairy industry. Milk with high levels of SCC cannot be consumed by humans. Therefore, must be dumped. Bulk milk tank somatic cell count (BMSCC) cannot reach over 750,000 cells/mL, otherwise it will not be bought by processors. Processors cannot produce as many milk products from milk with high SCC. Research focusing on the Ireland dairy industry shows that BMSCC has a negative effect on not only the farmers, but also the processors and industry levels as well (Geary et. al., 2013). This research discovered that the industry as a whole, dropped in revenue by 3.2% per year as BMSCC increased from <100,000 cells/mL to >400,000 cells/mL. This is a result of lower milk production, higher culling rates (the rate at which cows are removed from the herd for various reasons including problems related to mastitis), as well as higher farm costs, lower yields in product quality and quantity (Geary et. al., 2013). Cows with known mastitis issues must have their milk withheld from the bulk tank to ensure that the BMSCC is not increased. By doing so, the farm loses the profits from that cow's milk production. It is common for treated cows to have their milk withheld from the bulk tank for five days. Geary et. al. (2013) estimated that the price per liter of withheld milk was at .27 cents. The costs of milk cultures were around $5.59 per cow. They also estimated that the cost

of treatment was as follows: intramammary treatment was estimated at $9.95, injectable antibiotics were at $23.52, and pain relief medications were $18.25. One way for dairy farms to handle rising case of mastitis is to cull, or remove, cows who repeatedly become infected or who have sustained severe damage as a result of SCC from the herd. In doing so it can have great financial repercussions. Geary et. al. (2013) estimates on average, a farm loses $442 for every culled cow while the cost of buy and raises a replacement heifer is on average $1,550. During the duration of the study, from 1994 to 2004, the cull rate in Ireland increased from 3% to 14% as the BMSCC increased from <100,000 cells/mL to >400,000 cells/mL. Geary et. al. (2013) discovered that the net profit generated by dairy farms in Ireland decreased from $47,890 with a BMSCC of <100,000 cells/mL to $20,800 with a BMSCC of >400,000 cells/mL.

Furthermore, it was found that as BMSCC increased from <100,000 cells/mL to 400,000 cells/mL, the volume of milk delivered to processors decreased (Geary et. al., 2013). This is a direct result from the lowered quality of milk as producers prefer to purchase higher quality milk. Producers are greatly affected by high SCC because less milk and milk by-products can be produced with lower quality milk. Geary et. al. (2013) found that as BMSCC increased from <100,000 cells/mL to >400,000 cells/mL the price of milk increased.

Teat Health and the Effects on Mastitis

The teat end is the gateway for bacteria to enter the teat canal. Therefore, teat health is of upmost importance in combating mastitis. The teat skin is the first defense in protecting the deep tissue of the udders from infection, if it is not healthy than it leaves the tissue vulnerable to infection (Britt and Farnsworth, 2003). Teat ends are scored on a

scale of 1-4 for blemishes such as callousing, roughness, cracking, and hyperkeratosis (Britt and Farnsworth, 2003). It is best to use a visual chart when examining and issuing a teat end score to assure the assessment is correct. **Figure 3.** Studies show that each of the four teats can hold a different score. Teat end scores can vary among different breeds depending on several factors (Britt and Farnsworth, 2003). For example, Holstein cattle produce higher amounts of milk than a Jersey cow. They tend to score high on teat end condition. This is because their udders are exposed to more stress during milking periods due to the milkers being attached for a longer period. There are other factors that can cause changes in teat end scoring as well. Cleanliness of environment and weather have major impacts on teat end condition.

In a study conducted in the Netherlands, Barkema et. al. (2001) worked with 15 dairy herds to examine the relationship between teat end callosity (the amount of callous build up on teats) and the occurrence of clinical mastitis. This study focused on three different aspects of teat end conditions, teat-end callosity (TEC), teat-end callosity roughness (TECR), and teat-end callosity thickness (TECT). The focus was on clinical mastitis in the four separate quarters of individual cows. They found that cows who had clinical mastitis had high TECT and TECR scores in the months leading up to the study as well as during and after the study. This shows that teats with high TEC scores may have a precursor for clinical mastitis. TECT increased as the milk yielded of each cow peaked (Barkema et. al., 2001). This could correlate with cows being exposed to milking procedures for longer amounts of time during their lactational peak. During the milking process a cow's teats go through many stressors that could cause injury or changes to the teat end condition. If lesions or scabs form, this weakens the teat's ability to protect the

deep tissue of the udders, allowing bacteria to enter and cause mastitis (Barkema et. al., 2001).

During the study of Barkema et. al. (2001), it was determined that the relationship between TEC and the occurrence of clinical mastitis had not been previously studied with a focus on longitudinal quarters (the comparison of the teats that are beside each other). This prompted them to look further into the differences in TEC scores for each quarter of the udder. During the 1.5-year study 2157 cows were observed. Their results showed that the average TEC score ranged from 1.10 to 2.41 all together. The average for dry cows that showed signs of callouses in individual herds ranged from 22 to 58%. While the average for lactating cows in individual herds ranged from 24 to 65% (Barkema et. al., 2001). There are correlations between teat end health and mastitis cases. With teat ends having rough, calloused skin surrounding the teat opening, it is difficult for the teat end to completely close after milking. Quarters that were infected with clinical mastitis had a TECR score 8% higher than the non-infected quarters (Barkema et al., 2001).

Overall, Barkema et. al. (2001) further proved that there is a significant relationship between teat end health and clinical mastitis cases. Cows who tend to have thinker and rougher callouses on or around the teat end usually have a greater chance of becoming infected with mastitis. 40% of all the udders observed had clinical mastitis. Furthermore, since it is proven that teat health correlates with mastitis infections, TEC can potentially be used as an aid to predict and prevent future mastitis infections.

The Effects of Weather on Teat Health

When taking into consideration teat health, it is important to understand what environmental factors influence the teat conditions. Weather, for the purpose of this study, is a very important factor to consider. The winter months bring cold, dry air which cause teats to become dry, cracked, and sore (Casimiro et. al.). These factors can cause teat skin condition to deteriorate and increase the likely hood of a mastitis infection. It is not necessary to completely change the milking procedures during the winter however, it is necessary to implement appropriate products that can help combat the effects cold weather can have. Casimiro et. al. recommends not using an iodine base teat dip during this time as iodine will dry out the skin faster and aid in chapping and cracking. Nevertheless, it is important to examine the milking procedures currently in use to determine if changes would be beneficial; as well as observing cows and document the conditions of their udders as this is a great way to prevent future mastitis infections.

Human Risks Affiliated With Bovine Mastitis

Dairy products are one of the most important nutrition sources as they meet many nutrient requirements while also being economically viable around the world (Garvey, 2019). High levels of somatic cells can have potential risks on human health. Milk with a high SSC could potentially contain the bacteria that caused the elevation in somatic cells. This is particularly an issue among people who consume raw milk and raw milk products. One study found that out of 248 dairy farmers, 42.3% consumed raw dairy products (Brown et. al., 2006). Most dairy products being consumed by humans are pasteurized. However, while pasteurization rids milk of most pathogens, there are some that are heat

resistant. Garvey (2019) states that some pathogens, such as *Bacillus. Cereus* spores have been found in powdered infant formula because the spores are heat and chemical resistant and can withstand the pasteurization process.

When udders become infected with mastitis, the pathogens that caused the infection may be present in the milk after it leaves the teat. This could lead to contamination in the bulk tank. Brown took milk samples from each of the 284 farms in her study and found that the most common pathogens in the tanks were *Campylobacter jejuni*, shiga toxin-producing *Eschericia coli*, *Listeria monocytogens*, *Salmonella*, and *Yersinia enterocolitica*. The World Health Organization (WHO) released a list in 2017 that listed several pathogens that are commonly linked to mastitis that pose a threat to humans if consumed, among those listed was *Staphylococcus aureus* (Garvey, 2019). Furthermore, it is important for farmers to keep mastitis cases under control in an attempt to keep bacteria out of their bulk tanks as to not risk the transmission of foodborne illnesses in humans.

Objective

The objective of this project was to compare a powdered post-milking teat dip to a liquid, iodine-based post teat dip and determine effects on clinical mastitis, subclinical mastitis, and teat skin condition. By determining whether the powder teat dip has a greater effect on lowering mastitis rates and improving skin conditions, we will have a better understanding of different effective products that could potentially be implemented into milking procedures. This will give dairy farmers more options to consider when they are strategizing their procedures for the winter months.

Methodology

The MTSU Experiential Learning and Research Center dairy herd was used for this project. Sixteen lactating Holstein cows were evaluated over a period of 21 days starting January 10th and ending on January 31st, 2022. The average temperature during the study was 7.3°C (**Figure 1)** and relative humidity was 52.25% (**Figure 2**). Eight cows were randomly assigned the experimental group (treatment; Derma Soft n' Dry powdered post-milking teat dip, **Figure 3**), and the remaining eight cows received the normal, iodine-based post-milking teat dip (control). Cows in the treatment group were identified with a green Velcro leg band. For the 21-day period, cows with the green leg bands received the powered teat dip after milking and the control group received the iodine post dip. Daily records were collected on each cow for milk production, and activity levels through leg pedometers that are equipped on each cow's front leg (AFI® Milk).

Somatic Cell Count Data

On day 0, sterile milk samples were collected from all sixteen cows and tested using the Delaval Cell Counter (**Figure 4**) for somatic cell count (SCC). The tube containing the milk sample was slowly inverted two to three times to lightly disturb the sample. The Delaval Cell Counter cassette (**Figure 5**) then was inserted into the sterile milk sample to retrieve a small amount of milk. The cassette was then inserted into the Delaval Cell Counter where the SSC was measured shortly after. Any SSC that measured greater than 250,000 cells/mL was set aside to be cultured. This was repeated for days 7, 14, and 21.

Culturing

If a milk sample had a SCC of >250,000 cells/ml, then it was cultured using tri-plate agar to identify which bacterial species were present (Minnesota Easy Culture System User's Guide, 2013; **Figure 6**). Each culture was placed into an incubator with a temperature of 98° Fahrenheit and left to culture for 48 hours. After 48 hours the cultures were removed from the incubator and observed using the *Minnesota Easy Culture System User's Guide*. Pictures were then taken of each sample and the bacteria that was found was recorded. This was repeated for days 7, 14, and 21.

Observations for cow teat end scoring were assessed and recorded weekly on each cow (**Figure 7**; Britt & Farnsworth, 2003). Materials required were nitrile gloves, tri-plate agar, Delaval Cell Counter cassettes, sterile tubes for collecting milk samples, sterile cotton swabs for milk cultures, and WinterSet Powdered Teat Dip.

Data were compiled and analyzed using a mixed model with repeated measures using treatment as the main effect, and analyzed using SAS software (v9.4, SAS Institute Inc., Cary, NC). Production measures were reported as least squares means including standard error with significant differences noted at $P < 0.05$. The Chi-Square test and Proc Freq procedures in SAS were used to evaluate teat end scores and culture results.

Results

Milk Yield Results

The average Days in Milk (DIM) was 188 for the control group and 138 for the treatment group. Despite this difference in DIM, there was no significant difference in

milk yield (kg/d) between the control and the treatment groups. The control group produced on average 32.51 kg/day while the treatment group produced on average 32.43 kg/day ($P = 0.9794$; **Table 1**), indicating that the difference was insignificant.

Somatic Cell Score Results

SCC data were logarithmically transformed to Somatic Cell Score (SCS) using the following formula: SCS=log2(SCC/100) +3. The differences in SCS between the control group and treatment group were insignificant ($P = 0.9794$) (**Table 1**). The control group averaged a score of 10.14 and while the treatment group averaged 10.72. There was a slight numerical increase among the treatment group for the average SCS, however the Standard Error of Measurement (SEM) equaled 0.56, therefore the difference is not significant enough to indicate if the experimental powdered post dip was effective in the reduction of the SCS.

Conductivity Results

The differences in conductivity (mS/cm) in relation to the treatment group versus the control group was not significant ($P = 0.0676$; (**Table 1**). There did appear to be a significant difference by week ($P = 0.0459$). However, because the difference is representing both the control and the treatment group, it must be assumed that the difference is unrelated to the experimental powdered post-milking teat dip.

Activity and Rest Results

When examining the data collected on the cow's daily activity it was found that there were no significant differences as a direct result of the experimental powdered post dip. The overall steps per hour (steps/hr) resulted in a *P*-value of 0.6157 (**Table 1**). There was a significant difference by week ($P = 0.0001$). However, there does not appear to be

a direct correlation between this difference and the experimental powdered post dip. Furthermore, there were no significant differences between the two groups regarding the average rest bouts per day (times/d; $P = 0.9293$) nor was there a significant difference for average rest (minutes/d); $P = .5431$; **Table 1**).

Teat Score Results

When analyzing the overall teat end condition scoring according to treatment, there did not appear to be a significant difference between teat end scores of the control group and the scores of the treatment group (Table 2. 18.8% of the control group scored a 1 while 31.3% of the treatment group scored the same. This is a notable difference, however, with a *P*-value of 0.4914, the difference is not significant. 65.6% of the control group received a score of 2 whereas 53.1% of the treatment group also received a 2. Lastly, 15.6% of both the control and treatment group scored a 3 (**Table 2**). The overall difference between teat end condition scores was not significant.

Cultures Results

Over the twenty-one-day period, 21.9% of the cows assigned to the control group were cultured, while 31.3% of cows assigned to the treat group were cultured ($P = 0.4597$). Milk samples were swabbed and transferred to a Triplate agar (**Figure 6**) to test for bacteria such as: *Staphylococcus aureus* (**Figure 8**), other *Staphylococcus* species (**Figure 9**), *Streptococcus* species (**Figure 10**), and gram negative. There were no significant differences between the control group and the treatment group in relation to the frequency of cultures ($P = 0.4597$). **Table 3** indicates the bacterial growth that appeared on the cultures after 48 hours in the incubator. 3% of the control group and 3.1% of the treatment group showed no growth on their cultures. 6.2% of the treatment

group cultured *Staphylococcus aureus*, while 0% of the control group cultured this bacteria species. 15% of the control group cultured other *Staphylococcus* species, were 21.7% of the treatment group cultured the same. Lastly, 3% of the control group cultured *Streptococcus* species when 0% of the treatment group cultured this bacteria species. Overall, the *P*-value of 0.4597 showed that there was no significant difference referring to the types of bacteria cultured in relation to the experimental powdered post dip.

Discussion

The previous results show minimal correlation to previous studies. There has been an observed correspondence between teat end callosity and thickness, as reported by Barkema et. al. (2001). It was thought that callouses, thickening of skin, and lesions on or around the teat end made the teat more susceptible to mastitis causing pathogens. 31.3% of the treatment cows had a teat end score of 1 while only 18.8% of the control group scored a 1. However, the relationship between teat end health and SCC did not appear to be significant over the 21-day period of this experiment. This could, however, be because the small sample size as well as the average weekly temperatures being relatively high for the Derma Soft n' Dry (experimental powdered post dip; **Table 4**). The average temperature, overall, for the 21-day period was 7.3°C (45° F). The purpose of the Derma Soft n' Dry is to protect teat ends during extreme cold temperatures; therefore, the higher temperature was likely a hindrance in collecting sufficient data for the intended purpose of this project.

The data collected from the bacterial cultures showed no significant correlation between the Derma Soft n' Dry and the types of bacteria collected. Overall, both the control group and the treatment group had relatively the same culture results (**Table 3**).

However, it can be assumed that in more extreme temperatures and a larger sample size, there is a possibility that there would be a more defined trend in the relationship between the treatment group and temperature.

Conclusion

Mastitis remains the most prevalent disease among dairy herds. It is imperative that farmers continuously monitor both clinical and subclinical signs of mastitis. One of the most effective ways to prevent mastitis is to implement correct milking parlor procedures. Using a milking procedure that is formatted for specific herd needs would include analyzing the local climate and weather patterns. The study shows that use of products that are not best suited for herd needs can yield in no significant change in mastitis cases. The use of a powdered post dip in an area such as middle Tennessee, may not have as great of an effect on mastitis as it would in northern state that is more prone to harsh winters with extremely low temperatures.

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Appendix

Table 1. Least square means for production measures by treatment group. Control = iodine post-milking dip; Treatment = Derma Soft n' Dry.

Measure	Control	Treatment	SEM	*P*-Value
No. of cows	8	8		
DIM	188	138		
Milk Yield, kg/d	32.51	32.43	2	0.9764
Somatic Cell Score	10.14	10.72	0.56	0.9794
Conductivity	9.22	9.87	0.23	0.0676
Avg. Activity, steps/hr	89.9	84.1	8	0.6157
Avg. Rest Bouts, times/d	5.09	5.01	0.89	0.9293
Avg. Rest Time, min	283.5	312.6	32.9	0.5431

[a] Somatic cell count data were transformed using the following formula: SCS = log2(SCC/100) + 3.

* Significant difference at $Pp < 0.05$.

Table 2. Teat End Score[a] frequency according to treatment group.

Teat End Score	Control (%)	Treatment (%)	*P*-Value
No. of observation	32	32	0.4914
1	18.8	31.3	
2	65.6	53.1	
3	15.6	15.6	
4	0	0	

[a] According to the method devised by (Britt & Farnsworth, 2003), teat end scores are as follows: 1 = smooth bottom, no or smooth callous, no lesions; 2 = raised callous ring with slight roughness; 3 = rough callous with hyperkeratosis; and 4 = very rough callous with hyperkeratosis and radial cracking.

Table 3. Frequency (%) and type of culture after a 48-hour incubation period by control and treatment group.

SCC Reading	Control, %	Treatment, %	*P*-Value
Cows not cultured	78.1	68.8	0.4597
Cultured	21.9	31.3	
No bacterial growth	3	3.1	
Staph. aureus growth	0	6.2	
Other *Staph.* species	15	21.7	
Strep. species	3	0	
Gram negative	0	0	

[a] Milk samples were collected and cultured when SCC reading was ≥ 250,000 cells/ml.

[*] Significant difference determined at $P < 0.05$

Table 4. Average weekly temperature and relative humidity.

	Week 1	**Week 2**	**Week 3**	**Week 4**
Avg. Temperature (*C)	3.2	21	3	2
Relative Humidity %	66	58	50	35

[a] Temperatures and humidity data were collected from World Weather, 2022.

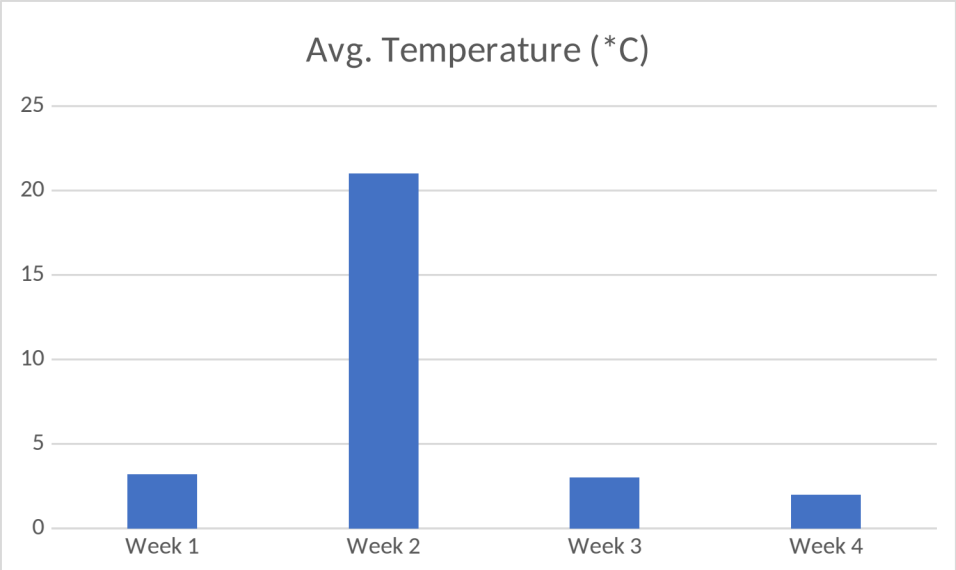

Figure 1. Weekly averages for temperature from January 10 – 31, 2022.

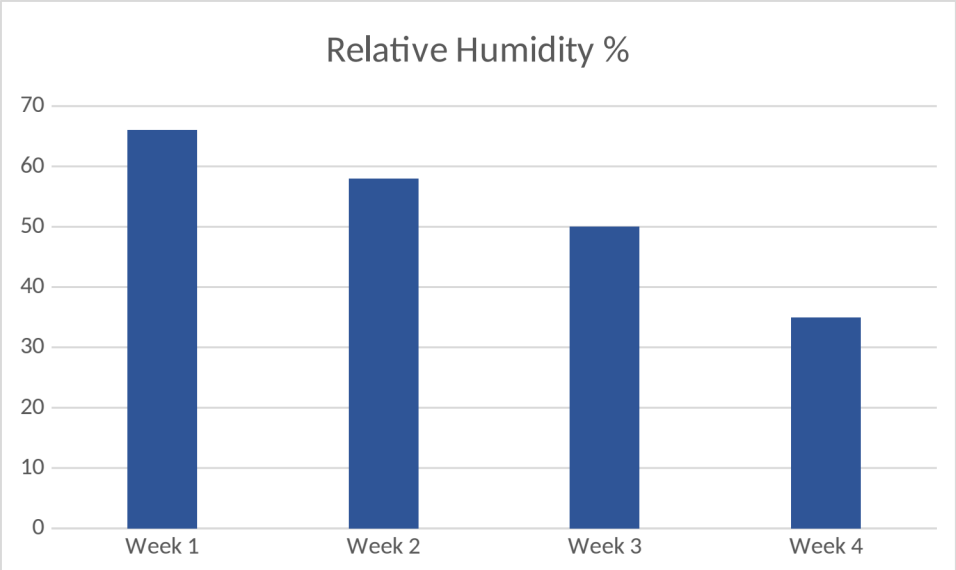

Figure 2. Weekly averages for relative humidity from January 10 – 31, 2022.

Figure 3. Derma Soft n' Dry, powdered post dip.

Figure 4. DeLaval Cell Counter used to calculate SCC of milk samples.

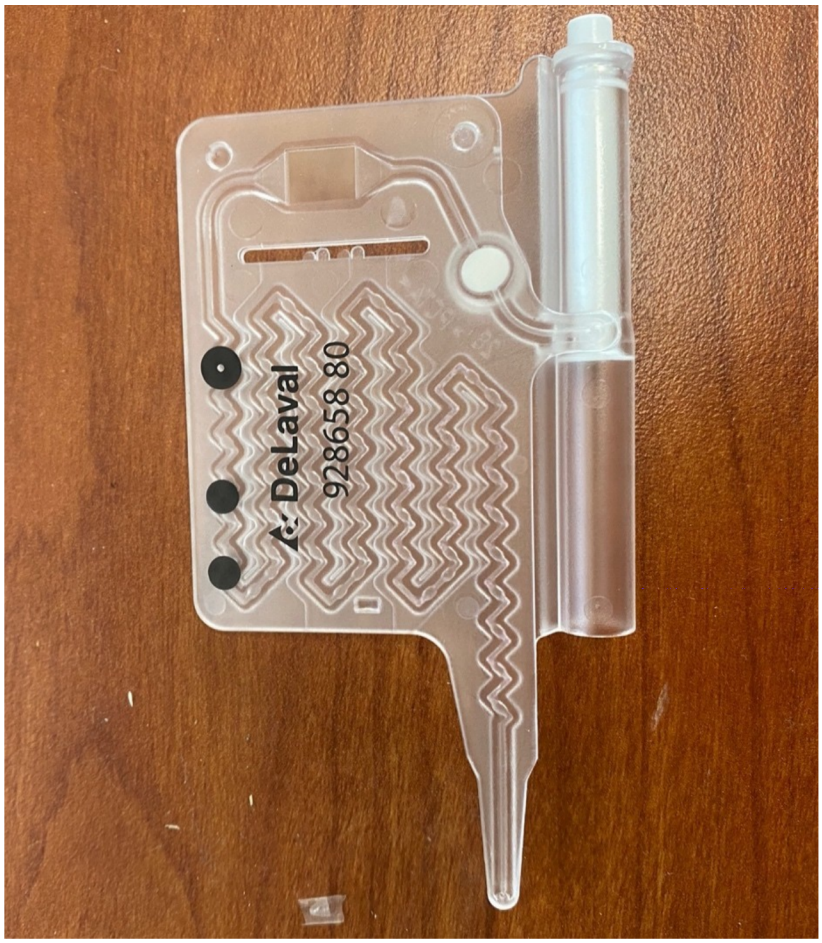

Figure 5. Cassette used to collect milk sample from sterile tube, and inserted into the DeLaval Cell Counter.

Figure 6. Example of a triplate agar used to culture milk samples with a SCC of >250,000 cells/mL. The light red on the top left represents the MacConkey Media, the dark red on the top left represents the Focus Media, and the red on the bottom represents the Factor Media (Minnesota Easy Culture System User's Guide, 2013).

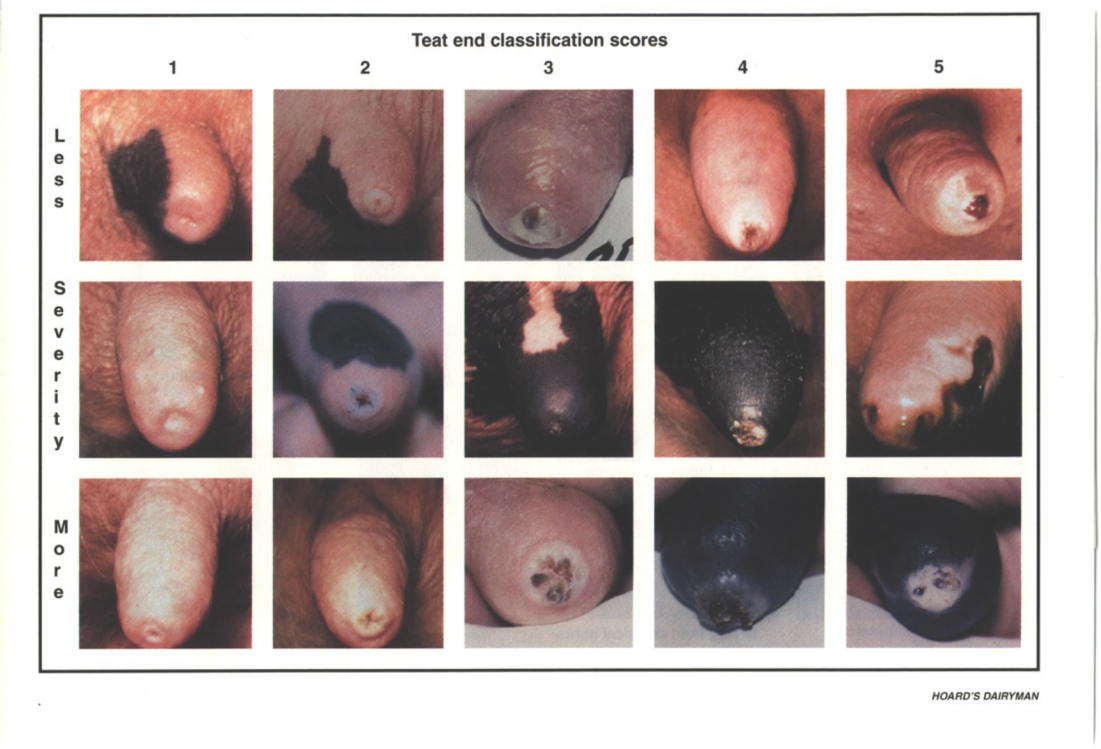

Figure 7. A teat end classification score chart which documents different levels of teat end roughness, callousness, and hyperkeratosis. (Britt & Farnsworth, 2003)

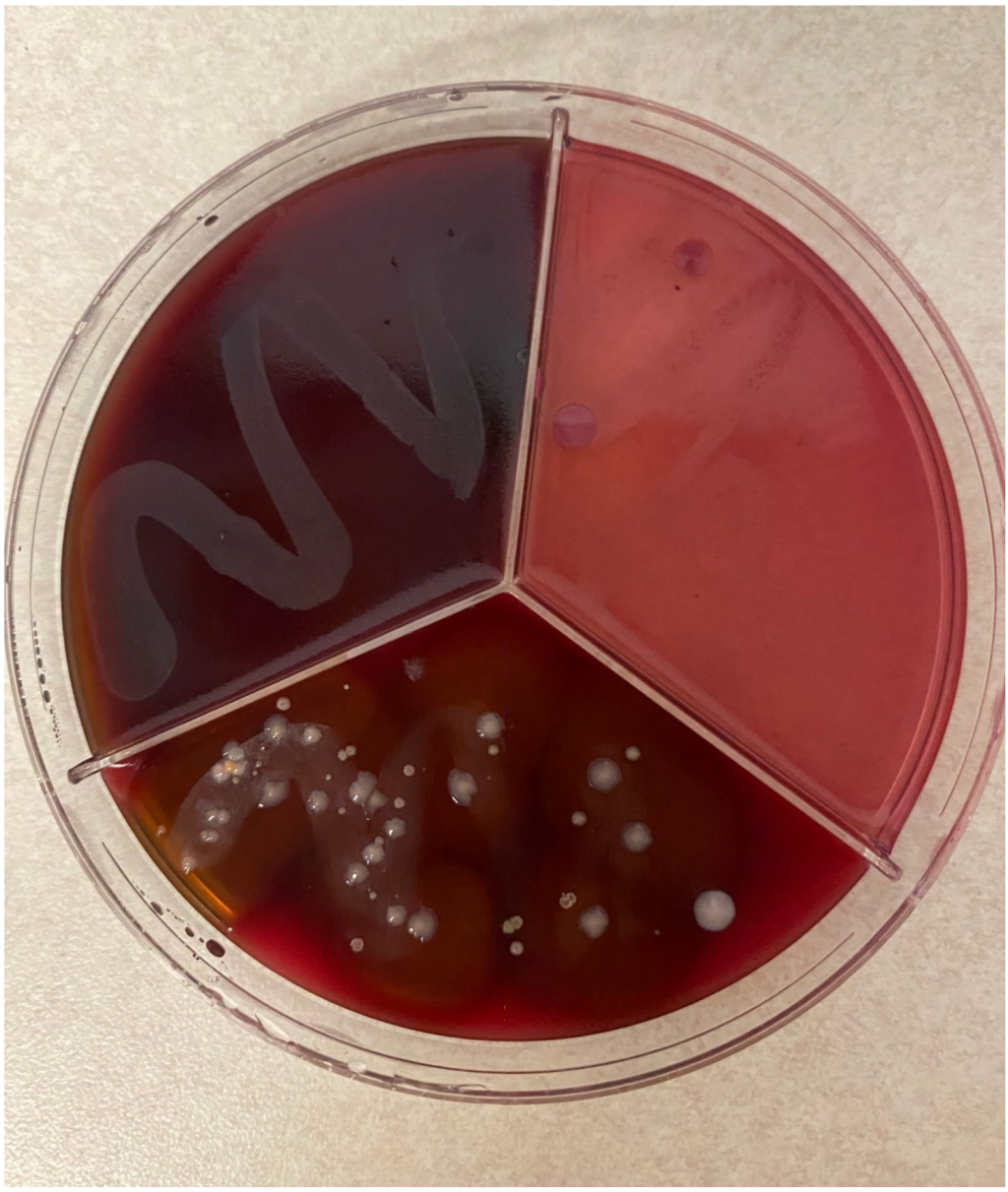

Figure 8. Culture from cow #608 on 1/31/22. This is culture shows *Staphylococcus aureus* bacterial growth, denoted by the zone of homologues in Factor Media.

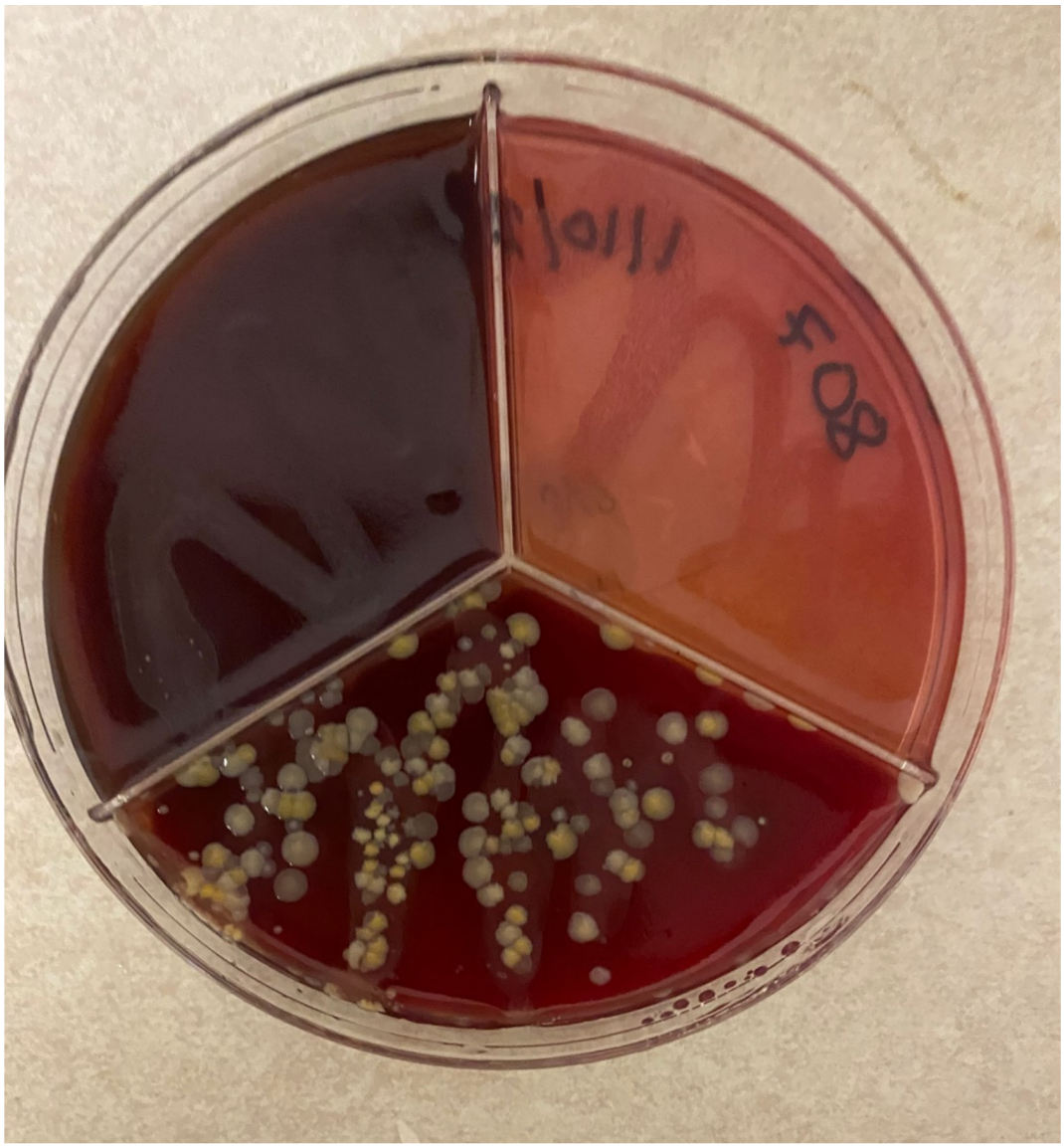

Figure 9. Culture from cow #807 on 1/10/22. This is culture shows Other *Staphylococcus* bacterial growth, denoted by bacterial growth in only the Factor Media.

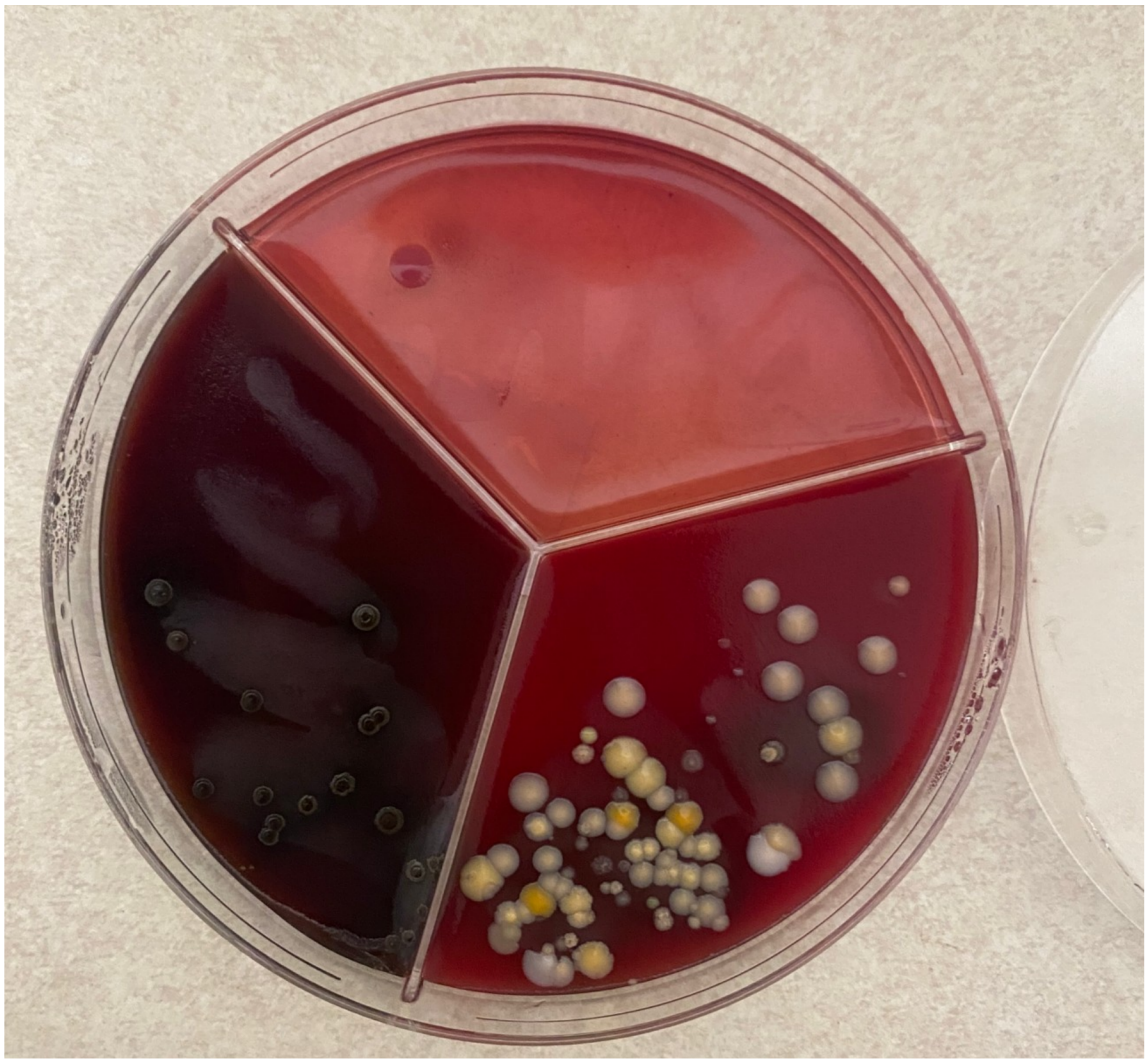

Figure 10. Culture from cow #808 on 1/24. This is culture shows *Streptococcus* bacterial growth, denoted by bacterial growth in both the Factor Media and the Focus Media.

IACUC
INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

IACUCN001: PROTOCOL APPROVAL NOTICE

Thursday, December 16, 2021

Senior Investigator	**Jessica Carter** (ROLE: Faculty Advisor)
Co-Investigators	Kacie Leonard
Investigator Email(s)	*jessica.carter@mtsu.edu; krl4n@mtmail.mtsu.edu*
Department	Agriculture
Protocol Title	***The Effects of Powdered Post Dip on Mastitis in Dairy Cattle in the Winter Months***
Protocol ID	**22-2008**

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Designated Member Review (DMR) mechanism*** and has approved your protocol in accordance with PHS policy. A summary of the IACUC action(s) and other particulars of this this protocol is tabulated as below:

IACUC Action	**APPROVED for one year**	
Date of Expiration	12/31/2022	Approval Date:: 12/15/2021
Number of Animals	SIXTEEN (16)	
Approved Species	**Bovine**	
Category Subclassifications	☐ **Teaching** ☐ Classroom ☐ Laboratory	☒ **Research** ☐ Laboratory ☒ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	Housing: 3211 Guy James Road, Lascassas, TN	
Restrictions	**1. Satisfy DMR requirements AND annual continuing review.** **2. Follow CDC guidelines and MTSU requirements to counter COVID-19 infection**	
Comments	NONE	

This approval is effective for three (3) years from the date of this notice (12/31/2024). This protocol **expires on 12/31/2022** The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved

by the IACUC prior to **12/31/2022** for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule: Refer tp the following table to request your CR:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	11/30/2022	TO BE COMPLETED
Second year report	11/30/2023	TO BE COMPLETED
Final report	11/30/2024	TO BE COMPLETED

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

Post-approval Protocol Amendments:

Date	***Amendment(s)***	***IRB Comments***
NONE	NONE	NONE

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office
(On behalf of IACUC)
Middle Tennessee State University
Tel: 615 494 8918
Email: iacuc_information@mtsu.edu (for questions) and
iacuc_submissions@mtsu.edu (for sending documents)