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Evaluation of colostrum on an immunological and microbiological level and its relation to calf health: a case study of calves at UVM Miller Research Center

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Interpretive Summary

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Colostrum is the first milk that is let down from the mammary gland of a mammal following birth. Calves are born with immature immune systems and need to consume colostrum to obtain immunoglobulins. Bacteria present within colostrum can cause adverse health effects in calves. This study evaluated colostrum fed to calves and its effect on calf health and explored the relationship between immunoglobulin and bacterial content of the colostrum. It was observed that the samples had high bacteria counts and the relationship between immunoglobulin and bacteria counts and their effect on average daily gain should be further explored.

RUNNING HEAD: ANALYSIS OF COLOSTRUM QUALITY

Evaluation of colostrum on an immunological and microbiological level and its relation to calf health: a case study of calves at UVM Miller Research Center

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ABSTRACT

The objective of this study was to evaluate the colostrum quality fed to calves at the UVM Miller Research Center on an immunological and bacteriological level as well as to explore the relationship between these factors and the health of the calves. Colostrum samples fed to twelve calves during their first and second feedings were collected. This colostrum was evaluated using a colostrometer, Brix refractometer, and was also plated on 3M Petrifilm coliform count plates, lactic acid bacteria plates, staph express count plates, and aerobic count plates. During the study the weights of the calves were taken once per week and their rectal temperatures were taken twice per week. Of the 18 colostrum samples, 7 of the Brix refractometer readings did not match the colostrometer readings. 9 of the 18 colostrum samples analyzed had coliform counts and total aerobic counts higher than the recommended levels. There was a significant relationship ($p=0.0092$) between a calf having a fever within the first 14 days of life and the average daily gain of the calf. The colostrum Brix values were also compared to the bacterial cfu/mL counts for coliform bacteria ($p=0.0145$), aerobic bacteria ($p=0.0381$), lactic acid bacteria ($p=0.0209$) and *Staphylococcus* bacteria ($p=0.0364$). Each showed a moderate linear negative correlation between the variables. The relationship between the sum of the lactic acid bacteria counts of the colostrum samples fed to the calves and the average daily gain of the calves was significant ($p=0.0406$). There was also a significant relationship between the average daily gain and the total sum of the aerobic counts ($p=0.0191$) as well as total sum of the coliform counts with ($p=0.0229$). Although significant relationships were found, more studies should be performed with larger sample sizes to further explore the immunological and bacteriological relationships as well as to compare the different methods of evaluating colostrum IgG to the gold standard of RID to make an accurate recommendation.

INTRODUCTION

Colostrum is the first milk that is let down from the mammary gland of a female mammal after giving birth. It is rich in nutrients and immunoglobulins (Metges et al, 2013). Colostrum plays an instrumental role in promoting calf health, growth, and survival rate. In utero, ruminants have no exchange of immunoglobulins across the placenta (Halleran et al, 2017). Therefore, at birth, calves do not have a fully developed immune system and must rely on colostrum for adequate passive transfer of immunoglobulins (Godden, 2008).

In a survey of 1261 US dairy farms, the most common way to evaluate colostrum on-farm was by visual assessment (45% of farms), while less subjective tools such as a colostrometer (hydrometer) or a Brix refractometer were only used on 11% and 4% of farms respectively (USDA, 2016). Hydrometers and refractometers measure colostrum properties that are proxies for IgG content (Heinrichs and Jones, 2016). Although refractometers and colostrometers are tools commonly used on-farm, neither is the gold standard to evaluate colostrum quality. Radial immunodiffusion assays (RID) are the gold standard for evaluating colostrum quality and are the most accurate method of evaluating IgG content. However, RID is an assessment that is performed in a laboratory and requires 18-24 hours to determine results. Therefore, it is not conducive to on-farm use (Bielmann et al, 2010).

Failure of passive transfer occurs when calves fail to absorb adequate amounts of immunoglobulins (Beam et al, 2009). Up to 19.2% of all dairy calves are estimated to suffer from failure of passive transfer (Beam et al, 2009) which has been liked to increased calf morbidity and mortality (Donovan et al, 1997) and a reduction in calf growth rate (Robison et al, 1988). Therefore, if calves are not receiving adequate colostrum, they can become more susceptible to disease.

Although colostrum is essential to calf health, it can also be one of the earliest potential exposures to infectious agents (Brady et al, 2015). Bacteria and pathogens can be transmitted in colostrum either by direct shedding from the mammary gland or through post-harvest contamination. (Stewart et al, 2005). Coliform bacteria in colostrum have been associated with reduced passive transfer of IgG from the intestine into circulation which results in reduced serum IgG concentrations in the calf (Johnson et al, 2007; Godden et al, 2012). It has also been shown that feeding low-bacteria colostrum may reduce the occurrence of scours in newborn calves (Gelsinger et al, 2015). On the other hand, lactic acid bacteria have been shown to have beneficial health effects and have been proposed as types of probiotics (Ljungh and Wadstrom, 2006). Therefore, having increased levels of lactic acid bacteria present in the colostrum may be beneficial to calves.

Since the health of the calf can be affected by the colostrum it receives, and because reduced health can result in reduced growth and development, it is important to find ways to monitor calf health. Calves that become sick may be asymptomatic during the early stages of illness and may not show clinical signs for several days to over one week (Schaefer et al, 2007). Monitoring rectal temperatures may be a way to spot illness in its early stages and to evaluate calf health (Vinson et al, 2014).

The aim of this observational study was to evaluate the immunological and bacterial quality of colostrum fed to dairy calves at the UVM Miller Research Center as well as to evaluate the potential association between calf health measures and bacterial contamination or immunoglobulin content of colostrum. It was hypothesized that calves fed colostrum with lower Brix values and higher bacteria counts would be at increased risk for adverse health events (rectal temperature above 103°F and reduced average daily gain pre-weaning). It was also

hypothesized that there would be a negative correlation between colostrum bacteria counts (cfu/mL) and colostrum Brix values.

MATERIALS AND METHODS

Study design, animal use, and farm colostrum management practices

In a prospective observational study, 12 calves from the UVM-CREAM herd born between July and September 2018 were enrolled. Animal use procedures were approved under University of Vermont Institutional Animal Care and Use protocol 18-007. Calves were followed for 8 weeks of life until each animal's approximate weaning date. Farm practices for colostrum harvest, testing, storage and feeding were not changed during the course of this study. The farm personnel (undergraduate students enrolled in the UVM-CREAM program) harvested colostrum from the first and second milking from cows who calved in. At the time of harvest, the date of colostrum harvest, cow of origin, and colostrometer reading prior to frozen storage were recorded. The calves in this study each received two feedings of colostrum, one given within the first 30 minutes after birth and the second feeding given 8 hours following the first. They were fed colostrum either fresh from their dam, or colostrum from another dam from thawed-frozen 4-liter aliquots where the colostrum quality had been determined using a colostrometer immediately prior to storage in the freezer. The calves were fed using a bottle to ensure that they each received at least 6 pints of colostrum during each feeding. 50mL aliquots were taken from the colostrum fed to the calves during the first and second feedings and stored in the freezer at the Miller Research Center at -20°C until they were picked up and brought to the lab for analysis. In addition to colostrum collection, body weights were taken at birth and then once per week until 8 weeks of age and rectal temperatures using a rectal thermometer were taken twice per

week until 8 weeks of age. Blood was also drawn from the calves and spun down to collect serum.

Sample analysis

Once taken to the lab, the 50mL aliquots of colostrum were stored in a -20°C freezer. Each colostrum sample was then thawed in a water bath at 40°C, which is the optimal temperature for thawing colostrum, prior to being analyzed (Balthazar et al, 2015). Once thawed and at room temperature, the colostrum was tested using a Brix refractometer to determine the total sucrose which can be equated to the IgG content of the colostrum. The blood samples from the calves were also tested for serum IgG levels using a Brix refractometer. A microbiological analysis of each colostrum sample consisted of plating 1mL of thawed colostrum on 3M Petrifilm plates including lactic acid bacteria (LAB) plates, coliform count plates (CC), staph express plates which are selective for *Staphylococcus* bacteria (STX), and aerobic bacteria (AC) plates. These plates were used to determine the colony counts of each type of bacteria within the colostrum samples (Moore and Sischo, 2015). The CC and STX plates were incubated for 24 hours, and the LAB and AC plates were incubated for 48 hours. The number of colonies were counted after incubation and the number of cfus/mL were determined for each sample of colostrum. Where plates demonstrated overgrowth of bacteria (number of colonies were > 300 or too numerous to count) 10-fold serial dilutions of samples were analyzed to achieve a countable plate (around 30-300 colonies). In some cases, the number of colonies on some plates were estimated using the method from the 3M Petrifilm Interpretation Guide, where a plate count was obtained by counting colonies within a defined number of sectors in the matrix. Statistical analysis was performed using the JMP software, including correlation analysis. All continuous data including the cfu/mL counts as well as the log 10 of the cfu/mL counts were checked for

normality using the Shapiro-Wilk Test on the JMP software. The raw cfu counts did not follow a normal distribution, however, taking the log 10 of the cfu/mL counts improved the normality of the data. Therefore, the log 10 of cfu/mL counts were used in the correlation analysis. All of the cfu counts also displayed a bimodal distribution. The residual plots were examined for the data, which showed random dispersal around the horizontal axis, supporting the fact that a linear model would be appropriate for the data.

RESULTS

Colostrum management evaluation

The measurements of colostrum quality taken at the UVM Miller Research Center with the colostrometer did not all match the measurements taken with the Brix refractometer in the lab. Of the 18 colostrum samples, 6 of the Brix refractometer readings did not match the colostrometer readings (Table 1). All 6 of these samples were measured to be "green" with the colostrometer, which equates to "good" quality. When using a refractometer, a Brix score equal to or above 22% indicates the colostrum is of good quality (Bielmann et al, 2010). The Penn State Extension Page states that a Brix value of 22% corresponds to 50mg/mL of IgG which results in a "green" reading on a colostrometer (Heinrichs and Jones, 2016). Therefore, all of the colostrum samples in the table that had a "green" colostrometer reading should have a Brix value that is greater than or equal to 22% which was not true for some of the samples.

Colostrum culturing and analysis

McGuirk and Collins (2004) have recommended that colostrum fed to calves should contain a total plate count of fewer than 100,000 cfu/mL and total coliform count of fewer than 10,000 cfu/mL. Of the colostrum samples fed to calves in this study, 9 of the 18 samples

analyzed had coliform counts higher than the recommended level of 10,000 cfu/mL (Table 2) and 9 of the 18 colostrum samples had total plate counts (aerobic counts) higher than the recommended level of 100,000 cfu/mL (Table 3). Currently there is no widely used threshold for recommended counts of lactic acid bacteria and *Staphylococcus* species in colostrum fed to calves. Descriptive analysis of cfu data demonstrated a bimodal distribution of bacteria counts for all types of bacteria tested including coliform, total aerobic bacteria, lactic acid bacteria, and *Staphylococcus* species, suggesting there were 2 distinct groups of colostrum samples, those over 1,000,000 (extremely high) and those below 1,000,000 (all others). The mean (stdev) log coliform cfu count, total aerobic cfu count, lactic acid bacteria count, and *Staphylococcus* counts of the extremely high colostrum samples were 6.76 (0.9), 8.32 (0.4), 8.03 (0.6) and 7.10 (0.2), respectively. In contrast, the mean (stdev) log coliform cfu count, total aerobic cfu, lactic acid bacteria count, and *Staphylococcus* counts of the other samples were 3.53 (0.9), 4.92 (0.4), 4.00 (0.7), and 3.47 (0.8), respectively.

Correlation analysis was performed comparing the log bacteria cfu counts to colostrum Brix % values. The correlation between all of the bacteria counts (coliform count, aerobic count, lactic acid bacteria count, and Staphylococcal count) and the colostrum Brix values were significant with p-values of 0.0146, 0.0381, 0.0209, and 0.0364, respectively (Figure 1). The relationship between the bacteria counts (coliform count, aerobic count, lactic acid bacteria count, and Staphylococcal count) and the colostrum Brix values all demonstrated a moderate linear negative correlation as evidenced by the following respective correlation coefficient values: -0.565, -0.492, -0.539 and -0.496.

A correlation analysis was also performed on the sum of the cfu/mL counts for coliform and lactic acid bacteria of the colostrum. This was a significant relationship with a-p value of 0.0060 and a correlation coefficient of 0.918 showing a strong positive correlation (Figure 2).

Effect on calf health

During the course of the study, 3 calves (Jinx, Leo, and Linus) had incidences of having a fever, or rectal temperature of 103°F or greater for two consecutive days in a row, however, all calves in the study had at least one day febrile during the 8-week window. Using correlation analysis, it was determined that a calf having a rectal temperature greater than 103°F for at least one day within the first 14 days of life affected the average daily gain of the calf and this relationship was significant (p=0.0092). The calves who had a fever during this time period ended up having a lower average daily gain (ADG) within the first 8 weeks of life (Figure 3). There was also a significant relationship between the sum of lactic acid bacteria counts of the colostrum samples fed to the calves and the ADG of the calves over the 8 weeks of the study (p=0.0406) and these variables displayed a moderate positive linear correlation as indicated by a correlation coefficient of 0.623. This relationship also displayed a relatively weak R^2 value, 0.388 (Figure 4). Although this R^2 value is low, the significant p-value still indicates that this relationship is significant. The low R^2 value may indicate that cfu counts are not a good predictor of Brix values of colostrum, however, other factors may be affecting the Brix value not determined in this study.

The quality of the colostrum fed during the first feeding, as measured by the Brix, did not have a significant relationship with the ADG of the calves (p=0.3801). The relationship between the Brix value of the first feeding of colostrum and the likelihood of the calf developing a fever within the first 14 days of life was also not significant (p=0.2639). There was no significant

relationship between the sum of the aerobic counts and a calf developing a fever within the first 14 days of life ($p=0.1477$) or between the total sum of the coliform counts and developing a fever within the first 14 days of life ($p=0.0501$). However, there was a significant relationship between the ADG and the sum of the aerobic cfu counts, as well as the ADG and the total sum of the coliform counts with p-values of 0.0191 and 0.0229 and correlation coefficients of 0.689 and 0.674, respectively . These both show moderate positive linear correlations (Figure 5).

DISCUSSION

Bacterial and immunoglobulin colostrum content

There was a significant relationship between the bacterial count (cfu/mL) of each of the different types of bacteria and the Brix (%) of the colostrum. All of the bacterial counts had a moderate negative linear correlation with the Brix colostrum values. In each of the graphs, there was a group of samples that clustered well above the regression line and had extremely high cfu/mL counts (Figure 1 A-D). These extremely high cfu data points that had evident grouping in Figure 1A-D were all from the same samples of colostrum. If these samples that were classified as having extremely high cfu/mL counts were excluded from the data set, the association between the bacteria counts and immunoglobulin content changes and there is no longer a significant correlation between the two variables. Although these few samples are different and have extremely high cfu counts, they should not be eliminated from the data at this time since they make up a relatively large portion of the samples collected (approximately 22%) and it is not clear that they are biological outliers. There was a great amount of variation in the bacterial counts of different colostrum samples, so these samples with extremely high cfu counts could just be part of the natural variation seen across samples. Although both bacterial counts and Brix values are very important ways to evaluate colostrum, the relationship between these two

variables has not been explored in the literature. However, some studies have shown that colostrum fed to calves on dairy farms in New Zealand followed a similar pattern to the colostrum collected, having sub-optimal Brix values, and high bacteria counts (Denholm et al, 2016). Although it is unclear why there is a significant relationship between the bacterial counts and the Brix readings for the colostrum, there are likely other factors involved that were not analyzed in this study that may be causing this.

There was a significant relationship between the sum of the coliform cfu/mL counts and the sum of the lactic acid bacteria cfu/mL counts ($p=0.0060$) of the first two feedings of colostrum which had a strong linear correlation (Figure 2). These results go against what would be expected. Lactic acid bacteria have been proposed as a type of probiotic (Ljungh and Wadstrom, 2006). They have been shown to exert inhibitory effects on the growth of enteropathogenic bacteria such as *Escherichia coli* (Kawakami et al, 2010). Some coliform bacteria are enteropathogenic, therefore it was thought that the lactic acid bacteria count and the coliform count would have a negative relationship as Kawakami et al (2010) have shown. This may not have been the case in this study since in general, the bacteria counts were very high for all types of bacteria. Since all of the counts were elevated, this could potentially have appeared as a false positive relationship. With elevated counts of all types of bacteria including the enteropathogenic bacteria or coliform bacteria, the lactic acid bacteria present may not have been able to inhibit these coliform bacteria since there may have been too many coliform bacteria present for the amount of lactic acid bacteria present to overcome.

Effect on calf health

Guidelines from the University of Wisconsin-Madison state that the normal body temperature for a bovine is between 100- 102.5°F. A calf that has a fever that exceeds 103°F for

one to two days in a row would be considered a sick calf which would typically warrant treatment (McGuirk, n.d.). It was determined that the relationship between a calf having a temperature greater than 103°F for at least one day within the first 14 days of life and the average daily gain of the calf was significant and calves who had a fever during this time period ended up having a lower average daily gain within the first 8 weeks of life (Figure 3). Only one calf (Dallas) was treated during the 8-week study due to a high fever (105.9°F). However, he was treated on day 22 of life which did not interfere with our temperature analysis which examined temperatures during the first 14 days of life. Calves that are morbid or have a disease such as respiratory disease have been shown to have a decreased dry matter intake, resulting in decreased average daily gain (Galyean and Hubbert, 1995). In this study, the exact cause of the increased temperature in these calves was not explored, but the type of illness they were affected by may put them at an even higher risk for having a lower average daily gain. For example, if the calves suffered from a gastrointestinal illness such as cryptosporidiosis, which is a common cause of diarrhea in calves, they may have a decreased average daily gain since they are not as able to absorb the nutrients in the food they are receiving.

There was a significant, moderate positive correlation between the sum of the lactic acid bacteria counts of the colostrum in the first two colostrum feedings and the ADG of the calves over the 8 weeks of the study (Figure 4). In other studies, oral administration of lactic acid bacteria to calves has resulted in improved body weight gain (Abe et al, 1995) which does support the results found in this study. One mechanism that may be involved in this is that probiotics, such as lactic acid bacteria, produce water soluble B vitamins which may improve nutrient metabolism in the gut, leading to an increased ADG (Roodposhti and Dabiri, 2012). There was also a significant relationship between the ADG and the total sum of the aerobic

counts, as well as the relationship between the ADG and the sum of the coliform counts. This positive linear relationship is not what would be expected. It was expected that the calves consuming colostrum with higher bacterial loads would experience decreased ADG. Studies have shown that increased bacteria present in colostrum can result in decreased serum IgG levels in calves (Johnson et al, 2007; Godden et al, 2012). This reduced serum IgG linked to increased bacterial loads could be the result of failure of passive transfer due to lack of immunoglobulins able to be transferred to the calf, which has been linked to reduced growth rate in calves (Robison et al, 1988). Although the results of this study suggest the opposite, this could be due to other factors not considered within this study.

Recommendation to management

Many of the refractometer readings did not match the colostrometer readings when evaluating the colostrum, and many of these evaluation disagreements occurred with colostrum fed during the calf's first feeding, which is the most critical feeding due to the short window in which passive transfer can occur. Passive transfer of immunoglobulins can only occur during the first 24 hours following birth, with transfer being optimal in the first 4 hours (Stott et al, 1979). Feeding colostrum of unknown quality could pose problems since the calves may actually be receiving lower quality colostrum than originally thought, resulting in potential failure of passive transfer. One reason for this disagreement between the colostrometer and Brix refractometer readings could be due to measuring the colostrum at an improper temperature. Colostrometers are temperature dependent and should be used to measure samples at room temperature. If the colostrum was measured at a temperature greater than room temperature, the colostrometer would underestimate the colostrum IgG and if it was measured at a temperature colder than room temperature, the colostrometer would overestimate the IgG content (Mechor et al, 1991). At the

Miller Research Center, there is currently no temperature protocol for using the colostrometer. One way to improve colostrum evaluation methods on farm would be to update the current colostrometer protocol to adjust for temperature. Another method which could potentially improve the evaluation methods would be to switch from using a colostrometer on farm, to a Brix refractometer. Brix refractometers function independently of colostrum temperature, yet are still user friendly (Bartier et al, 2015). Studies have shown that Brix refractometers provide a more accurate assessment as well as offer more precision in terms of repeatability than hydrometers do when assessing colostrum quality (Bartens et al, 2016).

The aerobic and coliform bacterial counts of most colostrum samples were much higher than the recommended bacterial counts. These high bacterial counts indicate that bacterial contamination is a serious issue on the farm. This could put the calves consuming this colostrum at an increased risk of becoming sick. The bacteria can come from either an infected mammary gland, or fecal contamination from the environment from post-harvest contamination. Studies have shown that the most significant bacterial contamination occurs during the harvest process. Bacteria counts were shown to be very low in colostrum samples collected directly from the udder of cows (Stewart et al, 2005). High levels of bacteria in colostrum are worrisome since these infectious agents could directly cause diseases such as enteritis or septicemia. It is also believed that increased levels of bacteria in the small intestine at the time of feeding of colostrum could interfere with systemic absorption of Ig molecules (Stewart et al, 2005). One possible mechanism for this could be competition between the intestinal microbes and Ig molecules to bind to common receptors on intestinal epithelial cells (Staley and Bush, 1985).

Feeding clean colostrum of good quality is very important to calf health and therefore measures should be taken to decrease contamination during the harvest and feeding process.

Possible sources of contamination include teat skin, milking cup liners, hoses, or floor buckets that the colostrum may be collected in (Stewart et al, 2005). In order to decrease the contamination of colostrum the procedure for sanitizing the udder prior to milking should be improved. The sanitation procedure of the milking equipment should also be improved, making sure to sanitize all of the components of the system including buckets and hoses. Methods could also be developed on farm to evaluate the cleanliness of equipment to see if cleaning procedures are working well. One tool commonly used in evaluating surface sanitation is an ATP Meter. Adenosine triphosphate (ATP) is present in all types of organic matter. An ATP meter can detect ATP levels via ATP bioluminescence which provides an indication of the level of surface contamination (Moore et al, 2010). This tool can obtain results in real time which would make it very easy to use in a farm setting. The rapid results that this test shows are also helpful because it will allow for easy monitoring of sanitation procedures and problems within the regimen can be identified and corrected quickly. This tool could be used to swab and test collection buckets, hoses, along with the other milk collection equipment.

Limitations and future prospects

One limitation to evaluating the data was the temperature of the colostrum samples were not recorded when colostrometer readings were taken at the Miller Research Center. Therefore, the colostrometer readings were not able to be corrected for temperature. At the Miller Research Center, in order to make a confident recommendation on how exactly to improve colostrum evaluation procedures, future studies should be completed analyzing batches of colostrum samples using both evaluation methods (refractometers and colostrometers) and comparing their readings to the results obtained by the gold standard method, RID, which has been explored in previous studies (Bartier et al, 2015). RID is an assessment that is performed in a laboratory and

requires 18-24 hours to determine results. Therefore, it is not conducive to on-farm use (Bielmann et al, 2010). However, by measuring samples with a gold standard test such as RID, we can use this information to determine the predicted value of the two tools (colostrometer and refractometer). By measuring a gold standard value, we can explore diagnostic sensitivity and specificity and positive and negative predicted values of the two tools used at this farm (Bartier et al, 2015). Another limitation within this study was the issue of missing samples. Colostrum sample collection was dependent on the farm personnel at the UVM Miller Research Center, which ultimately resulted in some samples not being collected resulting in missing data.

Although there were findings in this study that had significant p-values, the results of this study have limitations due to the small sample size. Having such a small sample size can increase the margin of error, and certain results may seem significant that are not due to the decreased power of the study. In order to have more conclusive results, more studies should be conducted using a larger sample size. Having a larger sample size can decrease the standard error and increase statistical power. The larger sample size will help to normalize the data and the data will likely behave more like the population which is important since studies are used to make inferences about the population as a whole.

CONCLUSIONS

In conclusion, this study provides valuable information to the UVM Miller Research Center about their current colostrum handling procedures and also provides a starting point to begin to look at updating on-farm procedures. The results of this study suggest that they should make adjustments to their colostrum harvesting procedure to minimize contamination and also improve their methods of evaluating colostrum quality. Decreasing the level of bacterial contamination would likely result in higher quality colostrum which would lead to improved

health of the calves. In order to further determine the best colostrum evaluation method for the Miller Research Center, the colostrum measurement tools discussed in this study could be directly tested against the gold standard, RID. Further studies could also be performed to determine the exact sources of contamination and pathogens present in the environment at the farm that could potentially compromise the heath of the calves. Additional studies with a larger sample size should also be carried out to re-evaluate the relationship between bacterial counts and colostrum IgG content along with average daily gain to get more conclusive results.

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Table 1. Colostrum sample evaluation results and comparison of colostrometer and refractometer readings of colostrum fed to calves during the first and second feedings. The highlighted rows indicate that there was not agreement between the colostrometer and Brix refractometer readings. Data that has been entered as "n/a" indicates missing values.

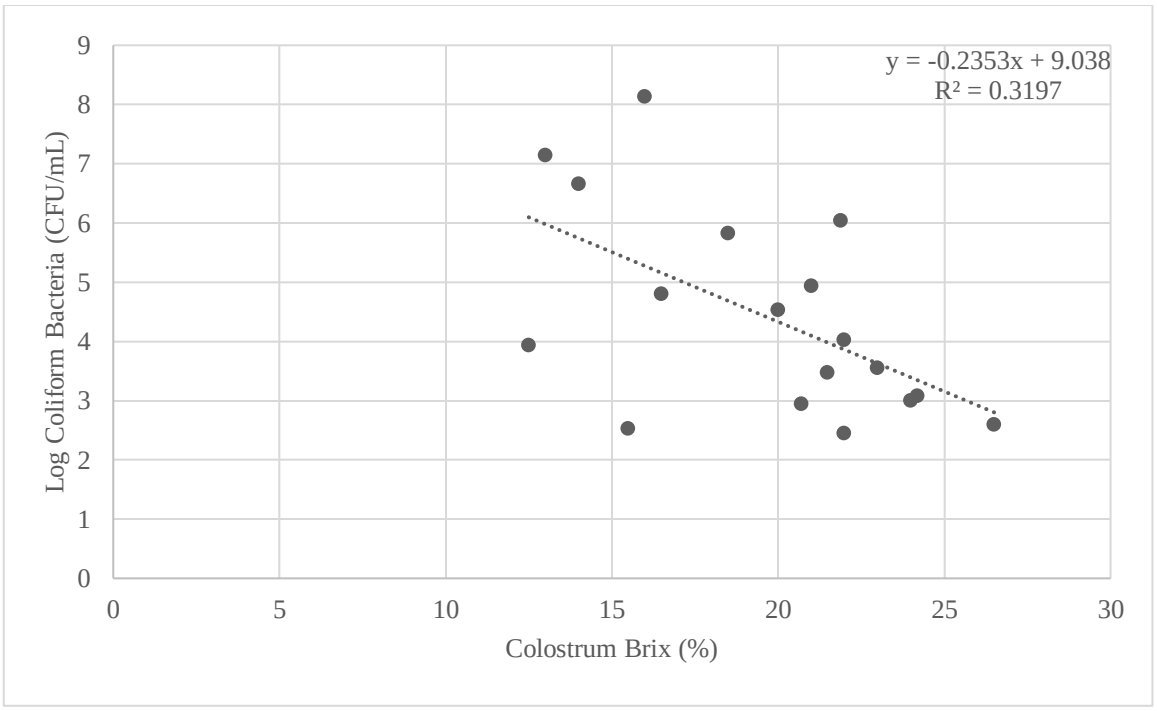
Calf Name	Colostrum Feeding Number	Colostrum Brix %	Colostrometer Reading	Agreement between evaluation methods
Lenox	1	20.7	green	no
Phoebe	1	22	green	yes
Louise	1	24.2	green	yes
Sadie	1	21.5	green	no
Leo	1	23	green	yes
lavender	1	13	green	no
Linus	1	24	green	yes
Jinx	1	16.5	red	yes
Lennox	2	20	green	no
Phoebe	2	21.9	green/red	no
Sadie	2	22	green	yes
Louise	2	15.5	n/a	n/a
lavender	2	18.5	green	no
Dolores	2	21	yellow	yes
Jemima	2	12.5	n/a	n/a
goose	2	14	yellow/red	yes
Linus	2	26.5	n/a	n/a
Jinx	2	16	red	yes

Table 2. Coliform bacteria count and Brix refractometer readings of colostrum fed to calves in the first and second feedings. Highlighted values indicate the cfu/mL is greater than the recommended industry level of 10,000 cfu/mL. Data that has been entered as "n/a" indicates missing values.

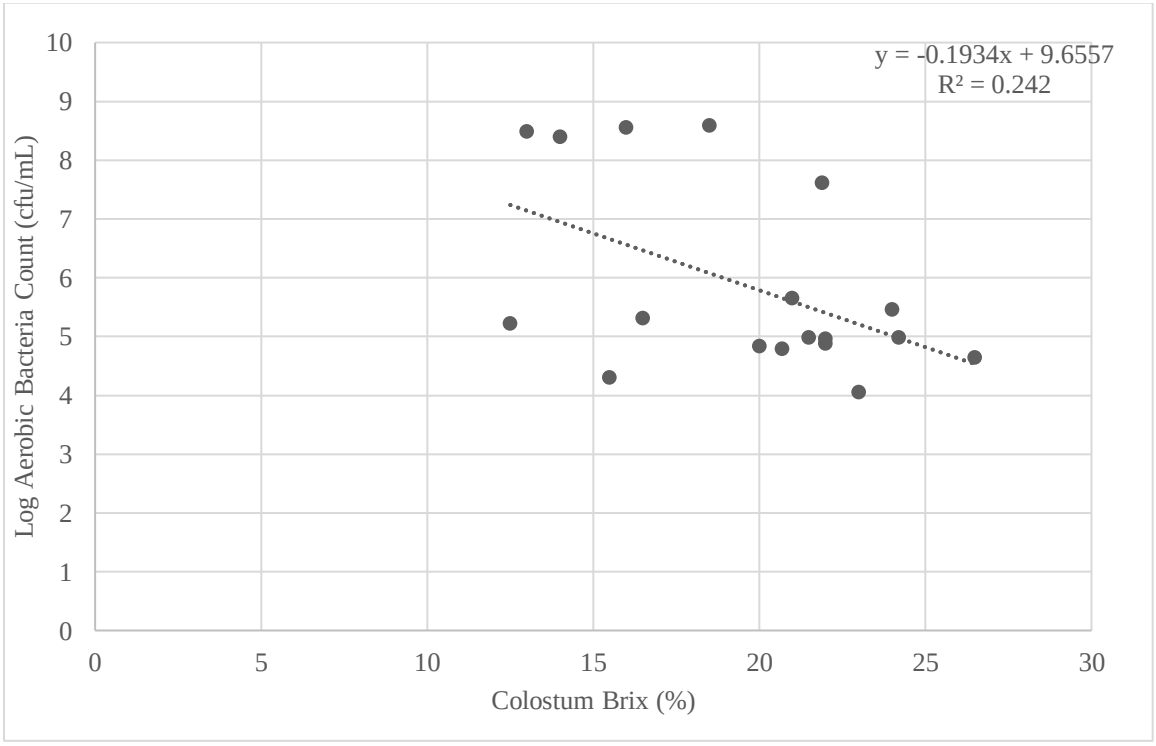
Cow that produced colostrum	Calf that received	Coliform cfu/mL	Colostrum Brix %	Feeding Number
Leena	Lennox	870	20.7	1
Leena	Lennox	34000	20	2
primrose	Phoebe	280	22	1
Dakota/Jubilee	Phoebe	1100000	21.9	2
Dakota	Sadie	3000	21.5	1
Dakota	Sadie	10500	22	2
Leena	Louise	340	15.5	2
Genevieve	Louise	1200	24.2	1
Leena/shadow	Lavender	13900000	13	1
Dakota	Lavender	670000	18.5	2
Genevieve	Leo	3600	23	1
n/a	Dolores	86000	21	2
Lakota	Jemima	8700	12.5	2
Genevieve/shadow	Goose	4600000	14	2
Lily	Linus	1000	24	1
n/a	Linus	390	26.5	2
Jubilee	Jinx	64000	16.5	1
Jubilee	Jinx	137000000	16	2

Table 3. Aerobic bacterial counts and Brix refractometer readings of colostrum fed to calves in the first and second feedings. Highlighted values indicate the cfu/mL is greater than the recommended industry level of 100,000 cfu/mL. Data that has been entered as "n/a" indicates missing values.

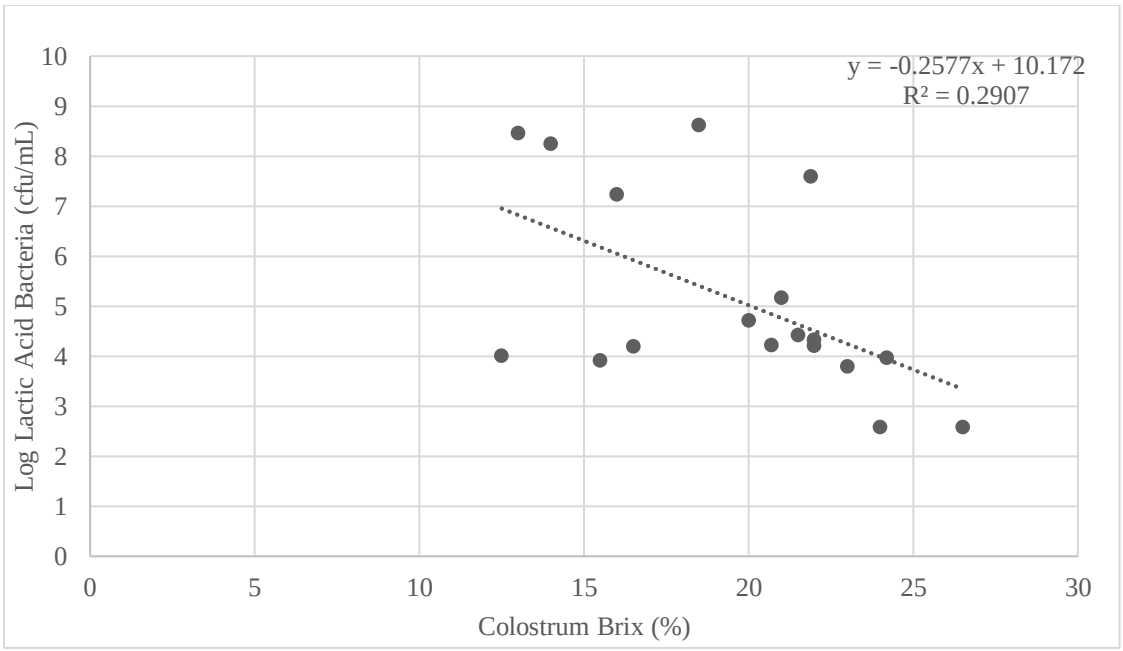
Cow that produced colostrum	Calf that received	Aerobic Count cfu/mL	Colostrum Brix %	Feeding Number
Leena	Lennox	60000	20.7	1
Leena	Lennox	67000	20	2
primrose	phoebe	89000	22	1
Dakota/jubilee	phoebe	40000000	21.9	2
Dakota	Sadie	95000	21.5	1
Dakota	Sadie	74000	22	2
Leena	Louise	19500	15.5	2
Genevieve	Louise	95000	24.2	1
Genevieve	Leo	11000	23	1
Leena/shadow	lavender	300000000	13	1
Dakota	lavender	380000000	18.5	2
n/a	Dolores	440000	21	2
Lakota	Jemima	164000	12.5	2
Genevieve/shadow	goose	244000000	14	2
Lily	Linus	286000	24	1
n/a	Linus	43000	26.5	2
Jubilee	Jinx	199000	16.5	1
Jubilee	Jinx	357000000	16	2


y = -0.2353x + 9.038
R² = 0.3197
Log Coliform Bacteria (CFU/mL)
Colostrum Brix (%)
0
1
2
3
4
5
6
7
8
9
0
5
10
15
20
25
30

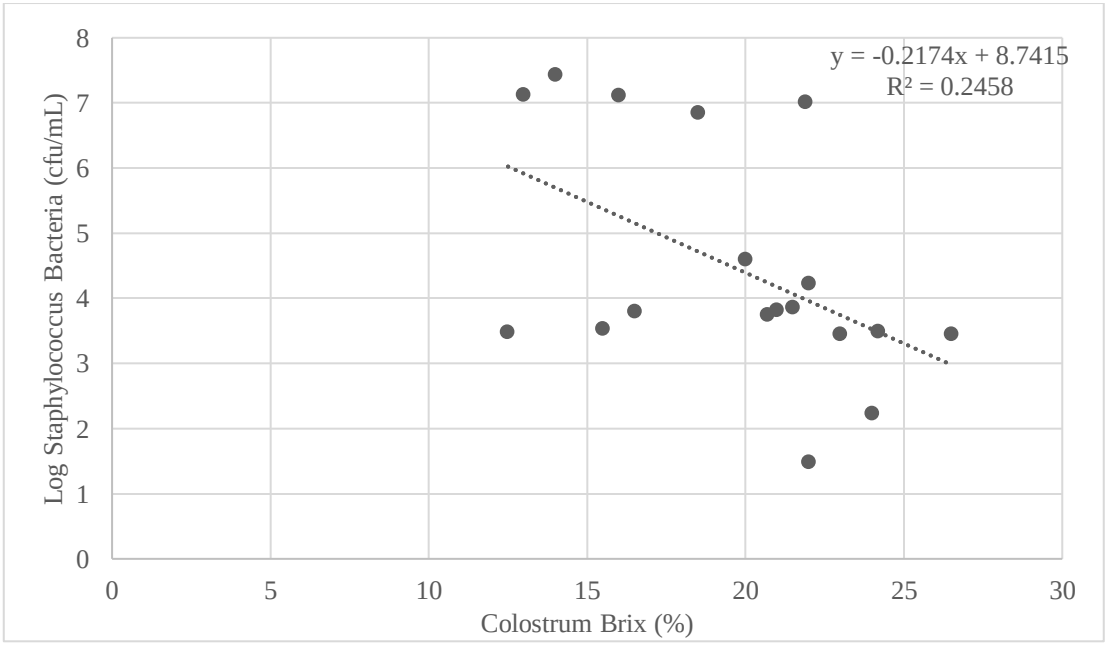
572 A)


y = -0.1934x + 9.6557
R² = 0.242
Log Aerobic Bacteria Count (cfu/mL)
Colostum Brix (%)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30

573 B)



C)



D)

Figure 1. Relationship between the four types of bacterial counts —coliform bacteria count (A), aerobic bacteria count (B), lactic acid bacteria count (C) and *Staphylococcus* bacteria count (D) — and the colostrum Brix value for the colostrum samples fed during both the first and second feedings to the calves.

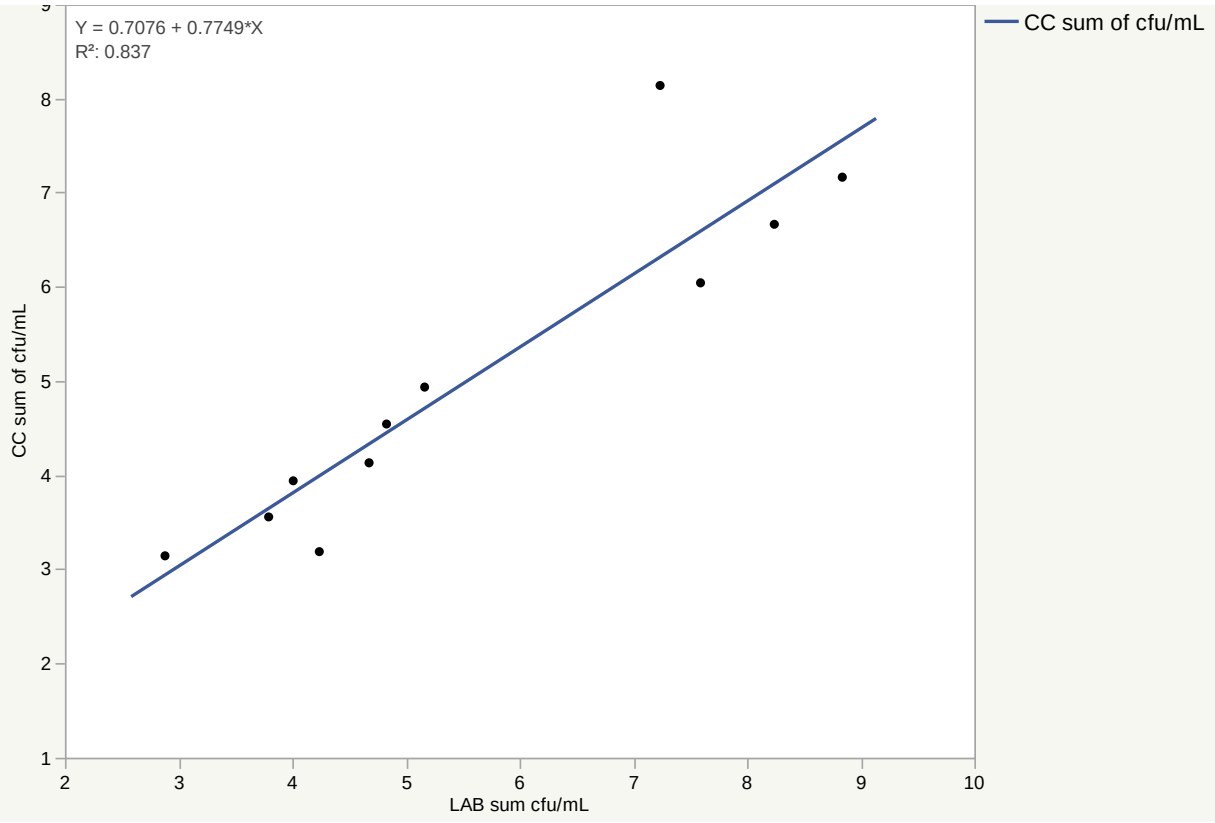


Figure 2. Relationship between the sum of the coliform bacteria counts (CC) of the first and second feeding and the sum of the lactic acid bacteria (LAB) counts of the first and second feeding.

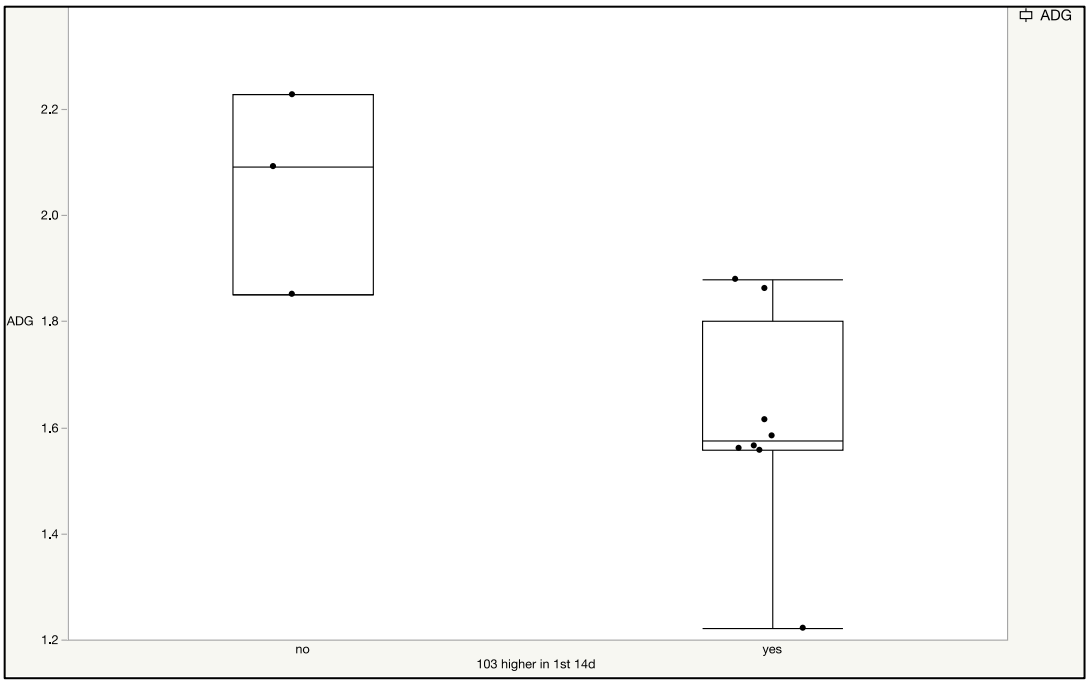


Figure 3. Relationship between a calf developing a fever (rectal temperature above 103°F) within the first 14 days of life and average daily gain (ADG) p=0.0092. Calves who developed a fever during the first 14 days of life had a decreased ADG.

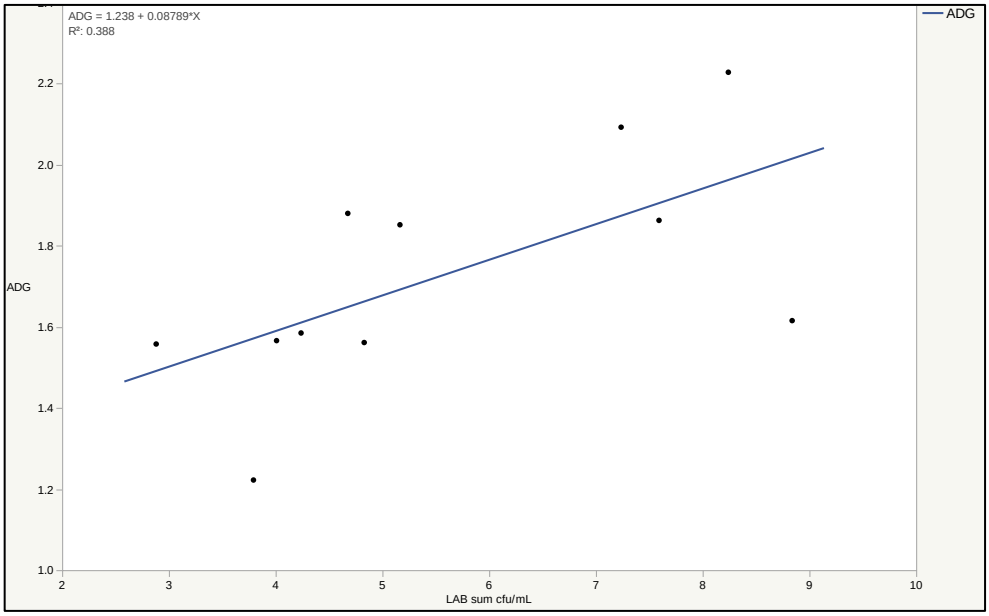
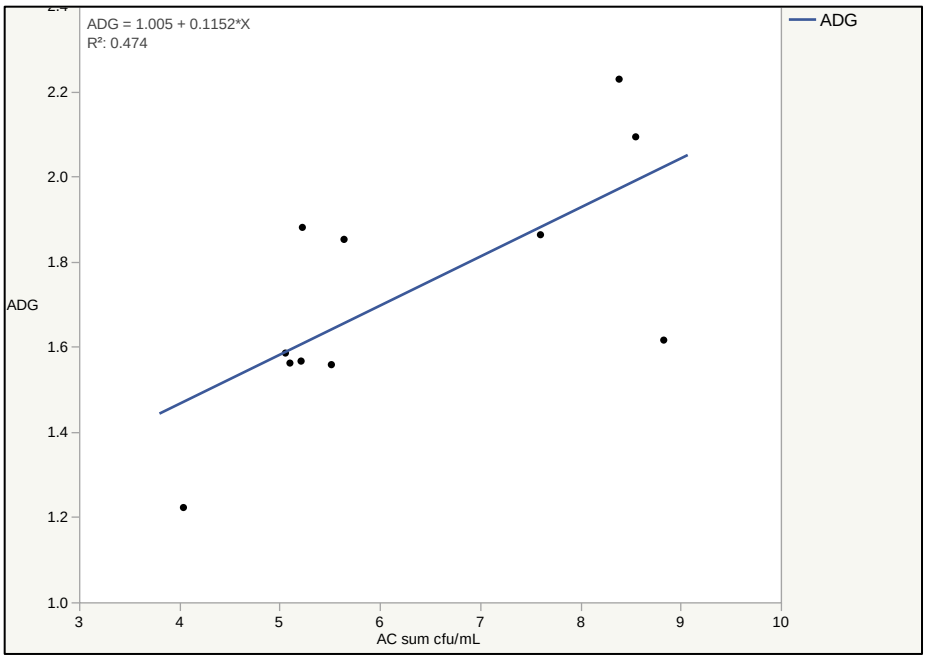


Figure 4. Relationship between the average daily gain (ADG) of the calves and the sum of the lactic acid bacteria (LAB) present within the colostrum fed during the first and second feedings.

A.



B.

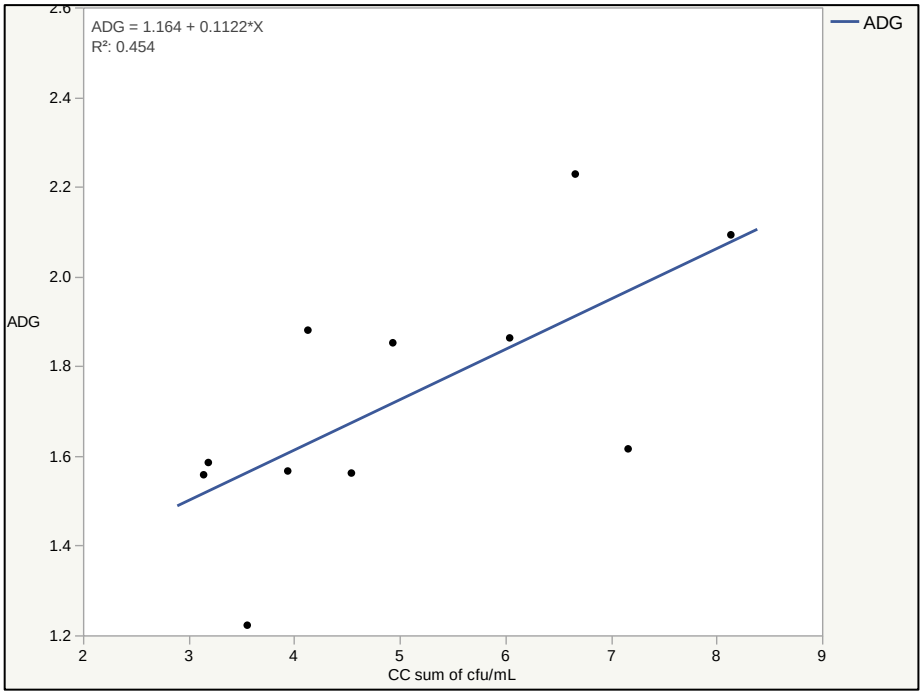


Figure 5. Relationship between the average daily gain (ADG) of the calves and the sum of the aerobic count (AC) bacteria present in both the colostrum from the first and second feedings (A) and the relationship between the average daily gain (ADG) of the calf and the sum of the coliform (CC) bacteria present within the colostrum from the first and second feedings (B).