A bi-seasonal evaluation of somatic cell count, hygiene scores, and bedding cultures of Holstein cows housed in a compost bedded pack barn

by
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Abstract

Cows housed on compost bedded pack barns are known to experience reduced somatic cell counts (SCC) compared to other types of housing. This study aimed to evaluate the relationship between SCC, bacterial cultures of milk and bedding, and hygiene scores of cows. Holstein cows (n=18) were monitored for 28 days during fall and spring seasons. Cows were sorted into high (H) and low (L) groups based on SCC. Weekly milk samples were collected, measured for SCC and those testing greater than 250,000 cells/mL were cultured. High cows had lower milk yields ($P=0.0001$), higher somatic cell scores ($P=0.0003$) and lower average activity ($P< 0.0001$) than L cows. Seasonally, cows exhibited lower milk yields in the fall ($P<0.0001$) while also having higher somatic cell scores ($P=0.0003$), average activity ($P<0.0001$) and average rest bouts ($P<0.0001$). These results suggest somatic cell score and season affect a cow's productivity and udder health.

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Introduction

Somatic cell count (SCC) is a calculated value that measures the amount of leukocytes, or white blood cells, per milliliter of milk. SCC data can be logarithmically transformed into somatic cell score (SCS) which is a reliable indicator of the level of infection of the mammary gland, commonly referred to as mastitis (Reneau, 1986). A high SCC often leads to lower productivity of individual cows (Jones et al., 1984) and also causes increased waste, as milk from individual cows with a high SCC can cause bulk tank somatic cell count (BTSCC) to exceed the U.S. legal limit of 750,000 cells/mL (Wenz et al., 2007). When this occurs, farms are forced to throw away several thousand dollars' worth of milk and spend even more money to prevent further loss of milk. By monitoring and controlling SCC, dairy farmers are able to improve cow comfort and productivity to maximize milk quality and monetary gains.

Factors Affecting Somatic Cell Counts

Somatic cell counts can be affected by many different factors. These include, but are not limited to: management practices, hygiene techniques, milking equipment, and environmental conditions. Bacteria levels in the environment and on the teat end can be linked to high SCC and increased mastitis incidences. When the udder and teat ends are exposed to pathogens, the pathogens are able to enter the streak canal of the teat and cause infections of varying severity (Smith et al., 1985; Natzke, 1981; Dodd, 1983). The level of cleanliness, particularly on the udder, is an indicator the level of exposure to infectious pathogens which commonly occurs when the cow lies down and the udder is exposed to the bedding of the barn. *Streptococcus* species, *Staphylococcus* species, other non-*Strep* gram-positive bacteria, and gram-negative bacteria cause increased mastitis incidence when they penetrate the teat-end orifice (Pyörälä and Pyörälä, 1997). With proper management practices, the compost bedded pack barn can help reduce the level of environmental pathogen exposure, thus decreasing the herd's risk for environmentally caused mastitis.

Compost Bedded Pack Barn

The compost bedded pack (CBP) barn was developed by Minnesota dairy farmers in order to improve cow living conditions and life expectancies (Barberg et al., 2007). This social type housing encourages natural cow-to-cow interactions while maximizing comfort. The CBP barns typically follow the design of a large open area meant for resting with a feed alley separated from the pack area by a concrete wall (Barberg et al., 2007). The resting area is commonly bedded with a deep layer of dry wood shavings or sawdust that is aerated 2-3 times a day to maintain proper surface moisture and incorporate waste matter into the pack (Black et al., 2014). This process promotes active composting, which breaks down waste matter. Maintaining a dry surface layer is vital in preventing the spread of bacteria found within the bedding (Black et al., 2014).

Seasonal Effects on SCC

Mastitis incidences can occur year-round; however, seasonal trends of SCC levels have been reported. In a study performed in 2012, Gillespie et al. found that SCC values are often at their highest in the summer months. This could be due to factors such as heat stress, stage of lactation or addition of new cattle as studied by Ferreira and De Vries (2015). In contrast, the SCC values were found to be the lowest in the winter months. In the same study, the SCC values for spring and fall months were reported to be very similar, which allows for a level of data consistency within a study completed over the course of the spring and fall seasons.

Objective

This project seeks to better understand the Middle Tennessee State University dairy herd's seasonal trends of mastitis and to increase the productivity of the herd through evaluation of the relationship between SCC and bacterial cultures of the bedding located within the MTSU CBP barn. By conducting a bi-seasonal study, we hope to elucidate a consistent correlation between bacteria found in the bedding and mastitis-causing bacteria found in the milk.

Methodology

This protocol was approved by the MTSU Institutional Animal Care and Use Committee (Protocol # 20-2002), and the investigator has completed the required online Collaborative Institutional Training Initiative (CITI) program.

This project utilized the MTSU Experiential Learning and Research Center dairy cattle and barn. At the beginning of the fall 28-day trial period, a group of 18 cows were identified and tagged based on recent Dairy Herd Improvement Association (DHIA) milk testing results. Cows with SCC greater than 250,000 cells/ml were assigned to the High trial group while those lower than 250,000 cells/ml were assigned to the Low trial group. Similarly, at the beginning of the spring 28-day trial period, a group of 12 cows were identified, sorted and tagged based on the previous month's DHIA milk testing.

Milk Sample Collection and Testing

Weekly milk samples were collected from all cows in the study during daily milkings (**Figure 1**). Each cow's udder was first prepped by routine pre-dip, strip, and drying process. Collectors wearing sanitary gloves then applied rubbing alcohol to all teat ends before collecting a composite sample of all 4 quarters into sterile disposable sample bottles labeled with the proper cow ID. The milk samples were tested utilizing the DeLaval Cell Counter (DCC) to obtain an on-site SCC (**Figure 2**). Milk that tested greater than 250,000 cells/ml was then cultured onto Minnesota Easy System Tri-Plates and incubated at 37°C (98°F) for 48 hours (**Figure 3)**. After washing hands and workspace, each milk sample was swabbed onto each of the 3 agar mediums of the tri-plate: Factor TM media, MacConkey media (Focus), and MTKT TM media. The plates were stored facing down and each swab was taken from a sterile packet to prevent any contamination of the culture plates and milk samples. At the completion of the 48-hour incubation period, any growth was recorded, and the species of bacteria identified utilizing the Minnesota Easy Culture System User Guide (**Figure 4, 5, 6, and 7**).

Bedding Sample Collection

The milk sample bacterial species were compared to the bacterial species found within the bedding of the MTSU CBP barn. Bedding samples were collected twice throughout the study: the first collection day (D0) and the last collection day (D28). Utilizing methods similar to those described by Black et al. (2014), equal amounts of bedding were collected from 10-12 evenly distributed locations throughout the barn (**Figure 8**). These samples were thoroughly mixed and then 2 cups of bedding were placed into a large plastic bag and frozen to preserve bacterial levels. Once all bedding samples were collected, the samples were sent to the Veterinary Diagnostic Laboratory at the University of Minnesota to be cultured and analyzed for bacterial species.

Hygiene scoring and Activity Data Collection

Along with sample collections, cow hygiene was assessed each week. Cows were scored on a 4-point cleanliness scale with 1 being very clean and 4 being very dirty, evaluating the udder, lower legs, and upper legs/flank separately (Cook, 2007, **Figure 9**). Each cow within the herd is equipped with an AFI Milk electronic pedometers and identification leg tags (**Figure 10**). These tags monitor each individual cow's daily activity level, milk production, and milk conductivity over the course of the study. This information allowed us to analyze the amount of time each cow spent lying down in the barn as well as the production level of the milk.

Data Analysis

Milk production, SCC, milk conductivity, average activity, average rest bout and average rest time behaviors were analyzed using the MIXED procedure in SAS (v. 9.4, SAS Institute Inc, Cary, NC). Somatic cell counts were logarithmically transformed to a SCS. Statistical significance was declared at $P \leq 0.05$.

Results

Production Data by Treatment Group

Conductivity, average rest bouts, and average rest time were not statistically different between Low and High treatment groups. Unsurprisingly, SCS was higher in high cows when compared to low cows with 14.1 and 12.4 ± 1.6 respectively. (P = 0.0001) Milk yield was also lower in high cows with 29 ± 1.6 kg as compared to low cows at 35 ± 1.6 kg. (P = 0.0003) The average activity of cows in the high group was found to be higher in low cows when compared to high cows with 101.6 and 90.4 ± 4.9

steps/hr respectively. ($P < 0.0001$). Production results as analyzed based on treatment group are summarized in **Table 1.**

Production Data by Season

When comparing production data based on season, average rest time was not different between fall and spring ($P = 0.3295$). Milk yield was greater in the spring at 35.3 ± 1.4 kg compared with 28.8 ± 1.4 kg in the fall ($P < 0.0001$). Similarly, conductivity was higher in the spring than the fall season with 9.8 and 9.4 ± 0.14 mS/cm respectively. ($P = 0.0003$) Somatic cell scores were lower in the spring season than fall at 13.7 and 12.9 ± 0.3 respectively. ($P = 0.0114$). Average activity was also higher in the fall than in the spring season at 102.8 ± 4.8 steps/hr vs 89.1 ± 4.8 steps/hr. ($P < 0.0001$) Similarly, average rest bouts were higher in the fall at 14.5 ± 0.77 times/d compared to the spring at 8.1 ± 0.77 times/d. ($P < 0.0001$) A summary of production data by season can be found in **Table 2.**

Production data was also analyzed by treatment group and season combined. A compilation of production data by treatment group in the fall can be found in **Table 3**. Similarly, production data by treatment group in the spring can be found in **Table 4**. Treatment group and season influenced Milk Yield (**Figure 11**, $P = 0.0021$), Somatic Cell Score (**Figure 12**, $P = 0.0044$), Average Activity (**Figure 13**, $P < 0.0001$) and Average Rest Time (**Figure 14**, $P = 0.0081$). There was no effect of treatment group and season on Conductivity (**Figure 15**, $P = 0.8541$) or Average Rest Bout (**Figure 16**, $P = 0.7214$).

Leg, Udder and Flank Hygiene Scores by Treatment Group

When analyzed for difference by treatment group, hygiene scores were not affected (leg, $P = 0.35$; udder, $P = 0.46$; flank, $P = 0.88$, **Table 5)**.

Milk Culture Results by Treatment Group

Milk sampled from low cows was not cultured 89.3% of the time while milk sampled from high cows was not cultured 48% of the time ($P < 0.0001$). Of the 10.7% of the low cow milk samples that were cultured over the course of the study, 4% showed no growth, 5.3% grew *Staphylococcus* species, and 1.3% grew gram negative bacteria. None of the milk samples from cows in the low treatment group exhibited a growth of *Streptococcus* species or *Staphylococcus aureus.* Of the 52% high cows cultured, only 2.7% of the milk samples exhibited no bacterial growth. 4% grew *Staphylococcus aureus*, 25.3% grew other *Staphylococcus* species, 13.3% grew *Streptococcus* species and 5.3% grew gram-negative bacteria. A summary of the culture data as analyzed by treatment group can be found in **Table 6.**

Bedding Culture Results

Bacterial species cultured from bedding samples taken on October 14, 2019, November 6, 2019, March 11, 2020 and April 8, 2020 were analyzed and a summary of the data can be seen in **Table 7**. *Bacillus* colonies were the most prevalent grown with each sample with 78.81%, 65.91%, 47.71% and 59.35%. This bacterial species was not able to be identified in cultures of milk samples. Gram negative bacteria included coliform colonies and non-coliform gram-negative bacteria. These results were summed to equal 0.14%, 5.37%, 5.99%, 0.76% for each sample date. *Streptococcus* species made up 21.06%, 28.72%, 47.71% and 39.89% of bacteria cultured from each bedding sample respectfully. No *Staphylococcus* colonies were grown from any of the bedding samples collected.

<u>Discussion</u>

Since their introduction to the dairy industry in 2001 (Barberg et al., 2007), CBP barns have been extensively studied in regard to effects on cow comfort, health, and productivity. As expected, there was a strong inverse relationship between somatic cell score and milk yield (**Figure 17**), and this result is consistent between the fall and spring seasons (**Figure 18**), which agrees with the findings of Gillespie et al (2012). Surprisingly, there was no difference in conductivity between low and high cows, contrasting the results found in the study published by Gröhn et al. (2004). When looking at the conductivity between seasons, we found there was a significant decrease from spring to fall (**Figure 19**). Conductivity has been known to decrease as protein and fat content increase (Henningsson et al, 2005) which indicates conductivity is a good indicator of milk nutritive content. As SCC increases, conductivity also increases providing producers with a quantitative value allowing for quick assessment of milk content. Since we observed a increase in SCC and conductivity in the spring season, more focus needed to be placed on increasing udder health to increase productivity.

In agreeance with Gröhn et al. (2004), we found there was a significant decrease in average activity between the low and high treatment groups (**Figure 20**) This can be attributed to the cow resting to allow their body to fight the infection in their udder. In the fall, cows lied down more frequently, were less active and had a higher SCC (**Table 2)**. More frequent lying bouts and less activity indicates greater exposure to bedding pathogens and thus a higher SCC than in the spring. Despite the increased exposure, cows resting more often is desired as it increases potential productivity. The observed increase in SCC as cows increase their rest time could indicate an issue with the composting in the pack.

Udder hygiene score is strongly correlated with the frequency of mastitis incidences, whereas leg hygiene has no significant correlation with mastitis (Schreiner and Ruegg, 2003). When analyzing the effect of cow hygiene scores on SCS, we found no correlation between leg, udder or flank hygiene score and somatic cell scores of cows studied. This result was consistent between seasons and contradicted the findings of Schreiner and Ruegg (2003) and Reneau et al. (2003).

While comparing the milk culture results between treatment groups, the presence of bacterial species in the udder increased with an increase in SCS (**Figure 21**). This is consistent with findings of Schreiner and Ruegg (2003) and Black et al. (2014) and supports the fact that SCS is a reliable indicator of the level of infection in the udder. When compared to bacterial species cultured from milk samples, we see that the presence of *Staphylococci* species likely did not come from contact with the same species of bacteria in bedding. These results are contrast the results observed by Black et al. (2014) where *Staphylococci* were the most prevalent bacteria in the bedding samples. The presence of *Staphylococci* in the milk samples could be attributed to environmental factors other than bedding or being passed from cow to cow within the milking parlor. In their study from 2014, Black et al. reported *Bacillus* bacteria rarely cause mastitis. Unfortunately, the culture plates used in this study are unable to distinguish *Bacillus* species from other gram-positive bacteria grown (**Figure 6**).

Conclusion

Udder health is imperative to the productivity and overall well-being of dairy cows. Somatic cell counts must be closely monitored and managed to maximize productivity and health of the herd. Cow productivity varies throughout the year. In the present study, cows were more productive and had improved milk quality in the spring vs. the fall. Bacterial species cultured in milk samples were not linked to exposure to bedding bacteria alone. There was no indication of the expected relationship between SCC and udder hygiene score. Future studies should focus on locating the source of *Staphylococcus* species found in the milk cultures.

References

Black, R. A., Taraba, J. L., Day, G. B., Damasceno, F. A., Newman, M. C., Akers, K. A., Wood, C. L., McQuerry, K. J., Bewley, J. M. 2014. The relationship between compost bedded pack performance, management, and bacterial counts. J. Dairy Sci. 97(5):2669-2679.

Barberg, A. E., Endres, M. I., Salfer, J. A., Reneau, J. K. 2007. Performance and Welfare of Dairy Cows in an Alternative Housing System in Minnesota. J. Dairy. Sci. 90(3):1575-1583.

Cook, N.B., University of Wisconsin-Madison. 2007. https://www.vetmed.wisc.edu/fapm/wp-content/uploads/2020/01/hygiene.pdf

Dodd, F. H. 1983. Mastiffs - Progress on control. J. Dairy Sci. 66:1773-1780.

Ferreira, F.C., De Vries, A. 2015. Effects of season and herd milk volume on somatic cell counts of Florida dairy farms. J. Dairy Sci. 98(6): 4182-4197

Gillespie, B. E., Lewis, M. J., Boonyayatra, S., Maxwell, M. L., Saxton, A., Oliver, S. P. Almeida, R.A. 2012. Short communication: Evaluation of bulk tank milk microbiological quality of nine dairy farms in Tennessee. J. Dairy Sci. 95(8): 4275-4279.

Gröhn, Y. T., Wilson, D. J., González, R. N., Schylte, H., Bennett, G., Schukken, Y. H. 2004. Effect of Pathogen-Specific Clinical Mastitis on Milk Yield in Dairy Cows. J. Dairy Sci. 87(10): 3358-3374.

Henningsson, M., Östergren, K., Dejmekll, P. 2005. The Electrical Conductivity of Milk—The Effect of Dilution and Temperature, International Journal of Food Properties. 8(1): 15-22.

Jones, G. M., Pearson, R. E., Clabaugh, G. A., Heald, C. W. 1984 Relationships Between Somatic Cell Counts and Milk Production. J. Dairy Sci. 67(8):1823-1831.

Natzke, R. P. 1981. Elements of mastitis control. J. Dairy Sci. 64:1431-1442.

Pyörälä, S. Pyörälä, E., 1997. Accuracy of Methods Using Somatic Cell Count and N-Acetyl-β-D-Glucosaminidase Activity in Milk to Assess the Bacteriological Cure of Bovine Clinical Mastitis. J. Dairy Sci. 80(11): 2820-2825.

Reneau, J. K. 1986. Effective Use of Dairy Herd Improvement Somatic Cell Counts in Mastitis Control. J. Dairy Sci. 69(6): 1708-1720.

Reneau, J. K., A. J. Seykora, and B. J. Heins. 2003. Relationship of cow hygiene scores and SCC. Proc. Natl. Mast. Coun., Madison, WI. 362-363

Schriener, D. A., Ruegg, P. L. 2003. Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis. J. Dairy Sci. 86(11): 3460-3465.

Smith, L. K., Todhunter, D. A., Schoenberger, P. S. 1985. Symposium: Environmental Effects on Cow Health and Performance. J. Dairy Sci. 68(6):1531-1553.

Wenz, J. R., Jensen, S. M., Lombard, J. E. 2007. Herd Management Practices and Their Association with Bulk Tank Somatic Cell Count on United States Dairy Operations. J. Dairy Sci. 90(8):3652-3659.

APPENDIX

Table 1. Least square means for production measures by treatment group as determined by preliminary somatic cell scores

Measure	**Low**	**High**	**SEM**	***P*-Value**
No. of Cows	15	15		
DIM	141.7	148.7		
Milk Yield, kg/d	35	29	1.6	0.0001*
Somatic cell score [a]	12.4	14.1	0.3	0.0003*
Conductivity	9.5	9.7	0.16	0.2802
Avg. Activity, steps/hr	101.6	90.4	4.9	<.0001*
Avg. Rest Bouts, times/d	11.6	11	0.8	0.4352
Avg. Rest Time	328.1	307.8	14.4	0.0612

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

*Significant difference determined at $p < 0.05$.

Table 2. Least square means for production measures by season

Measure	Spring	Fall	SEM	*P*-Value
No. of Cows	12	18		
DIM	138	150.1		
Milk Yield, kg/d	35.3	28.8	1.4	<.0001*
Somatic cell score[a]	12.9	13.7	0.3	0.0114*
Conductivity, mS/cm	9.8	9.4	0.14	0.0003*
Avg. Activity, steps/hr	89.1	102.8	4.8	<.0001*
Avg. Rest Bouts, times/d	8.1	14.5	0.77	<.0001*
Avg. Rest Time	321.4	314.5	13.7	0.3295

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

[*] Significant difference determined at $p < 0.05$.

Table 3. Production data for low and high treatment groups in the fall season

Measure	Low×Fall	High×Fall	SEM	*P*-Value
No. of Cows	9	9		
Milk Yield, kg/d	33.7	24.3	1.7	0.0021*
Somatic cell score[a]	14.2	14	0.4	0.0044*
Conductivity, mS/cm	9.9	9.5	0.2	0.8541
Avg. Activity, steps/hr	74.6	106.1	4	<.0001*
Avg. Rest Bouts, times/d	7.8	14.3	0.9	0.7214
Avg. Rest Time	301.6	314	15	0.0081*

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

[*] Significant difference determined at $p < 0.05$.

Table 4. Production data for low and high treatment group in the spring season

Measure	**Low×Spring**	**High×Spring**	**SEM**	***P*-Value**
No. of Cows	6	6		
Milk Yield, kg/d	36.8	33.3	1.8	0.0021*
Somatic cell score[a]	11.5	13.4	0.4	0.0044*
Conductivity, mS/cm	9.7	9.3	0.2	0.8541
Avg. Activity, steps/hr	103.6	99.5	5	<.0001*
Avg. Rest Bouts, times/d	8.5	14.7	0.9	0.7214
Avg. Rest Time	241.2	315.1	15	0.0081*

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

[*] Significant difference determined at $p < 0.05$.

Table 5. Hygiene score[a] frequency according to low and high SCS groups

Hygiene Score	N	Low (%)	High (%)	*P*-Value
Leg	149			0.35
1		0.7	0	
2		13.4	18.8	
3		20.8	19.5	
4		15.4	11.4	
Udder	149			0.46
1		16.1	18.1	
2		26.2	23.5	
3		8.1	6.7	
4		0	1.3	
Flank	149			0.88
1		6	6	
2		22.8	25.5	
3		10.7	9.4	
4		10.7	8.7	

[a] According to the method devised by Cook (2002), hygiene scores are classified as follows: 1= very clean; 2= slightly dirty; 3= moderately dirty; and 4= very dirty.
[*] Significant difference determined at $p < 0.05$.

Table 6. Frequency and type of culture growth after 48 hour incubation by treatment group

SCC Readings	Low (%)	High (%)	*P*-Value
Cows not Cultured[a]	89.3	48	<.0001*
Cultured	10.7	52	
No bacterial Growth	4	2.7	
Staph. aureus growth	0	4	
Other *Staph.* Species	5.3	25.3	
Strep. Species	0	13.3	
Gram Negative	1.3	5.3	

[a] Milk samples were cultured when DCC SCC reading was ≥250,000 cells/ml.
[*] Significant difference determined at $p < 0.05$.

Table 7. Species of Bacteria found in the bedding cultures

Date	Bacillus Colonies	Coliform Colonies	Environmental Step Colonies	Staph Sp. Colonies	Non-coliform Gram -	Total
10/14/19	78.81	0.11	21.06	0.00	0.03	100.00
11/06/19	65.91	0.47	28.72	0.00	4.90	100.00
03/11/20	47.05	1.20	47.05	0.00	4.70	100.00
04/08/20	59.35	0.76	39.89	0.00	0.00	100.00

Figure 1. MTSU milking parlor during an evening milking.

Figure 2. The DeLeval Cell Counter used for on-site somatic cell counts.

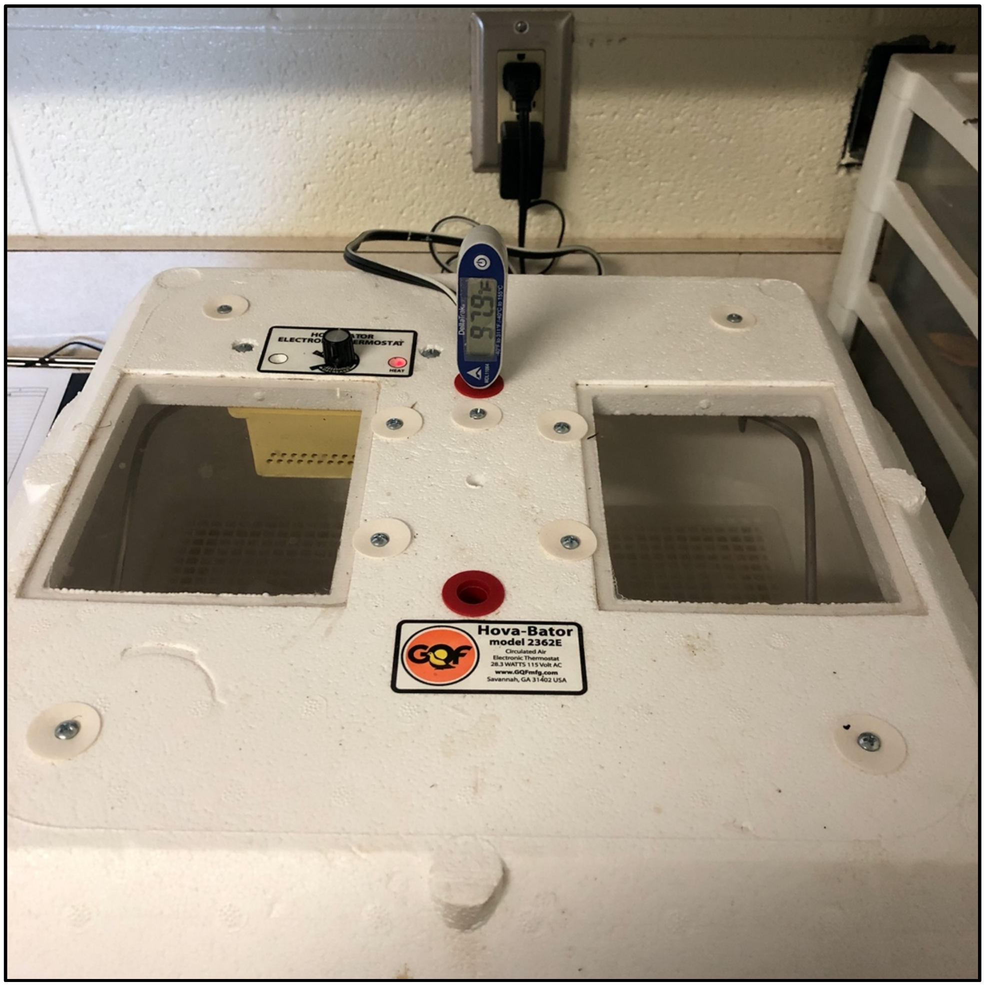

Figure 3. Egg incubator used to incubate milk sample cultures at 37° C for 48 hours. Cultures were checked at the end of each 48 hour incubation period each week and any growth was documented for each culture.

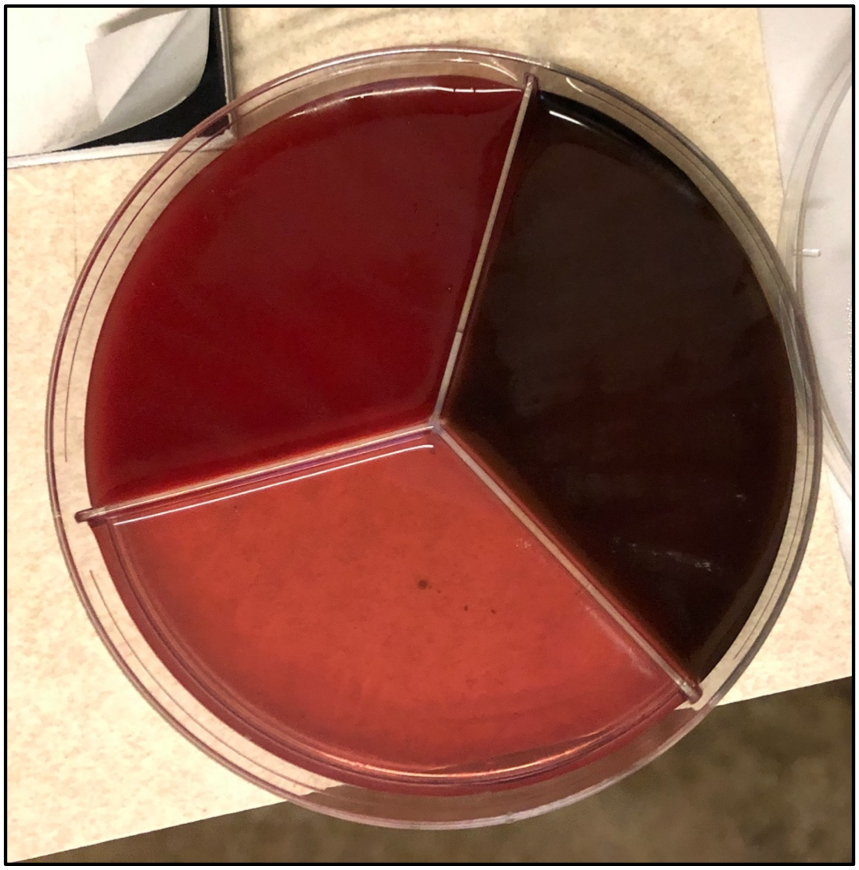

Figure 4. A tri-plate culture exhibiting no bacterial growth. The right, dark media is Focus media, the left, red media is Factor media, and the bottom, pink media is the MacConkey media.

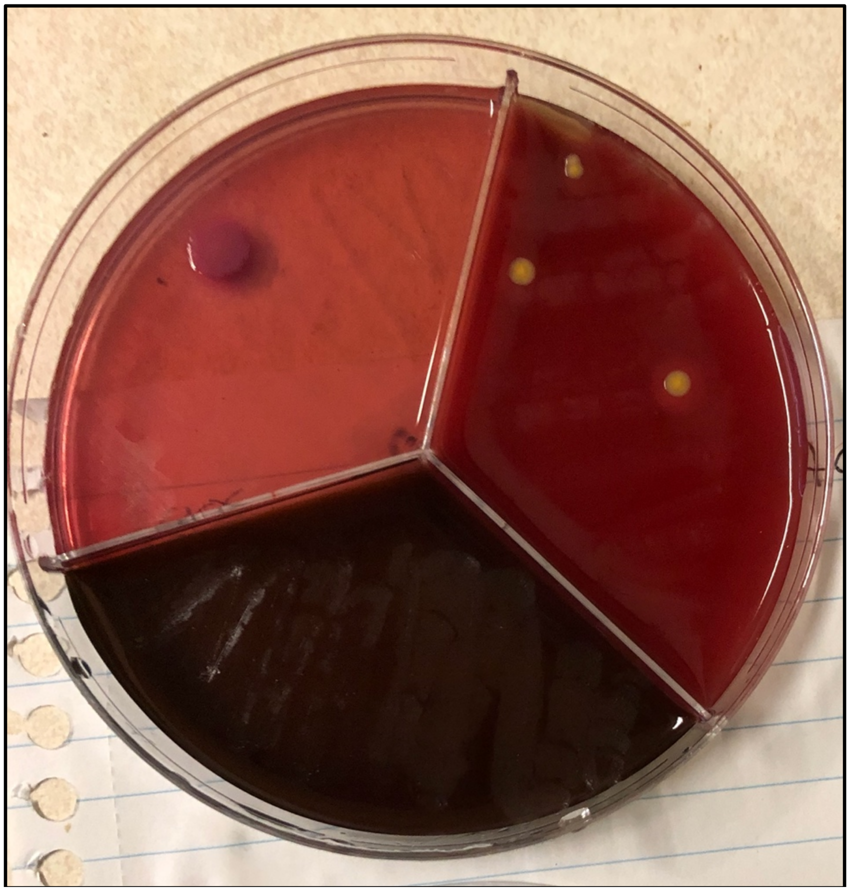

Figure 5. A tri-plate culture exhibiting *Staphylococcus aureus* growth. *Staphylococcus aureus* growth is limited to Factor media and contains zones of hemolysis around the bacterial colonies. The zone of hemolysis is specific to this species and allows for easy distinction from other species of bacteria.

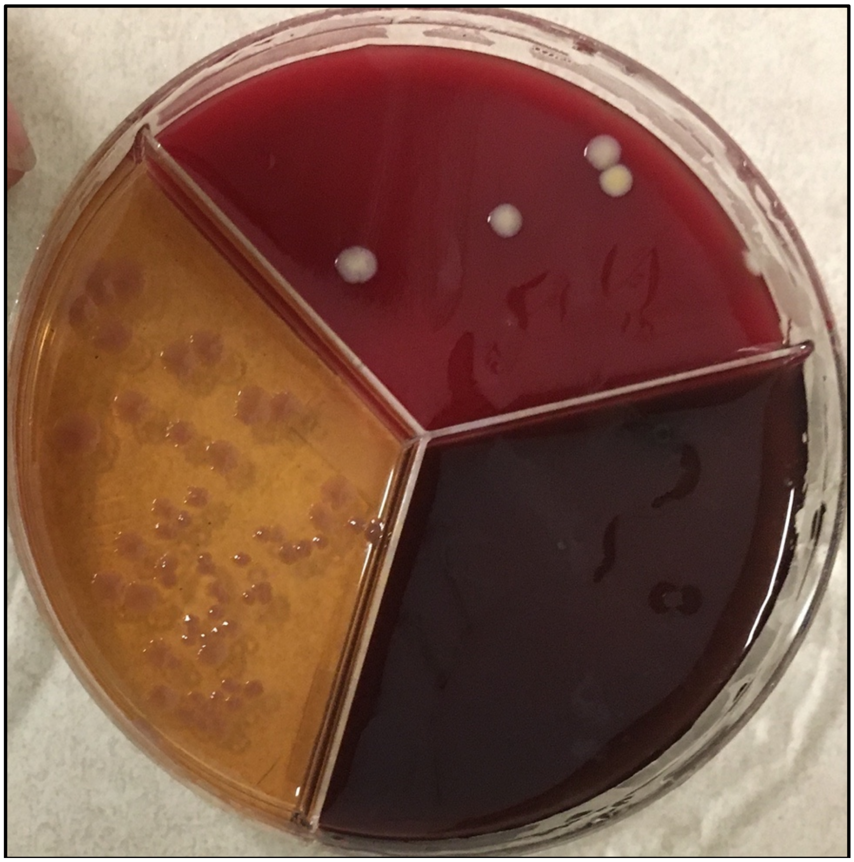

Figure 6. A tri-plate culture exhibiting growth of *Staphylococcus* species (top) and gram-negative (bottom left) species growth. Due to the lack of zones of hemolysis around the bacteria that had cultured after 48-hours this is not *Staphylococcus aureus*; however, concentration in the Factor media still indicate that this is a potential *Staphylococcus* species or other gram positive bacteria. The gram-negative bacterial growth can be observed in the MacConkey media only.

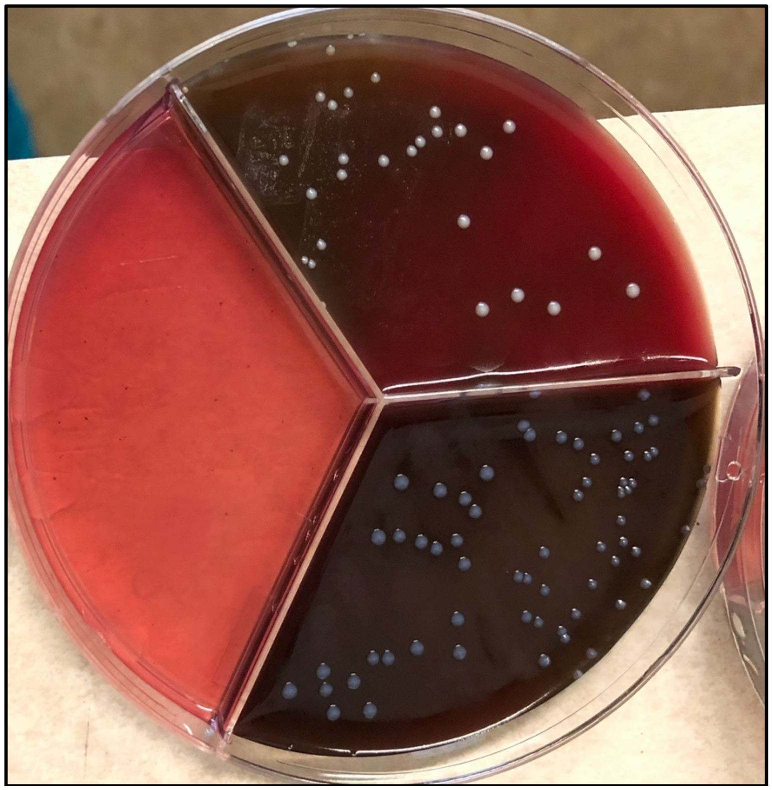

Figure 7. A tri-plate culture exhibiting *Streptococcus* species growth. Growth of colonies in both Factor and Focus media are indicative of *Streptococcus* bacterial species.

Figure 8. The MTSU compost-bedded pack barn. Bedding samples were taken from 12 evenly distributed locations throughout the barn.

Figure 9. A hygiene scoring card which documents the degree of manure contamination on a 1-4 scale for each of three zones, the udder, the lower leg and the upper leg and flank. Score sheet available at http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf.

Figure 10. Cow 405 standing in the milking parlor ready to be attached to the milking machine. AFI Milk control and identification system above showing cow number 405 and indicated by leg band worn on the front left leg of each cow.

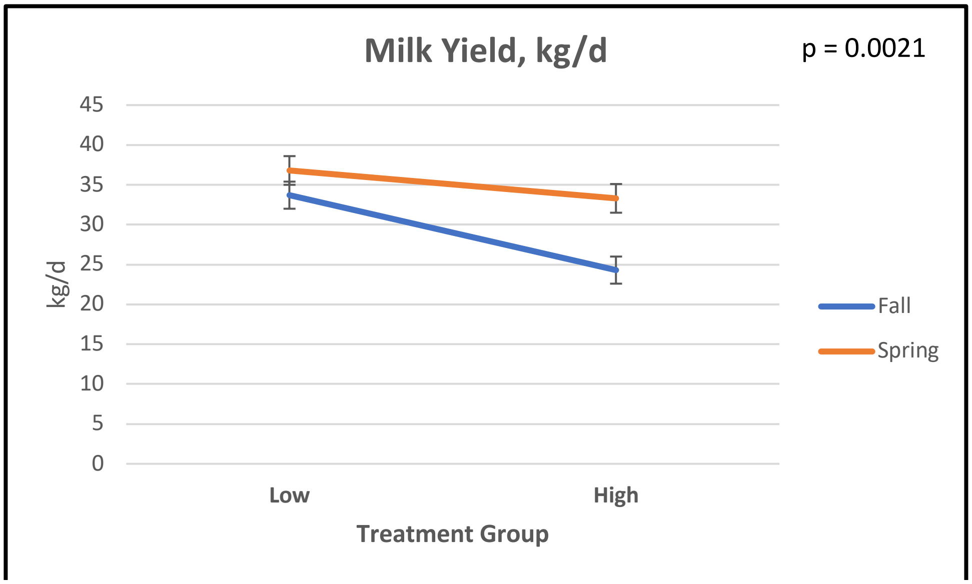

Figure 11. The average milk yield per day (kg/d) for fall and spring seasons by low and high treatment group. This relationship shows a strong correlation between treatment group and season in regard to milk yield.

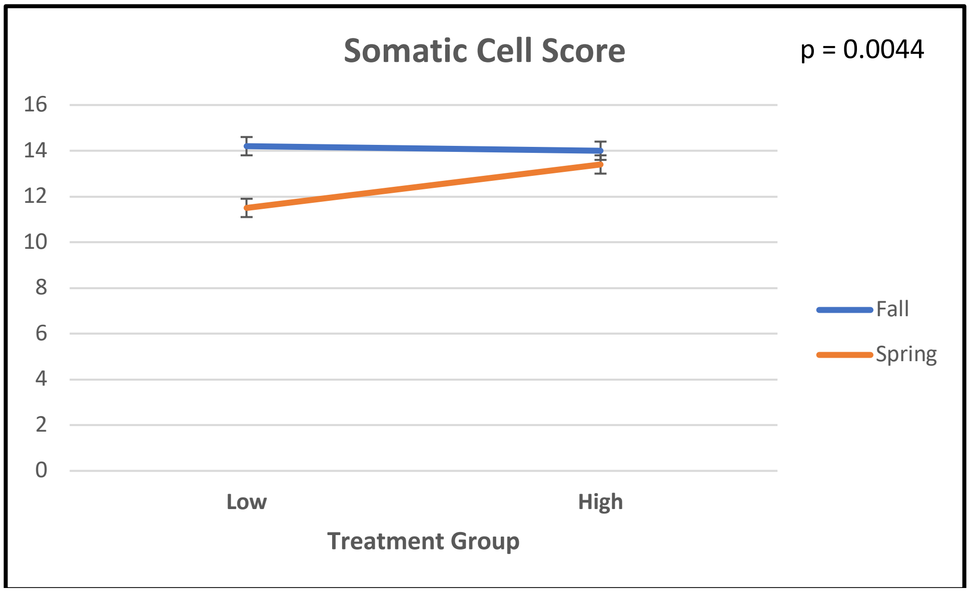

Figure 12. The average somatic cell score for fall and spring seasons by low and high treatment group. This relationship shows a strong correlation between treatment group and season in regard to somatic cell score.

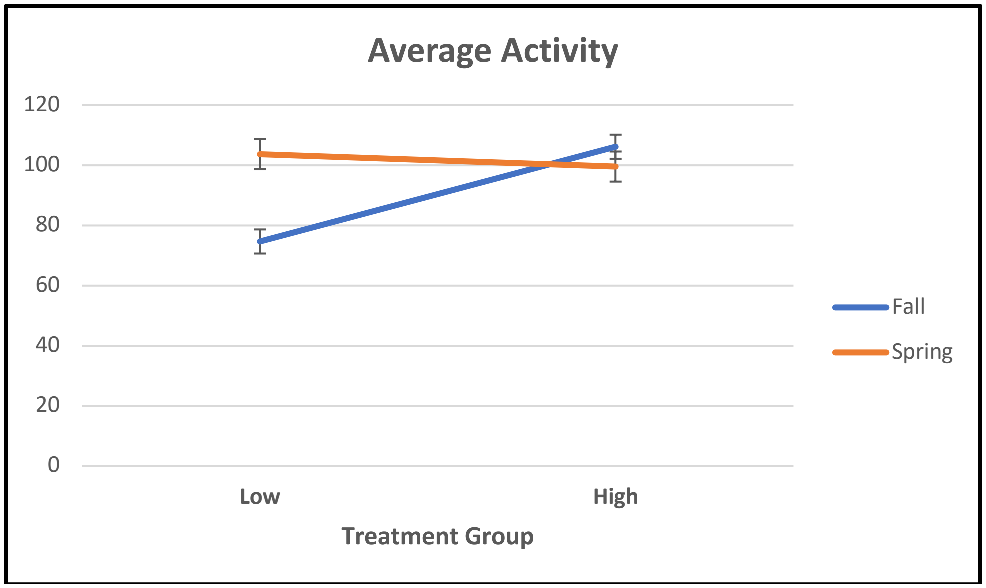

Figure 13. The average activity for fall and spring seasons by low and high treatment group. This relationship shows a strong correlation between treatment group and season in regard to average activity.

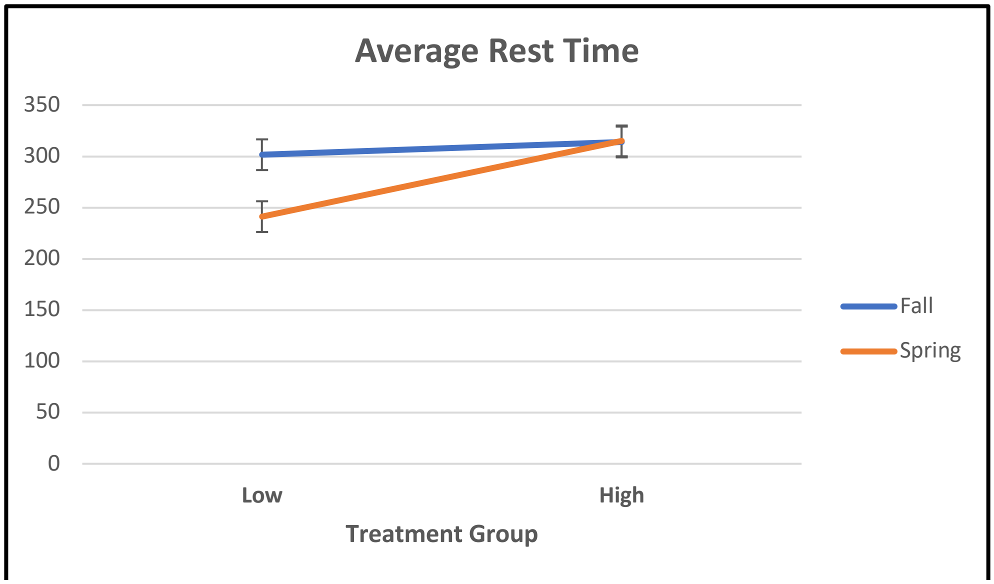

Figure 14. The average rest time for fall and spring seasons by low and high treatment group. This relationship shows a strong correlation between treatment group and season in regard to average rest time.

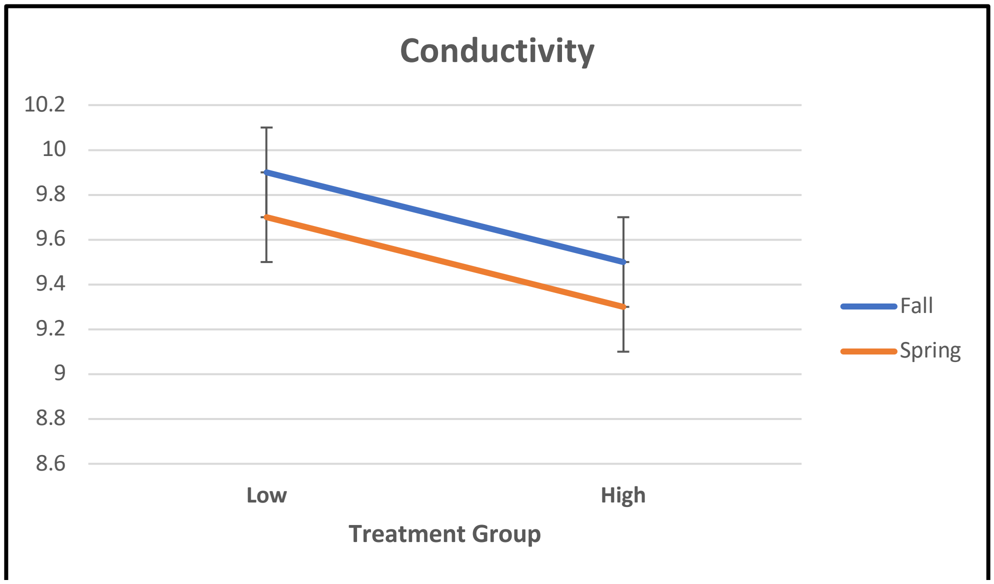

Figure 15. The average conductivity for fall and spring seasons by low and high treatment group. This relationship shows no correlation between treatment group and season in regard to average conductivity.

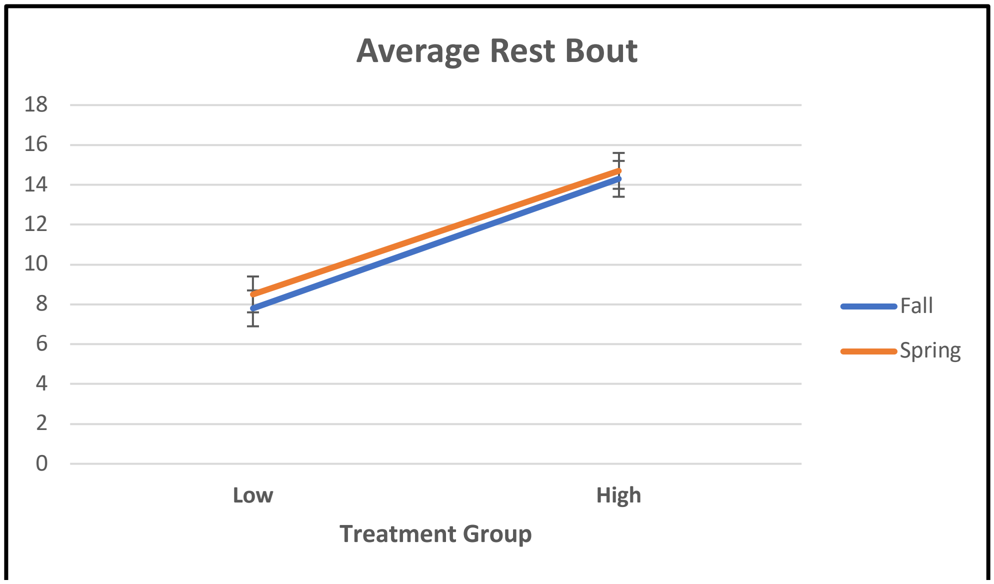

Figure 16. The average rest bout for fall and spring seasons by low and high treatment group. This relationship shows no correlation between treatment group and season in regard to average rest bout.

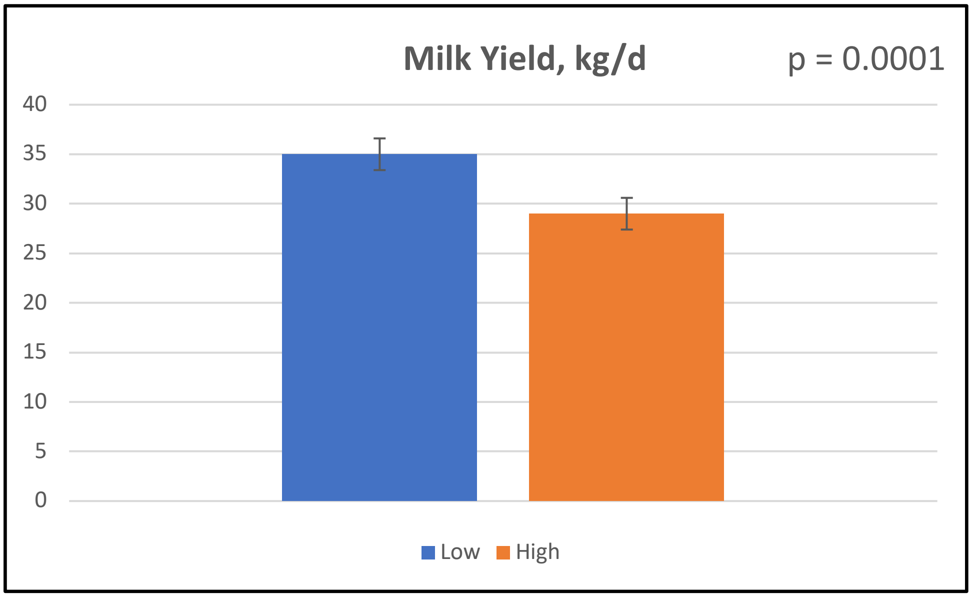

Figure 17. Milk yield in kilograms /day for cows in the low group (n=15) versus cows in the high group (n=15). Shows the inverse relationship between somatic cell scores and milk yield. Average Milk yield for low cows was 35 kg/d while milk yield for high cows was 29 kg/d.

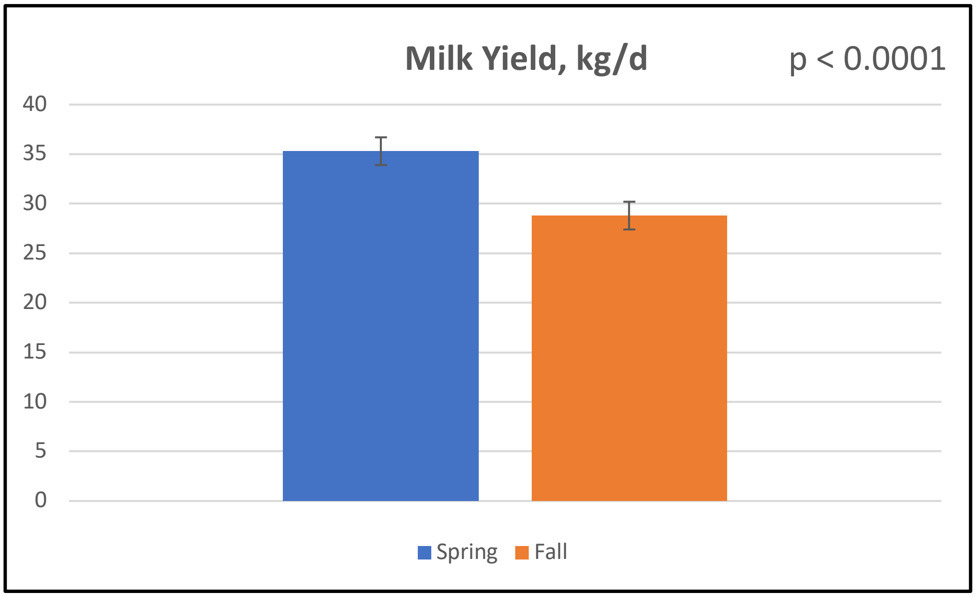

Figure 18. Average milk yield in kilograms of cows during the spring and fall study periods. Shows the decrease in milk yield from spring (35.3 kg/d) to fall (28.8 kg/d) associated with increased somatic cell counts.

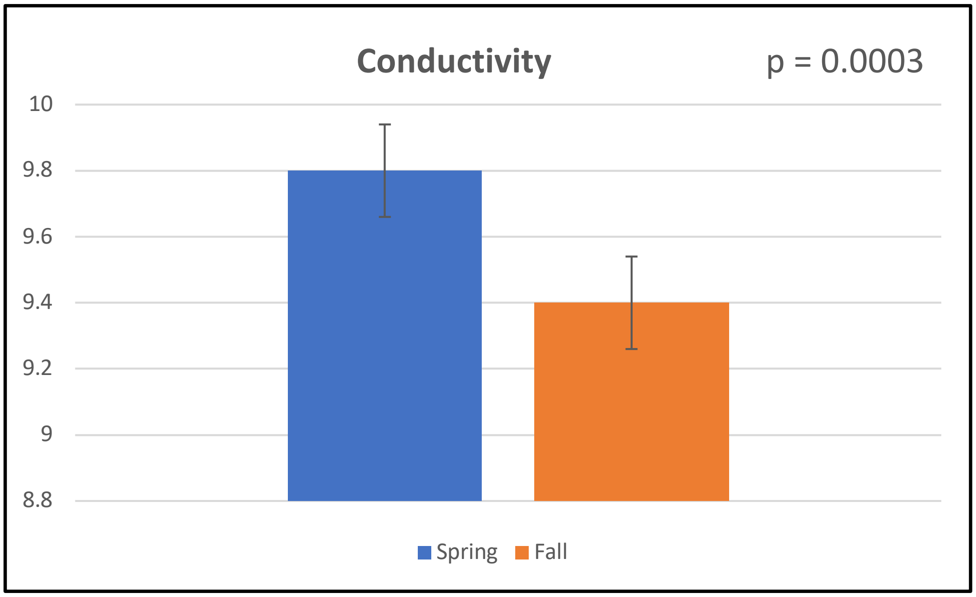

Figure 19. Average conductivity (mS/cm) of cows over the spring and fall study periods. Shows a decrease in conductivity from Spring to Fall with 9.8 mS/cm and 9.4 mS/cm respectfully.

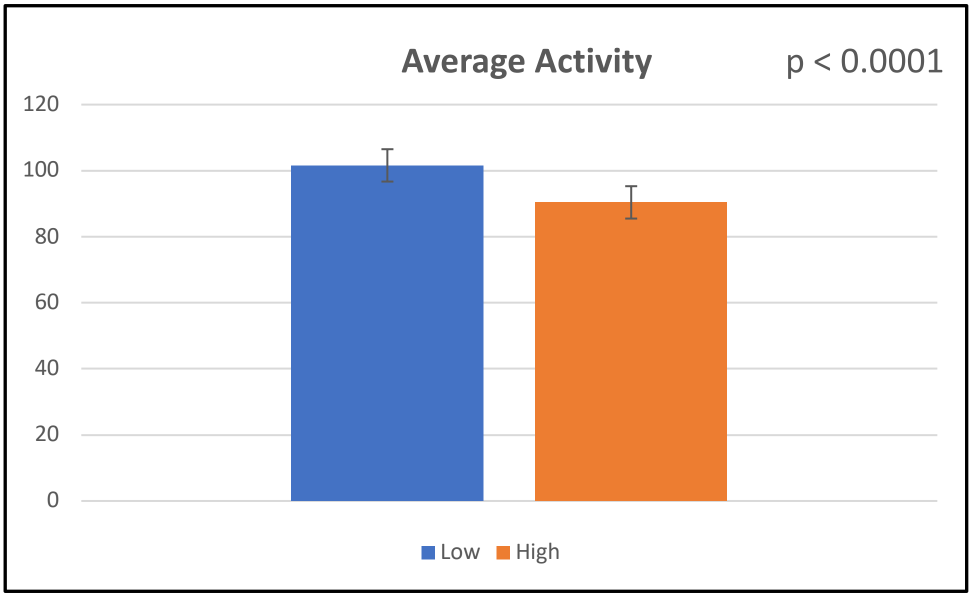

Figure 20. Average Activity of cows in the low and high treatment groups. Low cows had a greater activity level (101.6 steps/hr) when compared to those in the high group (90.4).

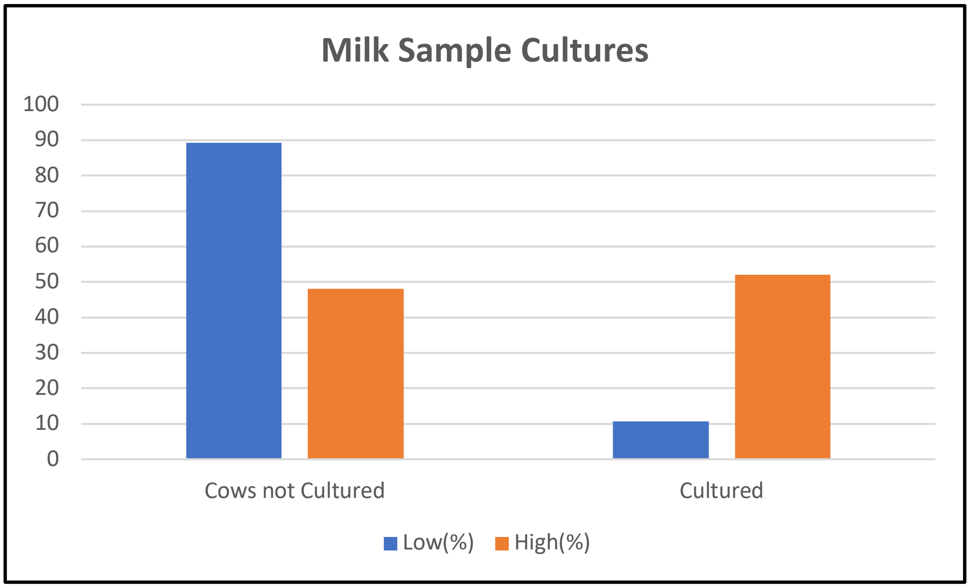

Figure 21. Frequency of milk samples cultured versus not cultured as analyzed by treatment group. Milk samples were cultured when DCC SCC was greater than 250,000 cells/ml. 89.3% of samples from the low treatment group were not cultured while only 48% of the high treatment group were not cultured. In comparison, 10.7% of the low treatment group samples were cultured and 52% of the high treatment group samples were cultured.

IACUC approval

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

Tuesday, October 01, 2019

Senior Investigator	**Jessica Carter** (ROLE: Faculty Advisor)
Co-Investigators	Gabrielle Mould (Student PI) and Maegan Hollis
Investigator Email(s)	*jessica.carter@mtsu.edu;gsm2x@mtmail.mtsu.edu; megan.hollis@mtsu.edu*
Department	Agriculture
Protocol Title	***The relationships between somatic cell count, bacterial cultures, and hygiene on milk production measures of cows housed in a compost bedded pack barn***
Protocol ID	**20-2002**

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	**9/30/2020**	
Number of Animals	30 (THIRTY)	
Approved Species	**MTSU bovine**	
Category Subclassifications	☐**Teaching** ☐ Classroom ☐ Laboratory	☒ **Research** ☐ Laboratory ☒ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	MTSU Dairy Farm	
Restrictions	**Satisfy DMR requirements AND annual continuing review**	
Comments	NONE	

This approval is effective for three (3) years from the date of this notice. This protocol **expires on 9/30/2020** 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

9/30/2020 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	8/31/2020	TO BE COMPLETED
Second year report	8/31/2021	TO BE COMPLETED
Final report	8/31/2022	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)