Alternative Feeding Practices for Dairy Calves and Beta-Casein Genotype and Its Effect on Dairy Cows

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ABSTRACT

These studies were conducted to compare how different genotypes and breed groups affect dairy calf and cow management. Specific focus was put on alternative management practices or niche markets that dairy producers face today. The objective of the first study was to compare crossbred dairy calves to Holstein calves for growth and milk consumption behaviors while being fed alternative milk allowances in an automated group feeding system. Calves fed *ad libitum* milk exhibited an increased growth rate and weaning weight without compromising the health of the calves, compared to calves fed 8 L. Breed groups exhibited no difference in weaning weight or health scores. Limousin crossbred calves exhibited a lower milk consumption, but increased growth rate compared to the other breed groups. The results of this study indicate feeding dairy calves *ad libitum* may increase milk cost but could be economically advantageous if increased growth rates are also realized. The objective of the second study was to compare the beta-casein genotype impact on production, fertility, and survival amongst purebred certified-organic Holstein cows. Herd had an effect on the production, fertility, and survival of the cows. Parity effected times bred, days open, and all production traits. The beta-casein genotype of the cows and the herd affected the percentage of cows that survived to the start of the subsequent lactation. This study indicated beta-casein genotype had no effect on the fertility and production of organic dairy herds. Meanwhile, survival may be biased against the A1A1 genotype as shown by lower survival rates to each lactation. This study may offer organic producers more flexibility in breeding programs and culling decisions to produce A2 milk. The results of both studies offer producers more insight into new management strategies to achieve their on-farm goals without compromising genetic or monetary input.

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CHAPTER 1. INTRODUCTION

Dairy calf care is an important aspect of dairy producer management. The loss of a calf means a loss in potential income (Henderson et al., 2011). It is important for dairy producers to understand and provide optimal conditions to raise calves efficiently and effectively. Traits such as growth, milk consumption behaviors, health and personality of the calf play an important role in calf care and management (Neave et al., 2017). Utilizing technology, such as an automated feeding system (**AFS**), may assist producers by off-setting labor input as well as collecting datapoints to track the calf's progress within the first few months of age.

A2 milk contains only the β-casein protein homozygous A2A2 allele and is gaining popularity in the dairy industry due to its potential to decrease the likelihood of milk intolerance and various health risks in humans (Laugesen et al., 2003). This viewpoint is still being debated as research remains in the early stages. The A1 and A2 alleles were first exclusively studied in the 1960's while scientists were evaluating the causes of Type-1 diabetes (Laugesen et al., 2003). Companies such as a2 Milk™ Company in New Zealand have created a niche market in the dairy industry by producing and marketing A2 exclusive milk to consumers (Mayer et al., 2021). Animal scientists are also interested in the effects of the A1 and A2 alleles on cow health, reproduction, and milk production.

AUTOMATIC FEEDING SYSTEMS FOR CALVES

Automated feeding systems for calves are gaining in popularity in the dairy industry to improve farm management and efficiency and allow for multiple feedings to each calf (Medrano-Galarza et al., 2018c). Automated feeders were first developed in Germany in the 1980s and were imported in Sweden in 1988 (Hepola, 2003). Since that time, AFS usage has slowly increased among dairy producers, even while most dairy calves are still housed individually. The AFS allow for calves to be housed in a group with the average group size ranging from 20 to 35 calves and the average age range typically within 14 days (Hepola, 2003; Jorgensen et al., 2017c). With the implementation of an AFS, calves can be fed multiple, small portions of milk throughout a 24-hour period at the optimum temperature and milk allowance per calf that can be personalized to fit the producer and calf's needs (Hepola, 2003). The AFS utilize an electronic transponder that is attached to the calf, commonly in the form of an ear tag, which allows the AFS to track the calf during each feeding. The technology and software for AFS has also developed over the years. The AFS are now able to measure milk consumption traits, time spent at the feeder, drinking speed, the number of rewarded visits to the feeder (calf is allowed milk upon entry to the AFS), unrewarded visits (calf is not allowed milk upon entry), and drinking speed; and collect calf weights (Conboy et al., 2021; Hepola, 2003).

Perception

The practice of individually housing calves has received public criticism over the past years for restricting movement of the calves and decreasing social interaction among calves (Jorgensen et al., 2017a). Many dairy producers are shifting to grouping their calves which has consequently led to some producers also implementing an AFS. Medrano-Galarza et al. (2018b) noted that, in North America, producers adopted the AFS due to its ability to create a more flexible work schedule and reduced the cost of labor. This study also distributed a questionnaire to Canadian dairy farms. From those results, the top factors that motivated producers to switch from a manual feeding system (**MFS**), farm personnel taking milk to each calf at set times each day, to an AFS were to “raise better calves,” offer a higher milk allowance per calf, decrease labor input and improve overall working conditions. Other responses included allowance of multiple feedings per day per calf, consistency of feeding quantity and quality, reduced stress during the weaning period, and better data management. Meanwhile, producers who were using a MFS reported choosing to stay with that system due to the high economic investment that would be required to change to an AFS, or the producers came from a small farm where it would not be efficient to use an AFS. These same producers also commented they wanted the “direct contact” with the calves at each feeding, perceived a decreased disease transmission between calves that were housed individually, or other factors such as family roles on the farm, were content with their feeding practices as is, or had previously tried an AFS and reverted to manual feeding.

In a Canadian questionnaire (Medrano-Galarza et al., 2018b), producers who used an AFS, were also asked to list their perceived advantages of having an AFS. The top advantages listed were allowing calves to express their natural behavior, ability to increase calf milk allowance while simultaneously decreasing labor, decreased cost of milk compared to MFS, improved working conditions and workers allocating less time for equipment cleaning compared to a MFS. Interestingly, another perceived advantage of an AFS from the Canadian questionnaire was earlier detection of illness through the AFS. However, producers who had a MFS indicated they could detect illness faster because of their increased direct contact with the calves. The MFS producers also noted their perceived advantages of the MFS were no competition among calves for milk, low capital cost of feeding milk by hand, “fast and easy” cleaning of buckets, and easier measurement of the weight of calves housed individually compared to group-housed calves. Conboy et al. (2021) also reported that an advantage to utilizing an AFS for calf rearing on a dairy farm is its ability to make labor input more efficient by offsetting some of the duties commonly seen with calf rearing such as automatically preparing and feeding the milk or milk replacer for the worker.

The Medrano-Galarza et al. (2018b) study also asked producers to list their top perceived disadvantages for their respective feeding system. Forty percent of producers who used AFS reported “less direct contact” with the calves as the top disadvantage of the system. Other disadvantages listed by producers included concerns about the cleanliness of the system, cross-sucking issues amongst calves, disease transmission, and increased calf training on the AFS. Most producers using MFS listed their top disadvantage of MFS as the difficulty to increase feeding frequency per day with the second most common disadvantage being the “unpleasantries” of feeding calves during the colder times of the year. Other less common disadvantages listed were concerns about consistency of feeding practices when multiple workers rotated feeding schedules, milk temperature, extra physical labor required, lack of calf socialization, restricted movement, and difficulty training calves to drink from a bucket.

Impact on Farm

Calf mortality is a crucial issue on dairy farms because this can impact not only the economics of the farm but also genetic gain on farm. Jorgensen et al. (2017b) conducted a study to investigate on-farm factors that may affect pre-weaned calf mortality rates. In 2017, the US calf mortality rate was between 6-8% which included all housing types. It was reported that the annual mortality rate on the farms using AFS in the study was 2.6%

with 13% of the farms reporting over 7% mortality and 57% reporting under 3% mortality. The authors concluded that this lowered mortality rate on AFS farms shows that a lower calf loss can be achieved using AFS. The authors also cautioned, however, that this may not be the case on every farm. Looking further, the study found a positive relationship between the age range of the calves housed in groups and the farm mortality rate. This would conclude that a wider age range between calves in groups could be detrimental to calf survivability. The majority of dairy producers limit age range from zero to 21 days between the youngest and oldest calves in a group (Jorgensen et al., 2017b).

Labor input is another prominent issue amongst dairy farms, i.e., lack of personnel or the amount of time needed for calf rearing. Sinnott et al. (2021) conducted a study comparing the differences in management styles while using either an AFS or a MFS. The study found that labor input was greater (+00:15:12 min/pen/day) for MFS than for AFS. Most of the extra time was attributed to time spent on milk inspections and preparations. However, the AFS required more labor time, in the study, for health inspections and calf training.

Calf health can also be affected by the feeding system. Sinnott et al. (2021) compared calf health traits to the type of feeding system being used. When compared to the calves in an AFS, MFS calves were more likely to score greater than zero for fecal appearance. Interestingly, the study found no interaction between the feeding system used and the breed of the calf or the number of days taken to achieve the target weaning weight. Similarly, Kung et al. (1997) conducted a study between calves reared individually in outdoor hutches and fed from a bucket versus calves reared in groups, in a three-sided barn and fed via an AFS. This study also found no significant differences in the growth rate between the two calf rearing methods.

AFS Capabilities

The AFS are known for their technological abilities that allow one machine to complete several tasks, simultaneously, to simplify the calf rearing process for dairy producers. Automatic feeding systems help aide in feeding customizable volumes of milk via a teat, as well as customizable feeding access to the calf dependent upon the parameters set by the dairy producer (Whalin et al., 2022). These systems can also help facilitate the weaning process by automatically decreasing either the amount or feeding schedule granted to each calf by the parameters set in the system (Schäff et al., 2018). Jensen (2006) investigated the effects of milk allowance and weaning method on calves fed via an AFS. The study concluded that increased levels of milk feeding (8 L/d) were shown to improve the efficiency of the AFS because it reduced the number of visits by calves to the AFS.

Another technological ability that can be added to an AFS is the use of a weight scale. Attaching a weight scale to an AFS reduces the amount of manual labor needed and can mitigate human error when weighing calves via a weight tape (Cantor et al., 2020). This study validated the use of a partial-weight scale that was attached to an AFS. There was a high precision from the scale with a high correlation coefficient when comparing the partial-weight scale to an electronic scale. A low bias was also calculated based off accuracy and precision analyses.

The AFS also serves as a central point of contact with calves and can be programmed to gather biological markers on calves. An example of this is the continual identification of traits shown in sick calves that can be recorded with an AFS to help identify when a calf is sick and appropriately notify the dairy producer. A trait associated with calf sickness is drinking speed which was shown to be the most sensitive parameter to identify calves with a respiratory or enteric disease faster than detection by the producer (Knauer et al., 2018). Farms that reported using drinking speed as an identification factor of calf sickness also had a lower annual mortality rate than those farms who did not use drinking speed as a disease identification trait (Jorgensen et al., 2017b). Milk allowance has been shown to be a confounding factor when identifying traits exhibited by sick calves. For instance, calves fed a low milk allowance (5 L/d) were more accurately identified as being sick based on fewer lying bouts, decreased lying time and number of rewarded visits to the feeder (Lowe et al., 2021). Sick calves fed a high milk allowance (10 L/d) were better detected via total visits to the feeder and lying behaviors. Jorgensen et al. (2017c) reported the majority (77.4%) of the dairy producers surveyed, used the percentage of milk consumed relative to the amount allotted as their primary identifier with drinking speed as the second most common trait (55.9%).

Maintenance

Medrano-Galarza et al. (2018c) conducted a study regarding management practices and prevalence of calf disease within pen using AFS. The study did not find an association between bacteria counts with cleaning practices but there was an association with cleaning frequency and calf diarrhea. When running the AFS automatic cleaning system three times per day, there was a reduction in the prevalence of calf diarrhea compared to running it fewer than 3 times per day. The labor input of cleaning and maintenance of the AFS and the pen in which the calves were housed was found to be significantly less than on farms that used MFS (Sinnott et al., 2021). This proportion was attributed to the amount of time needed to clean each individual pen per calf along with the feeders or buckets used. For pens with an AFS, only pen cleaning was required because the AFS was automatically cleaned via computer controls.

CALF FEEDING BEHAVIORS

Behavior of Calves on an AFS

The age of a calf plays a role in how successful and efficient an AFS can be. A study showed that calves that were introduced at six days old had 2.3 times greater odds of needing more training on an AFS, spent less time at the feeder and consumed less milk than calves who were introduced at 14 days of age (Medrano-Galarza et al., 2018a). Medrano-Galarza et al. (2018a) conducted a similar study comparing calves who were introduced to the AFS either immediately after their second colostrum feeding or after five days of age. Calves that were introduced very early required a greater number of assisted visits to the AFS than calves introduced later. This study also showed that birth weight tended to be associated with the number of assisted visits. For every kilogram that birth weight increased, the odds of requiring an assisted visit decreased by almost an entire visit. Another study also showed that heavier calves introduced to the AFS at six days of age had a shorter latency to their first unassisted meal (Fujiwara et al., 2014). This would suggest that age and weight should be considered when developing protocols for the time of introduction to an AFS.

Some studies have researched baseline behaviors of calves fed with AFS. Conboy et al. (2021) showed that calves spent on average, 40 minutes per day (range: 3 to 237 minutes) at the AFS. This study also reported that calves had, on average, 5 rewarded visits to the AFS per day (range: 1 to 29) and averaged 7 unrewarded visits per day (range: 0 to 81). Jensen (2004) showed that greater competition at the feeder increased the average drinking speed. Between groups of 24 or 12 calves with one AFS, the duration of rewarded visits was shorter in the larger group which caused the drinking speed to increase from calves in the group of 24 versus those with 12 calves per pen. It was also reported by Kung et al. (1997) that calves housed in a group pen with an AFS had fewer medicated days than calves housed individually in hutches and fed with buckets.

Impact of Milk Allowance

Prior to weaning, calves are fed whole milk or milk replacer, typically, for the first two months of age, at a total intake of 8 to 15% of the calf's body weight (Borderas et al., 2009b; Morrison et al., 2012). Historically, calves were recommended to be fed 4 liters (**L**) per day in two feedings at 2 L per feeding. Today, with more research available, producers are realizing that this limited milk allowance has underestimated the nutritional requirement of calves (Borderas et al., 2009b). Producers have now started to offer calves up to *ad libitum* milk allowance per feeding, offering the calf as much milk as it will naturally drink each day. Studies have shown that calves fed higher amounts of milk have increased growth rates compared to those calves that are fed with a lower milk allowance (Borderas et al., 2009b; Schäff et al., 2018). Borderas et al. (2009b) found that *ad libitum* access calves had a greater average meal size (2.68 L/meal) than calves fed a limited access allowance (1.72 L/meal). The average daily gain (**ADG**) of *ad libitum* access calves was also greater (0.80kg/d) compared to limited access calves (0.66 kg/d). Milk allowance can affect the behavior exhibited by calves as well. de Paula Vieira et al.

(2008) showed that calves fed restricted quantities of milk consumed the milk in two rewarded visits per day versus calves given *ad libitum* allowance that consumed milk in five rewarded visits per day. Milk restricted calves also had 12-times more unrewarded visits than *ad libitum* access calves. Calves fed restricted quantities of milk also stood one hour (**h**) longer per day than *ad libitum* access calves (6.4 h/d versus 5.5 h/d, respectively). Calves also have a greater energy expenditure when standing compared to lying down. Therefore, increased lying time, as exhibited by calves fed a greater milk allowance could improve the energy balance of calves and potentially the energy balance as a breeding heifer (Borderas et al., 2009b). Increased growth has also been shown to have long-lasting effects on health and mature cow milk production (Schäff et al., 2018). Soberon et al. (2012) noted that *ad libitum* feeding of calves increased milk yield during the first lactation compared to calves fed restricted feedings. This study reaffirmed that first-lactation milk yield was positively correlated with preweaning ADG and weaning weight. It was also reported that consequent cow milk production was positively correlated with ADG from birth to breeding and from weaning to breeding.

Past studies have suggested that increased milk allowance can lead to a decreased solid feed intake at weaning which can consequently decrease the growth rate of calves after weaning (Weary et al., 2008). Sinnott et al. (2021) noted that past research showed the speed milk will move through the digestive tract is stimulated by the frequency milk is being consumed, meaning milk empties at a faster rate in the abomasum to allow for new matter to enter. Feeding a greater volume of milk, therefore, would remain in the calf's abomasum longer which can decrease the frequency of abomasum emptying and consequently lead to an increased rate of digestive disorders. Therefore, the study suggests using AFS programming to decrease the portion size being consumed for calves that are allowed a greater milk allowance per day to counteract this problem.

Effect of Calf Personality

A calf's personality, or how they exhibit certain behaviors, can affect milk intake and consequent growth. For example, calves with lower vitality have a lower milk intake and consequent lower weight gain by 14 days of age and take longer to be trained on an AFS (Neave et al., 2019). Calves that have a calmer disposition, generally have better growth rates, improved immunity and decreased physiological responses to stressful events as calves and into maturity, as well as an increased milk production and meat quality as a mature cow (Neave et al., 2017).

To categorize possible personalities associated with calf behavior and growth, Neave et al. (2019) created five different personality factors to explain some of these differences. The factor categories were as follows: factor 1 (low vitality), factor 2 (fearful), factor 3 (strong drinker, fully consumed their first meal), factor 4 (slow learner), and factor 5 (exploratory-active). Factors 1 and 3 were negatively associated with total rewarded visits whereas factor 5 calves were positively associated with these measures. Factor 2 calves could be weaned at an earlier age and had reduced drinking speeds prior to weaning. However, factor 4 calves tended to wean later and had fewer total rewarded visits to the AFS, reduced drinking speed, and fewer unrewarded visits to the AFS. Calves that exhibited low vitality (factor 1) were associated as also exhibiting a reduced preweaning milk intake which led to reduced preweaning weight gains. For those calves with a strong drinking personality (factor 3), there was no association with weaning age, feed intake, or growth. These calves did however have fewer visits to the AFS and reduced weight gain during weaning which the authors note could be attributed to greater calf distress during the weaning process. Slow learners (factor 4) also exhibited behaviors such as reduced milk intake, drinking speed, and growth which could be consequent of “less persistence” in attempts to drink from the AFS and a reduced motivation to drink. Factor 2 calves (fearful) were not associated with any measures related to solid feed intake. These calves were more attentive to changes in their environment which may have attributed to their earlier weaning age. Factor 5 calves (exploratory-active) were also not associated with solid feed intake but did have an improved weight gain and greater final weight at weaning. However, this improvement in growth was not associated with increased milk or starter intake which, like factor 2 calves, could indicate these calves coped better during the weaning period. Whalin et al. (2022) conducted a similar study using three factor categories to describe calf behavior and personality. In this study, factor 1 calves were playful and exploratory, factor 2 calves were vocal and active, and factor 3 calves were interactive during a group test. These three factors explained 56% of the variance exhibited for feeding behaviors of the calves in the study. During the study, factor 1 calves had a higher milk intake preweaning per day and higher concentrate intake per day during the study and during and after the weaning period. Factor 1 calves also tended to have a faster drinking speed. Factor 2 calves had a lower preweaning milk intake per day and lower concentrate intake as well as a lower drinking speed. Factor 3 calves had a lower concentrate intake per day. This study did not find an association with any growth measures.

Calf Response to Disease

Sick calves will exhibit different feeding behaviors than their healthy counterparts, which can be detected by AFS and be programmed to alert the dairy producer of such events. In general, sick calves will have reduced feed and milk intake, slower drinking speed, and fewer unrewarded visits to the AFS (Borderas, Rushen, et al., 2009b; J. Morrison et al., 2021). On the peak day of an illness occurrence, sick calves had a significantly lower total milk intake however, the average milk consumption per visit was the same as healthy calves (Duthie et al., 2021). Additionally, sick calves consumed less total milk the day before the disease was detected, 2 days after the disease was detected and over the six days surrounding the diseased time. Sick calves drank 0.84 L less total milk than healthy calves. J. L. Morrison et al. (2022) found similar results where sick calves drank 2.16 L/d less than healthy calves. Diseased calves also drank less milk than healthy calves on days 5 to 1 before the dairy producer delivered treatment. Duthie et al. (2021) continued their discussion reporting that, compared to healthy calves, on the peak day of illness, sick calves had a lower total feed time and lower total feed time when milk was given than healthy calves. Consequently, in the 6 days surrounding the disease event, sick calves had 1.60 minutes less total feed time and 1.95 minutes less total feed time when milk was given compared to healthy calves. Similar results were also seen in a study where sick calves drank slower than healthy calves and specifically drank slower on day 4 (-129.70mL/min), day 3 (-179.31 mL/min), day 2 (-117.93 mL/min) and day 1 (-163.75 mL/min) before the producer delivered treatment and 158.4 mL/min slower on the day of disease diagnosis (J. L. Morrison et al., 2022). The study also found that the number of unrewarded visits to the AFS was lower in sick calves compared to healthy calves and specifically on day 7 (-2.276 visits/d), day 5 (-2.362 visits/d), day 3 (-3.236 visits/d), day 2 (-2.613 visits/d), and day 1 (-4.102 visits/d) before the producer delivered treatment. After treatment was administered, sick calves continued to have fewer unrewarded visits to the AFS on days 2, 4, 5, and 6. The number of rewarded visits was not different between the healthy and sick calves during the study, providing evidence that the number of rewarded visits would not be a valid tool in detecting disease because sick calves will typically still be motivated to drink, but they will only access the AFS a few times a day to eat compared to when they are healthy.

Milk allowance through an AFS influences the type of behaviors shown in sick calves. Sick calves fed a high milk allowance will access the AFS less, have a reduced milk intake, and spend an increased average time at the AFS the days before and on the day the illness was detected by the dairy producer (d -2 to 0) (Borderas et al., 2009b). After a disease was detected, high milk allowance (*ad libitum* or 12 L/d) calves that were sick continued to have a decreased milk intake, fewer total visits to the feeder, and shorter duration of visits to the AFS. Low milk allowance (4 L/d) calves exhibited no differences in the amount of milk consumed or in the frequency of visits to the AFS both before and after illness was detected by the producer. However, there was a reduction in the amount of time while visiting the AFS on the day the illness was detected and on the three days following detection.

The type of disease contracted by the calf can also affect a calf’s milk consumption behavior. For example, calves with Bovine Respiratory Disease (**BRD**) consumed less milk, had greater lying time with fewer lying bouts, and reduced step activity during the pre-diagnosis period (Cantor & Costa, 2022). A significant interaction between BRD status and day for changes in unrewarded visits before diagnosis was also shown with BRD positive calves deviating from their respective baseline behavior on day

4, day 2, and day 1 pre-diagnosis and on day 0, day the calf was diagnosed with BRD, when compared to healthy calves. Another study compared two of the most common calf diseases, BRD and neonatal calf diarrhea (**NCD**) (Conboy et al., 2021). This study showed that drinking speed was lower in calves with these diseases where BRD calves drank 152.6 mL and NCD calves drank 92.4 mL less per minute compared to healthy calves. Specifically, it was also shown that calves with BRD were associated with two fewer unrewarded visits to the AFS than calves without BRD.

CALF GROWTH

Milk Allowance Effect on Body and Organ Growth

It has been shown that increasing the milk allowance for a calf subsequently increases their body weight at weaning time. What is not yet known is how milk allowance affects the calf in terms of organ and body growth that consequently causes an increased weight. Schäff et al. (2018) reported that body and organ growth overall is regulated by the somatotropic axis which regulates the metabolism of mammals. By increasing the quality of milk feeding, this axis is stimulated in the pre-weaned calf. However, the calf's insulin-like growth factors (**IGF**) and insulin system also affect gastrointestinal (**GI**) development and growth, whereas these systems are controlled by nutritional factors in ruminating cows. Therefore, this study researched if milk allowance would affect the gene expression of the IGF and insulin system of the pre-ruminant calf. At the time of slaughter, body weight and carcass weight were greater in calves fed an *ad libitum* milk allowance compared to a restricted milk allowance. However, the weights of the rumen, omasum, abomasum and small intestine as well as the length of the small intestine were not affected by the milk allowance. There was also no effect on body and organ weight with breed or sex of the calf. This would lead to the assumption that milk allowance only affects body growth rather than intestinal growth.

Calf Body Weight

Calf body weight (**BW**) is an important trait that could add economic value to the calf in later years from carcass value and production efficiency (Yin & König, 2018). Weaning weight (**WW**) is another important weight benchmark that can be used to estimate calf stayability in the herd. In a study by de Passillé et al. (2012), weaning began when calves consumed a target weight of starter/d. The weight gain of calves from 20 days of age to 87 and their BW at 87 days of age was negatively correlated with the age a calf started and ended the weaning period but positively correlated with the length of time taken to wean. Conversely, van de Stroet et al. (2016) found no significant difference between calf BW and the chance of the heifer remaining in the herd until first lactation. Meanwhile, Henderson et al. (2011) reported WW class of calves had the greatest impact on mortality risk. Heifers with a WW between 34 to 50 kg had 3.17 times greater relative mortality risk than heifers with a WW between 60 to 68 kg, while heavier heifers, weighing between 81 to 126 kg at weaning, had 40% greater survivability rate than heifers in the average WW class. Therefore, heifers with a higher WW have a greater chance of surviving to maturity than lighter heifers. Similarly, van de Stroet et al. (2016) reported odds of survival to first lactation were 3.4:1 and 2.4:1 in the high growth rate group of calves when compared to calves from the low and medium growth rate groups, respectively.

The impact of calf weight also continues into the mature cow. Cows that were categorized as having a high growth rate as calves were 29.8 ± 8.3 kg heavier as cows, than those who were categorized in the low growth rate as calves. Likewise, cows from the medium and high calf BW weighed 22.4 ± 8.7 and 21.1 ± 8.5 kg, respectively, more than cows from the low calf BW category.

Milk production can vary and be impacted by calf BW. van de Stroet et al. (2016) did not find any significant associations between calf BW, growth rate and starter feed intake with the first lactation milk yield. However, cows that were classified as medium BW as calves, produced 2.2 ± 0.9 kg of milk/d more than cows categorized as high BW as calves during the first 10 weeks of lactation, across all lactations. Beam et al. (2015) showed similar results. Within the first 5 weeks of lactation, cows that were categorized as medium weight calves produced 5 kg more milk than light calves and 5.6 kg more than heavy weight calves. Within weeks 6 to 10 of lactation, medium weight calves produced 3.8 kg more milk as cows than light calves and 4.6 kg more milk than heavy calves. No differences were seen between weeks 10 to 20 of lactation.

Yin et al. (2018) researched the direct heritability of different calf BW traits. In the study, calf BW had a 0.47 direct heritability which was reported as similar to previous studies. The maternal heritability was 0.19 which was larger than in other studies. The direct and maternal heritability for BW at first insemination was smaller compared to calf BW (0.20 and 0.06, respectively). A decrease in the heritability between birth and weaning age was also seen in Coffey et al. (2006). Yin et al. (2018) also found low, negative genetic correlations between calf BW and the fat percentage and fat to protein ratio on test days. A high fat to protein ratio can indicate a negative energy balance in a lactating cow. A negative genetic correlation between calf BW and the fat to protein ratio would suggest that calves with a larger BW tend to prevent an energy deficit early on during lactation. It should be cautioned that this correlation was low so the magnitude of this effect may not be seen immediately. Positive and larger than 0.1 genetic correlations were also seen between calf BW and general disease status, respiratory disease, mastitis, claw disorders, and female fertility disorders. This would suggest that heavier calves have a higher risk for disease later in life, regardless of health status as a calf. There was a small, negative genetic correlation (-0.09) between calf BW and metabolic disorders, meaning heavier calves are less likely to develop any metabolic disorders during maturation.

Calf Average Daily Gain

In general, colostrum quality, feeding and housing practices, nutrient intake, climate, and disease can affect preweaning ADG (Shivley et al., 2018). Typically, calf ADG will be highest in calves with a high growth rate (van de Stroet et al., 2016). A study also reported that calves with greater milk allowance grew faster within the first 28 days of age but no difference was detected from days 28 to 56 (S. J. Morrison et al., 2012). The social environment and personality of the calf can also affect ADG. For example, drinking speed and a fearful personality were negatively associated with weight gain in calves (Gilbert et al., 2017). The ADG of a calf can also have an impact on later cow production. Beam et al. (2015) reported that heifer calf ADG was related to mature cow body weight. Specifically, it was noted that calves that had a low growth rate had lighter mature body weights as cows compared to those in the intermediate and high growth rate groups (45.3 kg and 52.7 kg, respectively). A similar study was conducted by Soberon et al. (2012) to study calf ADG and its correlating effect on later cow production. ADG was positively correlated with milk production from the birth of the calf to first breeding and from the weaning period to first breeding. The ADG in these two time periods were also highly correlated with each other (correlation coefficient = 0.94). The total days in milk (**TDM**) for the cows in the study was also analyzed with preweaning ADG and with every increase in ADG (kg), first lactation cows produced 850 kg more milk. The study continued to analyze TDM and the effect between the sire, dam, and individual calf's predicted transmitting ability and found no significant relationships between the animal predicted transmitting ability and TDM. This led the authors to conclude that an increase in milk yield was caused by environmental effects and had an equal effect on individuals with high or low genetic merit. When looking at 305-d first-lactation milk yields, as preweaning ADG increased, 305-d milk yield increased by 704 kg. This effect was also linear which leads to the assumption that the greater the ADG before weaning, the greater the potential first-lactation milk response could be.

Calf Hip Height

Calf hip height is an important measure of calf frame. Hip height has a positive effect on cow lactation milk yield (Beam et al., 2015; van de Stroet et al., 2016). In the first five weeks of lactation, calves with a low hip height produced 4.2 kg or 6.0 kg of milk less than calves in the intermediate and high hip height groups, respectively (Beam et al., 2015). There was no difference in milk yield between hip height groups during the tenth to twentieth weeks of lactation. However, another study found a difference in additional milk yield between medium and high calf hip height groups compared to the low calf hip height group during weeks 11 to 20 of the first lactation (2.7 ± 0.9 kg and 3.2 ± 1.0 kg more milk/d, respectively) (van de Stroet et al., 2016). No difference was shown in weeks 21 to 40 of the first lactation. In total 305-d first lactation milk yields, calves from the medium and high hip height groups had 782.1 ± 260.3 and 722.9 ± 260.3 kg, respectively, more milk than heifers that were from the low hip height group. Across all lactations, in weeks one through ten of lactation, calves from the medium and high hip height had 3.8 ± 1.1 kg and 3.5 ± 1.2 kg of milk/d more than calves from the low hip height. During weeks 11 through 20 of lactation, cows from the medium and high calf hip height groups had 3.4 ± 1.1 kg and 3.4 ± 1.2 kg of milk/d more than cows from the low calf hip height group. Total 305-d lactation yields were 699.44 kg of milk greater in cows from the medium calf hip height group than the low calf hip height group. Cows from the high calf hip height group tended to produce 715.6 ± 324.7 kg more milk than cows from the low calf hip height group. Heifer stayability in the herd to first lactation can also be affected by hip height. Of low, medium and high calf hip height groupings, medium hip height calves were the most likely to survive to the first lactation. Respectively, the odds of survival to first lactation for low, medium, and high hip height calves were 3.8 times, 10.7 times, and 3.4 times greater, respectively. Therefore, the survival odds ratio was 2.8 times greater for medium to low hip height calves and from high hip height to medium hip height 0.3.

Nutrition Effects on Heifer and Cow Fertility Traits

Breeding for replacement heifers is an important aspect of a successful dairy farm. However, the genetic makeup of a cow merely gives a possible upper threshold that could be attained when the cow is put in living conditions that best support those genetic effects. Nutrition is one of these conditions that must be met to reach that genetic potential. S. J. Morrison et al. (2012) studied how calf milk allowance subsequently affected the reproductive cycle and lactation traits of mature cows. The study found that there was a linear tendency with increasing milk allowance on a decreased age when the cow's first oestrus was observed. This did not affect the age or live weight at first calving. Interestingly, while Soberon et al. (2012) saw an increase in milk yield at first lactation for calves fed *ad libitum*, S. J. Morrison et al. (2012) saw no treatment effect on milk yield or milk composition during the cow's first lactation.

Health Effect on Growth

Respiratory infections and GI diseases, specifically bovine viral diarrhea (**BVD**), are the two most common ailments that affect pre-weaned calves and are a leading cause in calf mortality (Mahmoud et al., 2017). Specifically, GI diseases cause the most pre-weaned calf mortality cases and respiratory infections are the leading cause of postweaning calf mortality (Hansen et al., 2003; Henderson et al., 2011). For example, calves that experienced two or more disease occurrences requiring treatment were, at least, 1.2 times more likely to die before weaning than calves that had no disease occurrences pre-weaning. The occurrence of respiratory disease or diarrhea can also significantly decrease heifer growth. Rossini et al. (2004) reported that calves that had an occurrence of a GI disease had a two-fold increase in the probability of contracting a respiratory disease as well. The phenotypic correlation between calf bloat disease, specifically, and respiratory disease is close to zero, however, the genetic correlation was high (Henderson et al., 2011). The heritability for bloat and respiratory disease each are 0.095 and 0.139, respectively. Calf diseases also have an impact on mature cow performance. Research showed that decreased dry matter intake in mature cows was associated with calfhood disease (Mahmoud et al., 2017). Occurrence of disease during calfhood is associated with decreased milk, fat, and protein production at the first two test-days immediately after calving in first lactation cows. Heinrichs et al. (2011) also showed that the number of days a calf had either scours or coughing, during the first four months of age, had a significant negative effect on cow 305-d mature-equivalent and actual milk, fat and protein yield during first lactation.

The impact of respiratory disease during preweaning can have lasting effects as the cow matures. For example, calves that experienced respiratory disease multiple times, had a subsequent higher age at first calving (Mahmoud et al., 2017). Calf mortality rate can also be affected by the occurrence of respiratory disease. Calves who have been treated for pneumonia during the first 3 months of life have a 2.5 times higher probability of dying after 90 days of age compared to calves that were not treated for pneumonia (Waltner-Toews et al., 1986). Respiratory disease is also phenotypically associated with increased dystocia occurrence during a heifers first calving (Warnick et al., 1995).

Calf GI diseases can also have an impact on later cow production. If a calf is treated for diarrhea, it is 2.9 times more likely to calve after 30 months of age than heifers that are not treated for diarrhea (Heinrichs et. al., 2011). Similarly, calves that experience severe diarrhea during 3 to 7 months of age, have an increased likelihood of clinical mastitis as a lactating cow than healthy calves (Hultgren et al., 2009).

CALF GENETICS

Crossbreeding

The biological phenomenon of heterosis is a reason why producers may utilize crossbreeding in their breeding regime. Heterosis refers to the improvement of characteristics such as growth, fertility and yield of the offspring above its parents. Many examples of this can be found in the beef cattle industry. For example, Limousin sires on

Brown Swiss and Alpine Grey dams increased the value of calves by $126 in Italy (Dal Zotto et al., 2009). Another example is found in beef cattle crossbreeding. Angus x Nellore and Caracu x Nellore calves had a greater WW compared to purebred Nellore calves (Favero et al., 2019). Caracu x Nellore calves also had a greater ADG than purebred Nellore calves (Favero et al., 2019). In recent years, terminal crossbreeding dairy dams to beef sires (**BxD**) has been used in the dairy cattle industry to decrease production of replacement heifers and increase the market value of calves using heterosis effects. Beef on dairy crossbred calves are shown to have greater BW, price/kg, and market value than purebreds (Dal Zotto et al., 2009).

Calf Genomics

Little research has been conducted to compare genes that could be considered specific to the different stages of calf development, compared to research conducted on mature cow genetics. Understanding how particular calf traits and genes could impact later cow production and health is important to genomic selection because it offers the potential for early predictors for subsequent cow productivity (Mahmoud et al., 2017). Some genes have been identified that relate to calf growth and development. An example of a gene identified that could have implications on calf characteristics is the zinc finger and BTB domain containing 38 (ZBTB38) gene (Liu et al., 2013). This gene has been shown to regulate the production of IGF-II which consequently affects adult stature in cattle. In this study, it was shown that the single nucleotide polymorphisms (**SNP**) 2323 G > A and 2325 C > T were significantly associated with stature traits in cattle. Eberlein et al. (2009) studied *Bos taurus* chromosome (**BTA**) 6 which contains a subunit that is associated with the fetal growth rate of Charolais cattle. Another study found microsatellites on BTA 2, 6, and 14 which have significant associations with the birth weight of Holstein x Jersey crossbreds (Maltecca et al., 2009). Similarly, Cole et al. (2014) showed several regions on BTA 14 that were associated with increased birth weights. Body size, calving ease, daily gain, and stillbirth have corresponding markers on BTA 14 and 21 in the German Fleckvieh cattle breed as reported by Pausch et al. (2011). Other genes that have been identified correspond to mortality and disease traits. A total of 13.61% of additive genetic variance on the direct effect of pre-weaning mortality rates in calves was explained by SNPs located on BTA 2, 3, 10, 11, 14, 15, 16, 18, and 19 (Marín-Garzón et al., 2021). A gene located on BTA 14 was identified by Casas et al. (2015) that is associated with BVD. Other studies have shown gene identification for other calf traits. For example, Dreher et al. (2019) identified SNPs with the strongest signals associated with calf sucking reflex on BTA 7, 15, and 21. A strong sucking reflex is especially important for colostrum intake immediately after calving in order to supply the calf with the best transfer of passive immunity. Sucking reflex is also important for subsequent feedings by the calf to increase the amount of milk consumed which, in turn, will increase nutrient intake for growth and development. As explained above, certain calf traits can have effects on the mature cow. Therefore, by identifying genes associated with calf development, the subsequent mature cow can have an increased chance of proper development and efficient production.

BETA CASEIN PROTEINS IN MILK

A2 Protein Component in Cow Milk

Milk is an important commodity around the world and the sole purpose of the dairy industry. The composition of milk is what provides nutrients for life and the components needed for dairy biproducts such as yogurt or cheese. Milk consists of “86% water, 4.6% lactose sugar, 3.7% triglycerides, 2.8% milk protein, 0.54% minerals and 3.36% other constituents” (Priyadarshini et al., 2018). Protein is important for the development, maintenance, and repair of body tissues as well as acting as an energy source for the body (Lu et al., 2020). Most milk proteins consist of caseins and whey proteins (95%) and the remainder (5%) consist of peptones, low molecular weight peptides, and fat globule membrane proteins (Sodhi et al., 2021). Caseins contribute to 0.77 of the total protein and can be broken down into four categories: alpha-S1 ($\boldsymbol{\alpha s_{1}}$), alpha-S2 ($\boldsymbol{\alpha s_{2}}$), beta (**β**) and kappa (**κ**) (Daniloski et al., 2022; Givens et al., 2013; Park et al., 2021). The frequencies of these caseins have changed over time and with changing emphases in breeding programs (Mayer et al., 2021). β-casein accounts for one-third of the casein content in milk and consists of 12 genetic variants (Bisutti et al., 2022; Lambers et al., 2021). The most common variants of β-casein are A1 and A2 followed by A3, B and C (Daniloski et al., 2022; Mayer et al., 2021). A1 and A2 alleles are co-dominant and additive in their effect and the distribution of A1 and A2 alleles can vary by breed and region (Sodhi et al., 2021; Woodford, 2007). For example, the A1 frequency of Holstein Friesian cattle from North America and North Europe is 0.70 but Holstein cattle in Germany have an A1 frequency of 0.05 (Sodhi et al., 2021). Truswell (2005) summarized that the A1 allele is the most common allele found in dairy cattle of north European origin such as the Friesian, Holstein Friesian, Ayrshire, and British Shorthorn versus the A2 allele is more commonly seen in Guernsey, Jersey, Charolais, Limousin, and Zebu. Specifically, Woodford (2007) noted the Southern European breeds and the Jersey breed typically have an A1 frequency of 0.35 but the Guernsey breed A1 frequency was less than 0.10 and the Scottish Ayrshire is over 0.50. The genotype frequency can also vary but is generally the most frequent as A1A2 (0.43), A2A2 (0.33) and A1A1 (0.14) (Bisutti et al., 2022). Historically, the A2 allele was the only form of β-casein in the Genus Bos. However, a mutation occurred with a substitution at position 67 where the proline amino acid was substituted with histidine, consequently creating the A1 variant. The histidine substitution, in the A1 allele, is less resistant to "enzymatic digestions and is easily hydrolyzed" during protein digestion and subsequently releases β-casomorphin-7 (**BCM-7**), whereas A2 does not cleave at position 67 and releases β-casomorphin-9 (**BCM-9**) (Giglioti et al., 2020, 2021; Lambers et al., 2021; Sodhi et al., 2021). These BCMs are considered opioid-like peptides and are found in human and bovine milk during digestion in the intestine and is suggested to affect up to a quarter of the human population (Kaskous, 2020; Mayer et al., 2021). β-casomorphin-7 has a high affinity for the m-opioid receptor (**MOPr**) which is found in the brain and along the GI tract and immune cells (Osman et al., 2021).

Effect of A1 Verses A2 Alleles

In 1968, professors Bob Elliott from Auckland University and Murray Laugesen first noted that within the developed world, the majority (80%) of variation of Type 1 diabetes was explained by the per capita intake of A1 β-casein (Laugesen et al., 2003). Now, researchers are debating whether the binding of BCM-7, from the A1 variant, to MOPr, and its biproduct, is responsible for diseases such as Type-1 diabetes mellitus, chronic heart diseases, as well as GI motility and proinflammatory response and potentially milk intolerance (Bisutti et al., 2022; Mayer et al., 2021; Sodhi et al., 2021). Main areas of research are focused on the effects of A1/A2 allele milk on human health, “milk technological characteristics,” and its impact on cow performance.

Bisutti et al. (2022) studied the cheese-making abilities between A1A1 and A2A2 milk. It was reported that no significant differences were found except for a tendency seen with the curd syneresis instant rate constant that slightly worsened the coagulation properties for A2A2 milk. This led the researchers to conclude that the dairy industry should take caution when using milk containing the A2 allele because of its poor cheese-making abilities. Another study looked at the differences in Petit Suisse and Minas Frescal cheeses that were made with either A1A1 or A2A2 milk (Oliveira Mendes et al., 2019). The study asked a panel of consumers to taste the cheeses and report if the consumer noticed any differences. Consumers were not able to differentiate the Petit Suisse samples, however a substantial number of the consumers surveyed were able to differentiate the Minas Frescal samples. The consumers noted the A2 Minas Frescal sample was “softer and creamier” than the A1 sample, whereas the A1 sample was more “firm, rubbery, and drier.” Similarly, Park et al. (2021) reported that dairy goat farmers and cheese manufacturers preferred to use A1 milk because of its ability to produce firmer curds and overall higher cheese yield than A2 milk which produced a softer curd and lower cheese yield.

A1 and A2 milk derived casein hydrolysates (**CH**) were fed to rats to study its effects on diabetes (Thakur et al., 2020). This study showed that neither of the CH had any influence on the BW of the rats during the pre- and post-diabetic periods, blood glucose levels, insulin levels, affect the level of HbA1c, nor did it affect the total protein and albumin levels in the blood of the test rats. Low-density lipoprotein levels were also not significantly affected by A1CH or A2CH feedings, concluding that they did not influence cardiac health of the rats.

To genomically test a cow for its β-casein genotype, a producer can either test a milk sample or hair and skin follicle cells collected from the animal (Lu et al., 2020). From here, researchers, such as Lu et al. (2020), studied the effects of the A1 or A2 allele on cow performance, although previous literature has been inconsistent. Lu et al. (2020) reported that the β-casein genotype did not have a significant effect on any reproductive traits in the cows studied. A parity and β-casein genotype interaction also had no effect on reproductive traits but the interaction between farm and β-casein genotype did have an impact on the pregnancy rate at 42 days after the start of mating. β-casein also had no significant impact on total milk yield, fat yield, or protein yield. Conversely, Bech et al. (1990) and Ng-Kwai-Hang et al. (1990) reported A2A2 cows produced more milk than A1A1 cows. Morris et al. (2005) studied New Zealand dairy cows and reported that A2A2 cows had a significantly higher fat and protein yield than A1A1 cows. Lu et al. (2020) cautions that the differences reported from other studies could be attributed to the potential of gene linkage because casein genes on BTA 6 are closely linked, consequently making it difficult to distinguish the difference between the effect of linked genes or the β-casein loci itself.

Consumer Opinion

Some countries are now starting to see a trend, as more research is published concerning the effects of A2 milk on human health, in the increased use of A2 milk, milk that only contains the A2 homozygous alleles, which is driving producers and breeders to change their herd genetics to meet this growing demand (Bisutti et al., 2022). In New Zealand, the a2 Milk™ Company was developed in the early 2000's to provide milk and milk products, such as yogurt, cheese, milk powder, etc., that only contain the A2 allele to consumers (Mayer et al., 2021). Other countries such as Australia, Austria, America, China, Brazil, the UK, Italy, Germany, and the Netherlands have also created niche markets for A2 milk. Oliveira Mendes et al. (2019) surveyed Brazilian consumers concerning their opinions on selection criteria for milk and milk products as well as their knowledge on A2 milk. It was reported that Brazilian consumers did not read the labels of milk products, nor the information given about the milk type (A1 vs A2) and concluded that it would be important for the A2 milk industry to develop marketing strategies that publicize A2 milk benefits in order to promote this niche market.

CHAPTER 2. Response to *ad libitum* milk allowance by Holstein, crossbred dairy and dairy-beef calves in an automated feeding system

SUMMARY

The objective of this study was to compare 3-breed rotational crossbred calves sired by Jersey, Montbéliarde, Normande, Viking Red, Holstein and Limousin breeds with Holstein calves fed different milk allowances for growth and milk consumption in an automated group feeding system. Holstein (n = 16), crossbreds of Montbéliarde, Viking Red and Holstein (n = 24), crossbreds of Jersey, Normande, and Viking Red (n = 6), and Limousin crossbred beef x dairy (n = 45) calves were randomly assigned to one of two treatments from September 2019 to June 2020 at the University of Minnesota West Central Research and Outreach Center in Morris, MN. Treatment groups were randomly assigned to the first calf of each breed group at either 8 L/d (8L) or *ad libitum* (AL) milk allowance, then alternatingly assigned by breed group. Calves were introduced to the automated feeder at 5 d and were weaned at 56 d. Milk consumption behavior (drinking speeds) were collected from the automatic feeding system and analyzed by feeding group and breed group. Body weights were recorded at birth, weekly, and at weaning (56 d). Health scores of calves were recorded twice/wk. Data were analyzed using a linear mixed model in SAS. Independent variables for analyses were fixed effects of birthweight, season of birth, breed group, treatment group and the interaction of breed group and treatment group. Calves fed AL had a greater weaning weight (107.4 kg versus 91.4 kg) and greater average daily gain (ADG; 1.13 kg/d versus 0.88 kg/d) than calves fed 8L, respectively. The calves fed AL (1106 mL/min) had a slower drinking speed than calves fed 8L (1476 mL/min). Breed groups were not different for weaning weight or ADG across the 56 d. Milk consumption per calf per d was lower for Limousin crossbred calves compared with Holstein and crossbred dairy calves. As expected, AL calves had larger milk cost ($193.66) than the 8L calves ($138.82) during the 56-d study period, however the cost per day was similar between the two treatment groups. The Limousin crossbred calves had the least milk cost ($151.66) compared with Holstein ($171.66) and ProCROSS heifer calves ($181.32). The results from this study found that although feeding calves AL resulted in greater milk consumption than 8L calves, there may be economic advantage to feeding calves *ad libitum* if increased growth rates are realized. Furthermore, farmers may observe a difference in growth and feeding behaviors of different breeds of calves raised in an automated feeding system.

Key words: automated feeder, crossbreeding, milk allowance

INTRODUCTION

Rearing calves in groups has increased in popularity in the dairy industry, even while most calves are still housed individually (Hepola, 2003). With this change, the use of automated, or computer-controlled, feeding systems (**AFS**) has become more common in practice on dairy farms to efficiently feed calves housed in groups (Knauer et al., 2017). Computer-controlled AFS originated in Germany in the 1980s and were first imported to Sweden in 1988, followed by Norway in 1989 (Hepola, 2003). The advantages of these systems, as described by Hepola (2003), are to keep milk at a constant and correct temperature continuously, distribute the milk in small portions throughout a 24-h period, and track the daily habits of calves with the addition of a transponder attached to the calf. Automated feeding systems also utilize teat access which grants calves the opportunity to express natural behaviors, slow milk intake to improve milk digestion and reduces cross-sucking in grouped calves (Khan et al., 2011).

A few studies have compared the use of an AFS or a manual feeding system (**MFS**), feeding individually housed calves by calf workers at set times per day, on calf growth traits and found no significant differences between systems (Kung et al., 1997; Sinnott et al., 2021). Kung et al. (1997), additionally, reported no difference in milk-replacer intake between feeding systems. In contrast, Maatje et al. (1993), reported decreased milk intake and growth rate of grouped calves fed via an AFS versus calves housed individually and fed two times per d with buckets. Kung et al. (1997b) reported calves housed in a group pen with an AFS had fewer medicated days than calves housed in individual hutches and fed with buckets. Additionally, Sinnott et al. (2021) reported calves raised with a MFS were more likely to receive higher fecal scores than calves raised with an AFS. With calves in better health, mortality rates will also decrease. In 2017, the US calf mortality rate was between 6-8% which included all housing types (Jorgensen et al., 2017). The annual mortality rate on the farms using AFS was 2.6% with 13% of the farms reporting over 7% mortality rate and 57% reporting under 3% mortality rate.

Dairy farmers have typically fed calves 4 L of milk per d per industry recommendation. Currently, more producers are evaluating milk feeding levels of 8 or more liters per d. Soberon et al. (2012) reported pre-weaning average daily gain (**ADG**) and weaning weight (**WW**) were positively correlated with first-lactation milk production. However, producers may be apprehensive about increasing the amount of milk offered to calves due to potential for increased occurrence of scours and increased cost of production. Davis Rincker et al. (2011) reported that increasing heifer calf weight allowed heifers to achieve breeding weight earlier which may lead to decreased calving age and reduced costs for raising replacement heifers. Increasing milk allowance was also shown to improve the efficiency of the AFS by reducing the number of visits by the calves (Schäff et al., 2018).

While the Holstein (**HO**) breed has been the dominant dairy cattle breed in the U.S., some producers are turning to crossbreeding systems to take advantage of breed complementarity and hybrid vigor (Maltecca et al., 2006). There is little research reporting the effects of crossbreeding on pre-weaned calves. Dhakal et al. (2013) reported pure HO calves had significantly heavier calf birth weights (**CBW**) while pure Jersey (**JE**) calves had the lightest CBW. The results of the study also predicted purebred HO calves to weigh 9.3 ± 0.6 kg more than purebred JE calves. Ware et al. (2015) reported HO calves gained 5.5 kg more than JE calves at 42 d of age and 10.3 kg more at 56 d of age. Similarly, the HO crossbred group produced calves with higher CBW than HO x JE or JE crossbred groups but was similar to the JE x HO group (Dhakal et al., 2013). Maltecca et al. (2006) reported similar results whereas Hazel et al. (2014) reported Montbéliarde x HO had a greater body weight than HO cows. Hip height and heart girth were reported to be no different between HO and Montbéliarde x HO cows by Hazel et al. (2013, 2014). Interestingly, Maltecca et al. (2006) also reported on health scores compared between purebred and crossbred cattle. That study showed HO-sired calves tended to be scored worse for fecal consistency, from birth to 7 d, than calves whose sires were crossbred. There was no difference reported for respiratory disease scores between crossbred- or HO-sired calves.

No studies have compared whole milk feeding of HO, crossbred dairy calves, and crossbred dairy-beef calves for growth and profitability on an AFS. Furthermore, very little research has compared the pre-weaning effects of feeding calves *ad libitum* (**AL**) on an AFS. Therefore, the objective of this study was to evaluate growth, health and economics of HO, crossbred dairy calves and Limousin crossbred beef on dairy calves fed alternative milk allowances in an automated group feeding system.

MATERIALS AND METHODS

Recording of Data

All animal procedures involving animal care and management were approved by the University of Minnesota Institutional Animal Care and Use Committee (#1909-37379A). The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN dairy, where all calves were born. Data was collected on 46 heifer calves and 45 Limousin crossbred calves born from September to December 2019 (n = 49) and March to May 2020 (n = 42). Breed groups of calves were HO (n = 16), crossbreds composed of HO, Montbéliarde, and Viking Red (**MVH**; n = 24), crossbreds composed of Normande, JE, and Viking Red (**NJV**; n = 6), and Limousin-sired crosses from MVH and NJV cows (n = 45; **LH** = 22 heifer calves; **LB** = 23 bull calves). The unbalanced numbers of calves per breed group were because of sex ratios of Holstein and crossbred dairy calves and the dairy herd is two-thirds crossbreed (mostly MVH crossbreds) and one-third Holstein. Furthermore, Limousin was used for mating 40% of the dairy herd. Calf sex is unknown until birth, and calves that were used survived past 3 d of age for the study, so it can be very difficult to predict sex ratios by breed groups of calves before a study begins. The breed groups varied in the number of calves; however, these calves were spread across 2 calving seasons, so they contributed meaningful information for breed group comparisons. A power calculation was conducted with PROC POWER in SAS software before the beginning of data collection for the study. An analysis of variance with two treatments was used and the power estimate for 40 calves per treatment with an alpha level of 0.05 was 0.957, which is greater than 0.80, which is commonly used when conducting a power analysis.

Calves were separated from their dams within 12 h of birth, housed in individual pens (Calf-Tel I-Series 22|64 1.83 m pen; Calf-Tel, Hampel Corp.), fed (3.78 L/d in total) colostrum for two sequential feedings, then fed with transition milk until they reached 5 days old. At 5 d of age, calves were grouped into pens and introduced to the automated calf feeder (Holm & Laue GmbH & Co KG, Westerronfeld, Germany). Treatment assignment was blocked by breed, randomly assigning a treatment group to the first calf born for each breed group, then alternatingly assigned to one of two feeding treatments: 8 L/d (**8L**; n =47) or AL (n = 44) of whole milk based on birth order and breed group. Two pens of calves were formed during the fall and 2 pens of calves were formed during the spring. Each pen was filled within a 3 week timespan. Both 8L and AL calves were raised together in all pens. Each automated feeding pen had an indoor area of 12.2 × 4.9 m bedded with organic oat straw and access to an outdoor area that measured 10.7 × 4.9 m. Calves were weaned at 56 d of age. Organic, whole milk was fed at 13% total solids of pasteurized saleable and nonsaleable whole milk. The milk averaged 4.4% fat, 3.5% protein, and 5.6% other solids. Texturized calf starter and water were provided free choice in the automated group feeding pens. Calf starter consisted of organic corn, oats, expelled soybean meal, soybean oil, and minerals. The calf starter was mixed on site at the research dairy and contained (as a percentage of DM) 89.9% DM and 18.5% CP. Individual starter consumption was not recorded because calves were housed in the automatic milk feeding pens.

The Holm & Laue HL100 Calf Feeder was used to feed both treatments, and each pen of calves had 2 nipple feeding stations (4 stations in total). Only 2 calves (1 per pen) were allowed to drink at any time because the feeder did not allow calves to drink from all 4 nipple feeding stations at one time. From 5 to 56 d of age, calves in both feeding groups did not have a "ramp-up" or "ramp down" phase. Calves were allowed to consume 8L or AL from day 5. However, for calves in the 8L group, calves were allowed to consume 2.4 L per feeding and were not allowed to receive more milk until 2 h later. The 8L calves were allowed to consume 4 L per half day and a total of 8 L/d. The AL calves were allowed to consume as much milk as the calves chose to per day. If an AL calf consumed 9 L at one feeding, they were not allowed to consume more milk until 4 h later. All calves were allowed AL access into the nipple feeding station per day.

Body measurements of calves included CBW, WW, weaning hip height (**WHH**), weaning heart girth (**WHG**), total gain, and ADG. Total weight gain was calculated as the difference between WW and CBW. The ADG per calf was the difference between CBW and WW, divided by 56 d. Feeding behaviors, total milk consumed per calf per visit (L) and drinking speed (mL/min), were collected from the automated calf feeder each time a calf visited the feeding station. Individual health and hygiene scores were recorded on all calves once per week. The scoring method used for calf fecal and respiratory scores were adapted from McGuirk (2018) on a scale of 0 to 3. Fecal appearance was scored as 0 for normal to 3 as watery feces. Respiratory score was the combination of the scores for cough, nasal discharge, and eye or ear score depending on which was higher. These health indicators were also scored as 0 for normal appearance to scores of 3 as severe symptoms. Scours and general appearance scores were adapted from Heinrichs (2022) and scored from 1 to 5. A score of 1 would represent normal calf health, with each sequential score increasing in severity. The calf hygiene scoring method from Kellermann et al. (2020) scored the belly, side, and rear of a calf with dirtiness scores of 1 to 3. A score of 1 was used for minimal dirt and debris visible, while scores of 2 and 3 represented decreasing hygiene appearance. Milk feed cost was calculated by the total amount of milk consumed by each calf multiplied by a default milk price of $0.33/kg and summed for each calf. Health treatments were documented on an individual calf basis. Total health cost was the sum of the cost of treatments administered to each calf. Total cost was the sum of milk feed cost and total health cost per calf. Average cost per day was the sum of total cost per calf divided by the days on the AFS (56 d). Average cost per kg of gain was the sum of total cost divided by the total weight gain for individual calves. Sensitivity analyses were performed to evaluate the effects of changes in milk price on total feed cost and the average cost per kg of gain for dairy calves. An alternative milk price of $0.22/kg was used for a sensitivity analysis.

Statistical Analysis

Calf was the experimental unit for all analyses. For the analysis of calf body measurements, milk feeding behaviors, milk cost, health cost and total cost, the fixed effects were season of birth, breed group, treatment (8L vs. AL), and the interaction of treatment and breed group. Data were analyzed using PROC MIXED of SAS 9.4 (SAS Institute, 2018). For the analysis of calf health scores, the fixed effects were similar to the growth and economics models; however, PROC GLIMMIX of SAS was used for statistical analysis. Calf birth weight was a covariate in the statistical model. There were no repeated measures during analysis. Pen was not included as a fixed variable in the models because calf was the experimental unit and preliminary models indicated the effect of pen was not significant. All treatment results were reported as least squares means with significance declared at $P < 0.05$.

RESULTS AND DISCUSSION

8L vs. AL Milk Allowance

Each breed group was represented in each treatment group (8L, HO, n = 5, MVH, n = 15, NJV, n = 4, LH, n = 10, LB, n = 13; AL, HO, n = 11, MVH, n = 9, NJV, n = 2, LH, n = 12, LB, n = 10). Results for body measurements, feeding behaviors, and health and hygiene scores over both treatment groups are in Table 2.1. The AL calves had higher WW ($P < 0.0001$), WHH ($P = 0.02$), WHG ($P = 0.0002$), and total gain ($P < 0.0001$) compared to the 8L calves. Furthermore, the AL calves had higher (1.13 ± 0.03 kg/d; $P < 0.0001$) ADG compared to 8L (0.88 ± 0.03 kg/d) calves. Calves allocated 8L/d had a faster (1475.7 ± 56.1 mL/min; $P < 0.0001$) drinking speed than AL calves (1105.8 ± 63.3 mL/min). While there was a statistical difference ($P = 0.03$) between the fecal scores of AL and 8L fed calves, there was most likely no biological difference between the scores. There were no differences in scores for scours, respiratory, general appearance, belly, side, and rear between the treatment groups. The differences observed for WW and ADG among treatment groups were similar to previous studies (Jasper et al., 2002; Rosenberger et al., 2016; Suarez-Mena et al., 2021). Reardon et al. (1972) studied twin, male JE and Friesian x JE calves for the effect of preweaning nutrition on growth rates and carcass composition and reported an increase of 2 kg of carcass weight per every added kg of WW. While the limited access, 8L, calves in the current study drank faster than the AL access calves, Appleby et al. (2001) and Borderas et al. (2009a) reported calves who drank more also drank at a faster rate. The limited access calves in those studies spent more time at the feeder which could lead to the conclusion that limiting milk access to calves would render the AFS inefficient. However, in the current study, this was not the case which could be caused by one of two reasons. One possibility is the 8L access calves were hungrier and therefore drank at a faster pace than those given AL access. Jensen (2006) reported that calves with restricted access spent less time ingesting milk but they also spent more time at the feeder performing non-nutritive sucking. Calves in this study were healthy throughout, regardless of treatment group and fed at high milk levels. Other studies also noted an increase in fecal scores for calves that were fed a higher milk diet in the respective study (Jasper et al., 2002; Suarez-Mena et al., 2021) and de Paula Vieira et al. (2008) found no increase in scours compared to calves with lower milk diets. As calves consume more milk, it is expected that their manure will have a more liquid consistency compared to a more normal stool with firmer texture. Therefore, it is not surprising that the AL calves in this study had slightly higher fecal scores compared to the 8L calves. It is more important to note the decreased prevalence of scours as this is a main concern for dairy calves. Heinrichs et al. (2011) concluded that the number of days a calf had scours or coughing during the first four months of life had a negative impact on the subsequent first-lactation 305-d mature equivalent and milk, protein, and fat production.

Table 2.1 also has the means for milk cost, health cost, and economic and sensitivity analyses of treatment groups. Milk cost was higher ($P < 0.0001$) for the AL calves compared to 8L calves due to the increased amount of milk consumed. There were no differences for health costs for 8L and AL calves. The AL calves had higher ($P < 0.0001$) total cost per calf because of the higher milk feed cost. There was no difference in average cost per gain; however, average cost per d was higher ($P < 0.0001$) for AL calves compared with 8L calves. The sensitivity analysis also showed similar results between the treatment groups.

Breed Group Comparison

Least squares means for body measurements, milk feeding behaviors, health and hygiene scores, milk cost, health cost, and economics by breed groups are in Table 2.2. There were no differences ($P > 0.05$) for WW, total gain, and ADG among breed groups. The Limousin crossbred calves had lower milk consumption (444.13 ± 15.7 L for heifers and 451.64 ± 14.8 L for bulls) compared to the other breed groups. Notably, the Limousin crossbred calves had the lowest milk consumption while having similar WW and ADG compared to the other breeds groups, which may be an indicator of heterosis for feed efficiency of beef on dairy crossbreds (Berry, 2021).

The economic analysis within breeds is also shown in Table 2.2. Limousin crossbred calves consumed less milk and consequently had the lowest ($P < 0.05$) milk feed cost compared to HO and MVH. Health costs were not different among the breeds due to the low incidence of disease observed during the study. Total cost therefore was lowest ($P < 0.01$) in the Limousin crossbred calves compared to the MVH calves and numerically the lowest total cost compared to HO and NJV calves. During the study, Limousin crossbred calves drank the least milk but still gained similar weights to the other breeds. Meanwhile, the average cost per gain for Limousin bull calves was lowest ($P < 0.05$) compared to the other breed groups and the NJV had numerically the highest cost per gain. The same trend also occurred in the average cost per d, however the MVH calves had numerically the highest cost per day.

In addition to Reardon et al. (1972), there are few studies that also compare milk allocation or drinking behavior with crossbred dairy calves. In 1992, Khalili et al. compared three different milk allocation schedules using Friesian x zebu dairy calves and saw similar general results compared to the current study. The live-weight gain during the study was significantly higher for the calves given the most milk throughout the study compared to the lower milk allowance treatments ($P < 0.05$). Bjorklund et al. (2013) studied three different weaning ages using HO, 1964 breed-average level-maintained HO (**H64**), crossbred combination between HO, Montbéliarde, and Swedish Red (**HMS**) and a crossbred combination between New Zealand Friesian, JE and Swedish Red cattle (**HJS**). This study reported HJS calves had the lowest ($P < 0.05$) CBW and the HO and HMS calves had similar ADG, but HO calves had a higher ($P < 0.05$) ADG than H64 and HJS calves. The H64 calves had similar measurements to HJS calves for WW, hip height and heart girth and both had lower body weights than HO calves pre-weaning. Additionally, Groenendijk et al. (2018) compared AL milk allocation with low allowance (10% initial live weight) and high allowance (20% initial live weight) using New Zealand crossbred HO x JE dairy calves. The AL calves had the greatest ADG pre-weaning whereas the low allowance calves had the lowest ADG pre-weaning. This is similar to the results seen in the current study comparing purebred HO and crossbred calves.

CONCLUSION

Feeding an AL milk allowance showed an increase in WW, skeletal size, and ADG in calves without compromising health and hygiene of the calves when compared to calves allowed 8L/d. Increasing the milk allowance also increased the milk feed cost, total cost, and average cost per d. However, it did not increase the average cost per gain. Both the positive effect of an increased WW and decreased health concerns could potentially counteract the increased production costs associated with an increased milk allowance for calves. The breed comparison analysis showed that in the dairy breeds used, HO, MVH, and NJV, while there were statistical differences amongst the different traits analyzed on the AFS, there was no particular dairy breed that outperformed the other dairy breeds in all traits. There was a noticeable difference between the beef on dairy, LH and LB, and the dairy breeds just listed. The LH and LB calves drank less milk while continuing to gain enough weight for a final WW that was similar to the dairy breeds. This study offers producers more insight into feeding different milk allowances and offering a comparison on different breeds used on dairy farms.

Table 2.1. Least squares means and standard errors of means for body measurements, milk feeding behaviors, health and hygiene scores, and milk cost, health cost, and economic and sensitivity analysis of dairy calves by milk treatment during the first 8 weeks life.[1]

	8 L/d feeding (n = 47)		Ad-libitum feeding (n = 44)	
Measurement	LSM	SE	LSM	SE
Birth weight (kg)	39.2	0.9	39.1	1.1
Weaning weight(kg)	91.4[a]	1.6	107.4[b]	1.8
Weaning hip height (cm)	94.8[a]	0.5	96.5[b]	0.6
Weaning heart girth (cm)	104.3[a]	0.8	109.3[b]	1.1
Total gain (kg)	51.3[a]	1.6	67.3[b]	1.8
ADG (kg/d)	0.88[a]	0.03	1.13[b]	0.03
Drinking speed (mL/min)	1,475.7[a]	56.1	1,105.8[b]	63.3
Total milk consumed (L)	406.5[a]	11.7	567.1[b]	13.2
Milk consumed per calf (L/d)	7.4[a]	0.2	10.3[b]	0.2
Fecal score	0.32[a]	0.03	0.42[b]	0.04
Scours score	1.36	0.07	1.51	0.08
Respiratory score	1.00	0.06	1.05	0.07
General appearance score	1.05	0.06	1.03	0.07
Calf belly score	1.00	0.11	1.00	1.11
Calf side score	1.01	0.11	1.02	1.11
Calf rear score	1.11	0.12	1.07	0.11
Milk feed cost ($)	138.82[a]	4.00	193.66[b]	4.51
Health cost ($)	4.07	1.76	4.67	1.98
Total cost ($)	142.89[a]	4.46	198.32[b]	5.02
Average cost/gain ($/kg)	2.86	0.09	3.03	0.10
Average cost/d ($/d)	2.55[a]	0.08	3.54[b]	0.09
Lower milk cost ($)	92.54[a]	2.67	129.10[b]	3.01
Lower total cost ($)	96.62[a]	3.27	133.77[b]	3.69
Lower cost/gain ($/kg)	1.94	0.06	2.04	0.07
Lower cost/d ($/d)	1.73[a]	0.06	2.39[b]	0.07

[1] Reported means and SE are based on feeding group averages.

[a,b] Means within a row without common superscripts are different at $P < 0.05$.

Table 2.2. Least squares means and standard errors of means for body measurements, milk feeding behaviors, health and hygiene scores, milk cost, health cost, and economic and sensitivity analysis of dairy calves for breed groups during the first 8 weeks life.[1]

	Holstein (n = 16)		MVH crossbreds[2] (n = 24)		NJV crossbreds[3] (n = 6)		Limousin-crossbred dairy-beef heifer (n = 22)[4]		Limousin-crossbred dairy-beef bull (n = 23)[4]	
Measurement	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Birth weight (kg)	38.3^{abc}	1.6	40.2^{b}	1.2	33.3^{c}	2.5	40.0^{ab}	1.3	43.9^{d}	1.2
Weaning weight(kg)	98.1	2.7	99.8	2.1	98.2	4.3	99.4	2.1	101.6	2.1
Weaning hip height (cm)	97.0^{a}	0.9	97.4^{a}	0.7	96.0^{ab}	1.4	94.2^{b}	0.7	96.6^{b}	0.7
Weaning heart girth (cm)	106.8	1.2	106.7	1.0	106.3	2.6	107.0	1.0	107.2	1.0
Total gain (kg)	58.0	2.7	59.6	2.1	58.1	4.3	59.3	2.1	61.5	2.1
ADG (kg/d)	0.96	0.04	1.02	0.04	0.99	0.07	1.01	0.04	1.05	0.04
Drinking speed (mL/min)	1,192.9	92.5	1,322.2	72.5	1,426.6	151.3	1,275.8	74.8	1,236.1	74.7
Total milk consumed (L)	502.7^{a}	19.2	531.0^{a}	15.2	504.6^{ab}	30.4	444.1^{b}	15.7	451.6^{b}	14.8
Milk consumed per calf (L/d)	9.1^{a}	0.4	9.7^{a}	0.3	9.2^{ab}	0.6	8.8^{b}	0.3	8.2^{b}	0.3
Fecal score	0.38^{a}	0.06	0.26^{b}	0.04	0.50^{a}	0.10	0.31^{ab}	0.05	0.41^{a}	0.05
Scours score	1.46	0.12	1.29	0.09	1.64	0.20	1.35	0.10	1.46	0.09
Respiratory score	1.00	0.10	1.01	0.08	1.06	0.16	1.05	0.09	1.01	0.08
General appearance score	1.07	0.11	1.03	0.08	1.02	0.16	1.07	0.09	1.03	0.08
Calf belly score	1.00	0.21	1.00	0.15	1.00	0.23	1.00	0.09	1.00	0.12
Calf side score	1.01	0.21	1.05	0.16	1.00	0.23	1.01	0.09	1.00	0.12
Calf rear score	0.04	0.22	1.04	0.16	0.07	0.24	1.13	0.10	1.17	0.13
Milk feed cost ($)	171.66^{a}	6.57	181.32^{a}	5.20	172.31^{ab}	10.37	151.66^{b}	5.36	154.23^{b}	5.04
Health cost ($)	3.56	2.89	2.15	2.28	0.00	4.55	8.74	2.36	7.40	2.22
Total cost ($)	175.22^{ab}	7.32	183.48^{a}	5.79	172.31^{ab}	11.55	160.40^{b}	5.98	161.62^{b}	5.62
Average cost/gain ($/kg)	3.10^{a}	0.15	3.06^{a}	0.10	3.17^{a}	0.23	2.75^{ab}	0.12	2.65^{b}	0.11
Average cost/d ($/d)	3.13^{ab}	0.13	3.28^{a}	0.10	3.08^{ab}	0.21	2.86^{b}	0.11	2.89^{b}	0.10
Lower milk cost ($)	114.44^{a}	4.38	120.88^{a}	3.47	114.87^{ab}	6.91	101.11^{b}	3.58	102.82^{b}	3.36
Lower total cost ($)	118.00^{ab}	5.38	123.04^{a}	4.25	114.87^{ab}	8.49	109.85^{b}	4.39	110.21^{b}	4.13
Lower cost/gain ($/kg)	2.08^{a}	0.11	2.06^{a}	0.08	2.11^{ab}	0.17	1.89^{ab}	0.09	1.80^{b}	0.08
Lower cost/d ($/d)	2.11^{ab}	0.10	2.20^{a}	0.08	2.05^{ab}	0.15	1.96^{b}	0.08	1.97^{b}	0.07

[a-d]Means within a row without common superscripts are different at $P < 0.05$.

[1] Reported means and SE are based on feeding group averages.

[2] MVH = crossbreds of Montbéliarde, Holstein, and Viking Red. [3] NJV = crossbreds of Normande, Jersey, and Viking Red.

[4] Limousin-sired crossbred from MVH and NJV cows.

CHAPTER 3. Relationships of beta-casein genetics with production, fertility, and survival of purebred organic Holstein dairy cows.

SUMMARY

The objective of this observational study was to compare beta-casein genotypes of purebred certified-organic Holstein cows, and their effect on production, fertility, and survival to first and second lactation. Holstein cows (n = 1,982) from 13 certified-organic dairy herds from western, midwestern and northeastern USA were genomically tested for A2 genotype. Two-hundred fourteen cows were A1A1 (11%), 848 cows were A1A2 (43%), and 920 cows were A2A2 (46%). In total, 2,249 lactation records consisting of 1,025 records during first parity and 1,224 records during second parity and greater were used. Test-day milk, fat, and protein production (305-d) and somatic cell score were obtained from DHIA. Days open records were analyzed starting at 50 days open up to 250. Cows with more than 250 days open had days open set to 250 d. Independent variables for statistical analysis were the fixed effects of herd, parity, beta-casein genotype (A1A1, A1A2, A2A2), and beta-casein genotype by parity interaction. Cow nested within parity was the random effect in the statistical models for fertility and production traits. A linear mixed model was used to analyze all variables except survival which was analyzed with a generalized linear mixed model. Herd had a significant effect on all fertility, production, and survival variables. Parity affected times bred and days open, milk, fat, and protein production, and somatic cell score. Beta-casein genotype and herd had an effect on the percentage of cows surviving to first and second lactation. Results indicate no difference in production and fertility with regard to beta-casein genotype for organic dairy cows. Survival may be biased against the A1 allele which is indicated by lower survival rates during first lactation. These results may offer organic producers more flexibility in breeding and culling decisions to produce A2 milk.

Key words: beta-casein, A2, milk production, fertility, organic

INTRODUCTION

Protein constitutes 3.5% of milk components (Davoodi et al., 2016) and is imperative to development, maintenance, and repair of body tissues in addition to providing an energy source for cattle (Lu et al., 2020). Caseins and whey proteins account for 95% of protein in milk with the remaining 5% consisting of peptones, low molecular weight peptides, and fat globule membrane proteins (Sodhi et al., 2021). Caseins in milk are biologically found in four variants: alpha-S1, alpha-S2, beta (**β**), and kappa, and the frequencies at which these are found, within a population, have changed over time (Daniloski et al., 2022; Givens et al., 2013; Mayer et al., 2021; Park et al., 2021). Beta-casein accounts for one-third of milk casein content and consists of 12 genetic variants (Bisutti et al., 2022; Lambers et al., 2021). The most common forms of β-casein are A1 and A2 (Daniloski et al., 2022; Mayer et al., 2021). The A1 and A2 alleles are co-dominant and additive in their effect and the frequency distribution of A1 and A2 alleles can vary by breed and geographic location (Sodhi et al., 2021; Woodford, 2007). Truswell (2005) reported the A1 allele is the most common allele found in dairy cattle of Northern European origin such as the Friesian, Holstein Friesian (**HO**), and Ayrshire versus the A2 allele which is more commonly observed in the Channel Island breeds of Guernsey and Jersey (**JE**) cattle. The genotypic frequency of the A1 and A2 alleles may also vary and Bisutti et al. (2022) reported genotypic frequencies of 0.43 for A1A2, 0.33 for A2A2, and 0.14 for A1A1 from 1,133 Italian Holstein cows. The remaining 0.1 were animals where complete genotype identification was not possible. The A2 allele, historically, was the only predominant form of β-casein in Genus Bos. However, a mutation occurred which caused a histidine substitution for proline at position 67 and consequently created the A1 variant. The A1 allele is less resistant to enzymatic digestion and subsequently releases β-casomorphin-7, whereas A2 does not cleave at position 67 and releases β-casomorphin-9 (Giglioti et al., 2020, 2021; Lambers et al., 2021; Sodhi et al., 2021). These β-casomorphins are considered opioid-like peptides and are found in human and bovine milk during digestion in the intestine and is suggested to negatively affect up to a quarter of the human population (Kaskous, 2020; Mayer et al., 2021).

Very few studies have evaluated beta-casein genotypes and their effects on cow production and fertility. A New Zealand study in 2020 evaluated the effects of the A1 and A2 allele on cow production and fertility traits in HO, JE, and HO x JE crossbred cows (Lu et al., 2020). The study reported that β-casein genotype did not have a significant effect on fertility in cows. The β-casein genotype within lactation number had no effect on fertility, however, herd and β-casein genotype did have an impact on the pregnancy rate at 42 days after the start of the breeding season. Furthermore, β-casein genotype did not affect total milk, fat, or protein production. Conversely, Ng-Kwai-Hang et al. (1990) reported HO A2A2 cows had more milk production than A1A1 cows. Morris et al. (2005) reported that A2A2 cows had higher fat and protein production than A1A1 cows in New Zealand dairy cattle. Lu et al. (2020) concluded that the differences reported from research studies may be attributed to the potential of gene linkage because casein genes on *Bos taurus* chromosome 6 are closely linked, consequently making it difficult to distinguish the difference between the effect of linked genes or the β-casein loci.

Interest in A2 milk is growing and given the heightened health consciousness of organic dairy consumers, quantifying the frequency of the A2 allele in the US organic population is important. This may provide insight on how quickly the organic dairy industry could adapt to market shifts if demands for A2 milk continue to grow.

Many genomic testing panels include the marker that distinguishes between the A1 and A2 β-casein types. Most research with the A2 allele has focused on human health aspects; however, few studies have compared the effect of β-casein genotype on dairy cow production and fertility from the allele itself or other closely linked genes. Most studies investigating β-casein genotype in dairy cows have also been conducted on non-organic dairy herds. Therefore, the objective of this study was to evaluate organic HO cow milk production, milk components, and somatic cell score (**SCS**) and fertility from cows genotyped for the A1 and A2 alleles.

MATERIALS AND METHODS

Recording Data

All animal procedures involving animal care and management were approved by Penn State Institutional Animal Care and Use Committee (protocol #47560). Data were collected from HO cows on 13 USDA certified-organic dairy herds located in the northeastern, midwestern, and western portions of the United States between 2017 to 2019 (Hardie et al., 2021).

Holstein cows and heifers (n = 1,982) were genotyped with CLARIFIDE® Plus from Zoetis (Zoetis Inc., Parsippany-Troy Hills, NJ) for A2 status and had breeding events recorded from on-farm software (Hardie et al., 2021). The total 2,249 lactation records collected included 1,025 records from first parity and 1,224 records from second parity and greater. Data collected on-farm were from either Dairy Comp305 (Valley Ag Software, Tulare, CA), PCDart (Dairy Records management Systems, Raleigh, NC), DHI-Plus (Amelicor, Provo, UT), or handwritten records. Due to the nature of the records obtained, reasons for departure from a herd were not collected. From the herds in the current study, 7 herds (n = 881) had test day milk records for milk, fat, and protein production and SCS from DHIA milk recording. Herds were selected based on industry personnel feedback of herds that recorded production, fertility, and survival (Hardie et al., 2022).

Fertility of cows included days open (**DO**), times bred per pregnancy (**TBRD**), and first-service conception rate (**FSCR**). The DO and TBRD were continuous variables and FSCR was recorded in a binary manner as either conceived or not conceived at first service. For DO, a lower limit of 50 d was applied and those with more than 250 d DO had DO set to 250 d. The maximum of 250 d for DO is used by the Council on Dairy Cattle Breeding for routine genetic evaluations for cow fertility in the United States. All 1,982 genotyped animals were used in the analysis for the percent of heifers who survived to first lactation (**SURV1**) and the percent of cows who survived to second lactation (**SURV2**). These two variables were recorded in a binary manner as did or did not survive to the respective lactation. Production traits were 305-d milk, 305-d fat, 305-d protein, and SCS from DHIA milk recording.

Statistical Analysis

For the analysis of FSCR, DO, TBRD the fixed effects were herd, parity group (primiparous or multiparous), β-casein genotype, and the β-casein genotype by parity interaction with cow group nested within parity as a random variable. Data were analyzed using PROC MIXED of SAS 9.4 (SAS Institute, 2018). The variables SURV1 and SURV2 were analyzed with the fixed effects of herd and β-casein genotype and PROC GLIMMIX of SAS 9.4. The 305-d milk, fat, and protein production and SCS were analyzed with the fixed effects of herd, parity group, β-casein genotype, and the interaction between β-casein genotype and parity group, with cow group nested within parity as a random variable. PROC MIXED of SAS was used for the statistical analysis of all production traits. All variables were reported as least squares means and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Beta-Casein Genotype Frequency

Two-hundred fourteen cows were A1A1 (11%), 848 cows were A1A2 (43%) and 920 cows were A2A2 (46%). The genotypic frequencies of the cows in the current study were similar to those reported by Bisutti et al. (2022), and the A1A1 genotype was the least frequent.

Herd Comparison

Least squares means for fertility and survival by herd are in Table 3.1. Herd significantly ($P < 0.0001$) explained variation for all fertility and survival traits. The DO ranged from 113 d to 252 d which was fall around the mean DO of 135 d in the United States reported by Pszczola et al. (2009). The range for TBRD in the current study was 0.78 to 2.81 times bred. First service conception rate ranged from 28% to 65% which is similar with the range reported by Grimard et al. (2006) of 25.9% to 63.6% for herds. Survival to first lactation was high for all herds with the lowest survival rate of 85% for

Herd K. The percentage of survival to second lactation was lower for all herds with the lowest survival rate of 65% for Herd K.

Milk, fat, and protein production for herds are displayed in Table 3.1. Herd significantly (*P* < 0.0001) explained variation for the production traits. The 305-d milk production ranged from 6,243 kg to 9,855 kg, 305-d fat from 254 kg to 367 kg, 305-d protein ranged from 206 kg up to 325 kg. The average SCS ranged from 2.25 to 3.11. While there were significant differences among herds within each production trait, no single herd had the highest or lowest yields for 305-d milk, fat, and protein. In a study of 8,000 HO cows, 305-d production averages were 7,000 kg milk, 250 kg fat, and 220 kg protein (Ng-Kwai-Hang et al., 1990). For the current study 305-d milk results were similar to Ng-Kwai-Hang et al. (1990); however, 305-d fat and protein were higher than Ng-Kwai-Hang et al. (1990). Differences for production among herds was most likely caused by differences in herd management practices such as breeding protocols, environment and weather, grazing time, health treatment protocols, percentage of dry matter from pasture during the grazing season, winter feed rations, or production goals.

Beta-Casein Genotype Comparison

Table 3.2 includes results for fertility and survival traits of each β-casein genotype. Beta-casein genotype had no significant effect on FSCR, TBRD, and DO which was also reported by Lu et al. (2020). Genotype did significantly affect SURV1 and SURV2. The A1A1 cows had a lower (93% ± 2.0, *P* = 0.015) percent SURV1 than the A1A2 cows (98% ± 1.0). Reasons for culling were unknown at the time of the study. The SURV2 was higher for the A1A2 cows (87% ± 2.0) compared to A1A1 (80% ± 3.0; *P* = 0.032) and A2A2 cows (82% ± 2.0; *P* = 0.017). As the niche market for A2 milk becomes increasingly popular, the demand for A2 milk, milk that contains the A1A2 or A2A2 genotype, has increased. Therefore, some producers may wish to make culling decisions based on this growing demand to meet the needs of the producer's milk cooperative.

Beta-casein genotype had no effect on 305-d milk, fat, or protein, or SCS as shown in Table 3.2. In 2007, Woodford (2007) reported A1 and A2 were co-dominant and additive. However, in the current study this was not the case for the production traits measured. The A1A1 cows had numerically higher production than A1A2 and A2A2 cows for 305-d milk and 305-d fat. Dairy cow studies are inconsistent when reporting β-casein genotypes and production. Lu et al. (2020) found no difference for production and β-casein genotype for HO, JE, and HOxJE crossbred cows. Çardak (2005) also reported β-casein genotype did not affect daily milk and protein or fat and protein percentages for HO cows. However, fat production of HO cows was different by β-casein genotype and A1A1 cows had higher ($P < 0.01$) fat production than A1A2 and A2A2 cows (Çardak, 2005). Meanwhile, Morris et al. (2005) reported A2A2 HO cows were superior ($P < 0.05$) to A1A2 HO cows for fat production. These inconsistencies could be caused by differences in the environment, feed rations, health treatment protocols or breeding protocols.

Beta-Casein by Parity Comparison

Results for fertility with the interaction β-casein genotypes and parity are in Table 3.3. The interaction between parity and genotype had no significant effect on FSCR. First-service conception rate was numerically higher for A1A2 cows for both primiparous and multiparous cows. Lu et al. (2020) reported a FSCR of 45.7% and 54% for second and third and later parity cows, respectively, which was similar to the FSCR for multiparous cows in the current study. Furthermore, the authors reported A2A2 cows had a numerically lower FSCR than A1A1 and A1A2 cows; however, β-casein genotype had no effect on FSCR (Lu et al., 2020). Parity had a significant ($P < 0.0001$) effect on TBRD. The A1A2 primiparous cows numerically had the lowest TBRD compared to the other β-casein genotype cows in first lactation. Furthermore, the A1A1 multiparous cows had numerically the lowest TBRD compared to the other β-casein genotype multiparous cows. Similar with the current study, Gebeyehu et al. (2007) reported parity had a significant effect ($P < 0.05$) on number of services per conception. Parity had an effect ($P = 0.0057$) on DO but β-casein genotype did not affect the DO of cows. The A1A1 primiparous and multiparous cows had numerically the highest DO. The DO differences may be caused by environmental factors due to the low heritability of fertility traits. The current study was similar to Lu et al. (2020) who reported no difference for β-casein genotypes for DO of New Zealand HO, JE, and HOxJE crossbred dairy cows.

Table 3.3 also contains least squares means for 305-d milk, fat, and protein production and SCS by the β-casein genotype and parity interaction. Parity had a significant ($P < 0.0001$) effect on all production traits; however, the interaction between β-casein genotype and parity did not affect production. Lu et al. (2020) also reported parity had a significant effect ($P < 0.05$) on milk, fat and protein production for multiparous HO, JE, and HO x JE cows but the interaction between parity and β-casein genotype was not significant with production. Conversely, Ng-Kwai-Hang et al. (1990) reported HO A2A2 cows had higher milk production than A1A1 cows during the first through third lactation, while the A1A2 cows were intermediate for milk production.

CONCLUSION

The results from this study found that fertility and production was not affected by β-casein genotype or by the β-casein genotype interaction with parity of organically raised cows and heifers. However, β-casein genotype was a significant factor in survival. This study offers producers who use an organic management system insight into how β-casein genotype may or may not affect the cow. The niche market for A2 milk has the potential to increase in demand based on consumer opinion or if more studies can confirm the A1 allele is responsible for milk intolerance in humans. With this increase more producers would be pushed to produce A2 milk to meet the demand. Similar production and reproduction traits of β-casein genotypes may offer producers more flexibility in breeding and culling decisions to produce A2 milk.

Table 3.1. Least squares means and standard errors of means for fertility indicator traits and production measurements of Holstein dairy cows by herd.[1]

	Days Open (d)		Times bred		First Service Conception Rate (%)		Survival to 1st lactation (%)		Survival to 2nd lactation (%)		305-Milk (kg)		305-Fat (kg)		305-Protein (kg)		Somatic Cell Score	
Herd	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
A	120^{cegh}	6	1.61^{a}	0.17	46^{ace}	4.0	93^{ab}	2.0	80^{acf}	5.0								
B	116^{fg}	4	1.55^{a}	0.15	55^{abef}	4.0	97^{a}	2.0	90^{ef}	4.0	9855^{a}	104	367^{ag}	4	309^{a}	2	2.69^{a}	0.10
C	131^{dh}	5	1.77^{a}	0.19	53^{abef}	5.0	98^{a}	2.0	79^{ac}	4.0								
D	113^{ef}	4	2.51^{bc}	0.15	37^{c}	3.0	89^{b}	1.0	71^{bc}	3.0	8732^{bf}	68	346^{b}	2	285^{bf}	1	3.11^{b}	0.06
E	131^{dg}	7	1.81^{a}	0.24			99^{a}	4.0	91^{ade}	8.0	6302^{cd}	177	254^{cd}	6	206^{cd}	4	2.36^{acd}	0.16
F	121^{def}	9	1.93^{ace}	0.29			99^{a}	5.0	88^{acde}	9.0	6243^{d}	204	271^{d}	7	220^{d}	5	2.60^{acd}	0.19
G	140^{d}	6	1.55^{a}	0.20			99^{a}	3.0	86^{ade}	5.0	7283^{e}	119	294^{e}	4	239^{e}	3	2.55^{ac}	0.11
H	139^{d}	6	2.72^{bef}	0.30	39^{acd}	7.0	99^{a}	3.0	97^{de}	6.0								
I	141^{d}	6	2.02^{acf}	0.28	65^{f}	6.0	94^{a}	2.0	84^{ade}	4.0	8638^{f}	95	355^{fg}	3	290^{f}	2	2.30^{cd}	0.09
J	137^{d}	3	0.83^{a}	0.09	47^{ae}	3.0	97^{a}	1.0	85^{ade}	3.0	9546^{g}	67	359^{g}	2	325^{g}	1	2.25^{d}	0.06
K	135^{cd}	6	0.78^{dg}	0.12	43^{ac}	5.0	85^{b}	1.0	65^{b}	4.0								
L	164^{b}	3	2.81^{bh}	0.11	28^{d}	3.0	96^{a}	2.0	71^{bc}	3.0								
M	252^{a}	10	1.85^{acfgh}	0.72	61^{ef}	7.0	95^{ab}	3.0	89^{ade}	6.0								

[1] Reported means and SE are based on lactation by genotype averages.

[a,b] Means within a column without common superscripts are different at $P < 0.05$.

Number of lactation records used: DO, 2249; milk, fat protein and SCS, 1774.

Number of animals analyzed: FSCR and TBRD, 1733; SURV1 and SURV2, 1982.

Table 3.2. Least squares means and standard errors of means for fertility, survival, and production measurements of organic Holstein dairy cows by beta-casein genotype.[1]

Beta-Casein Genotype		A1A1		A1A2		A2A2	
	n	Mean	SE	Mean	SE	Mean	SE
First-service conception rate (%)	1733	44	4.3	50	2.2	48	2.0
Times Bred	1733	1.94	0.14	1.88	0.09	1.89	0.09
Days Open (d)	2249*	146	5	139	2	142	2
Survival to 1st lactation (%)	1982	93^{a}	2.0	98^{b}	1.0	96^{ab}	1.0
Survival to 2nd lactation (%)	1982	80^{a}	3.0	87^{b}	2.0	82^{ab}	2.0
305-Milk (kg)	1774*	8162	114	8098	61	8000	61
305-Fat (kg)	1774*	325	4	321	2	318	2
305-Protein (kg)	1774*	268	3	269	2	267	4
Somatic Cell Score	1774*	2.59	0.10	2.51	0.06	2.55	0.06

[1] Reported means and SE are based on lactation by genotype averages.

^{a,b} Means within a row, without common superscripts are different at $P < 0.05$.

*Denotes lactation records. Otherwise n = animals.

Table 3.3. Least squares means and standard errors of means for fertility and production measurements of organic Holstein dairy cows by parity and beta-casein genotype. [1]

	Primiparous						Multiparous					
Beta-casein genotype	A1A1		A1A2		A2A2		A1A1		A1A2		A2A2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
First-service conception rate (%)	41	5.0	49	3.0	47	3.0	47	6.0	51	3.0	49	3.0
Times bred	2.33	0.19	2.11	0.11	2.15	0.11	1.55	0.20	1.64	0.11	1.63	0.11
Days open (d)	141	6	133	3	135	3	149	6	144	3	147	3
305-Milk (kg)	7388	145	7388	74	7244	71	8935	144	8806	73	8754	73
305-Fat (kg)	290	5	286	2	283	2	360	5	355	2	353	2
305-Protein (kg)	242	3	243	2	241	1	294	3	293	2	292	2
Somatic Cell Score	2.31	0.12	2.25	0.06	2.31	0.06	2.87	0.13	2.77	0.06	2.78	0.06

[1] Reported means and SE are based on lactation by genotype averages.

[a,b] Means within a row, within parity, without common superscripts are different at $P < 0.05$.

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