Effect of glycerol supplementation in early lactation on metabolic health, milking activity, and production of dairy cows housed in automated milking system herds.

by

Clayton John McWilliams

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Animal Biosciences

Guelph, Ontario, Canada
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ABSTRACT

EFFECT OF GLYCEROL SUPPLEMENTATION IN EARLY LACTATION ON METABOLIC HEALTH, MILKING ACTIVITY, AND PRODUCTION OF DAIRY COWS IN AUTOMATED MILKING SYSTEM HERDS.

Clayton John McWilliams
University of Guelph, 2023
Advisor(s): Dr. Trevor DeVries

The objective of this thesis research was to quantify the effects of supplementing early-lactation dairy cows with a dry glycerol product, delivered through automated milking system (AMS) concentrate, on metabolic markers, milking behaviour, and milk production. Cows either received a control pellet or the treatment pellet for the first 21 days in milk (DIM) and were observed until 150 DIM. Overall, glycerol supplementation improved milk yield, milking frequency, and AMS concentrate intake during the 21-d supplementation period and also from 22-150 DIM. Over-conditioned glycerol cows were not at an elevated risk of high β-hydroxybutyrate (BHB; BHB ≥ 1.2 mmol/L), as opposed to over-conditioned control cows, and glycerol cows maintained better body condition to peak lactation. In summary, this research indicates that supplementing glycerol through the AMS concentrate to early lactation dairy cows may be an effective way to improve milking behaviour and yield until mid-lactation.
ACKNOWLEDGEMENTS

First, I owe the last 3 years of academic and career achievements to my advisor, Trevor DeVries. It has been an absolute privilege to work with you over the last 3 plus years. Your encouragement, guidance, and criticism have helped shape my future more than I could have hoped. I always looked forward to our meetings where we spent the majority of the time talking about cows, hunting, and fishing. It was a huge benefit that you answer emails and send back edits faster than I could handle them. I also appreciated your patience and understanding when outside factors were pulling my attention away from my work. As I step into the next phase of my life I am looking forward to taking advantage of those fast responses whenever I have a question. I would also like to thank Dr. Todd Duffield and Dr. Katharine Wood for their recommendations and guidance on how to conduct the trial and with data interpretation. My research would not have been possible without the generous funding from Priobiotec International and the Natural Sciences and Engineering Research Council of Canada.

I also owe a huge thank you to everyone in Cowcrew. Thanks to Anna Schwanke and Brandon Van Soest for guiding me through the weeds of grad school and department SOPs. Special thanks to Sarah Bruner, Jess Brasier, Maddy McLennan, Michelle Brower, Kris Lutz, and Larissa Baker for their help over the 8 plus months to collect the data, and sorry for the tardiness. I would not have been able to get this study finished without all of your help, seriously. All of the talks during the long car rides between farms, the jokes, the cats, bird fatalities, and frequent coffee breaks with you all were the most fun times during the thesis. Huge shout out to Sarah and Jess who handled the trial while I was away for a week after the storm that destroyed our farm in May 2022.

I also owe a huge thank you to each of the 5 farms who allowed me to conduct this research at their farms. I sincerely enjoyed the bulk tank talks over the months and enjoyed learning from each of you. You all made us researchers feel like we were part of the team.

Lastly, I would like to thank my parents, brothers, family, friends, and girlfriend who were all there to support me through the project. None of this would have been possible without all of you.
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LIST OF ABBREVIATIONS

AMS – automated milking system
DIM – days in milk
DMI – dry matter intake
NEB – negative energy balance
SARA – sub acute ruminal acidosis
PMR – partial mixed ration
SCC – somatic cell count
DM – dry matter
SCK – sub clinical ketosis
BHB – β-hydroxybutyrate
NEFA – non-esterified fatty acids
KET – ketosis
BCS – body condition score
VFA – volatile fatty acids
NFC – non-fibre carbohydrates
ATP – adenosine triphosphate
CLA – conjugated linoleic acid
CON – control treatment
GLY – glycerol treatment
TMR – total mixed ration
PSPS – penn state particle separator
ADF – acid detergent fibre

NDF – neutral detergent fibre

LS – lameness score

SD – standard deviation
1 CHAPTER 1: GENERAL INTRODUCTION

Since the first installation on a Canadian commercial dairy herd in the late 1990’s, there are now as of 2023, an estimated 1500 Canadian dairy herds that are milking with automated milking systems (AMS) (Lactanet, 2023). The rapid adoption of this new technology has sparked a new wave of research focusing on the benchmarking, behaviour, housing, and management of cows in these systems. The increased understanding of cow behaviour, production, and health in AMS has also identified gaps in the knowledge, including that of fresh cow management in AMS. Fresh cows in AMS can increase their daily milkings beyond the conventional 2 or 3 milkings per day. The subsequent increase in milk yield, and energy demands, may further exacerbate negative energy balance (NEB) in this period. AMS units can offer concentrate to individual cows, both to promote voluntary visits and individually supplement cow nutrient needs. However, typical AMS concentrates contain high concentrations of starch, which may have adverse ruminal effects in early lactation. The consumption of energy dense concentrates individually, coupled with the transition from a low to highly digestible ration, leave AMS fresh cows at an increased risk of sub-acute ruminal acidosis (SARA). Nevertheless, fresh cows must consume high amounts of energy to mitigate NEB. Thus, offering energy dense ingredients that are not starch based has been suggested to be a solution. Glycerol, a gluconeogenic precursor, has previously been studied as a treatment for ketosis, although this was largely abandoned due to the high cost. As crude glycerol is a byproduct of the ethanol industry, the availability has increased, and the cost is more favourable. Crude glycerol can be refined into pure glycerol to remove harmful impurities such as catalysts, salts, and methanol (Donkin, 2008). Further,
glycerol can be included at high concentrations in the ration without adverse ruminal effects. Offering glycerol to fresh cows, delivered through the AMS concentrate, may be an effective method of mitigating NEB, without negatively affecting the rumen environment. This review will outline the unique challenges and opportunities of AMS, transition cow ailments and management strategies, and the use of glycerol as a dietary supplement in lactating cows.

1.1 Introduction to Automated Milking Systems

1.1.1 AMS Adoption and Benchmarks

The Canadian dairy industry has experienced a rapid adoption of modern technology in the last couple decades. AMS, also known as the robotic or voluntary milking system, is one such technology. The first AMS unit was installed in the Netherlands in 1992, and were introduced into Ontario, Canada in 1999 (Barkema et al., 2015). There are currently 12.7% of Canadian herds participating in DHI milking with AMS, representing 15.6% of cows in Canada (Lactanet, 2020). On a global scale, it was estimated in 2017 that there were approximately 35,000 AMS herds worldwide and that number is expected to rise (Salfer et al., 2017). With this rapid adoption of the AMS, additional research is required to improve management strategies (Lyons et al., 2014). The exponential growth in popularity of AMS units globally along with the increase in cow-level data generated by the AMS may both be contributing to the demand for additional research to improve AMS management.

Benchmarking AMS herds has been a complicated process, due in part to the ability of the AMS system to create cow-level data, which may prompt some producers to withdraw from a
DHI program in an attempt to reduce costs, which inadvertently limits the public access to the data. There have been studies conducted in North America to create benchmarking data for AMS herds. In a recent study involving 197 Canadian dairy herds, the average # of cows/AMS unit was 47.5. This is slightly lower than other AMS studies in North America that reported 49 (King et al., 2016), 51 (Tremblay et al., 2016), and 56 (Siewert et al., 2018) cows per AMS respectively. These field observations all fall below the perceived capacity of 60 cows per AMS that has been suggested (Jacobs and Siegford, 2012). An important parameter for the productivity of AMS herds is the number of successful milkings per day. This is commonly referred to as visits/d, but there is an important distinction between successful milking visits and milking refusals. A successful milking visit is when a cow enters the AMS and is milked, whereas a milking refusal is when a cow enters the AMS, but has not exceeded her minimum milking interval, and is forced to exit the AMS without being milked. Recent studies across North American AMS herds reported that average successful milking visits per day were 2.8 (Siewert et al., 2018), 2.91 (Tremblay et al., 2016), and 3.01 (Matson et al., 2022) respectively. An analysis of 635 North American AMS dairy farms reported the mean refusal frequency was 1.86 ± 1.38 (Tremblay et al., 2016). These researchers also observed that an increase in refusals was associated with a decrease in milk production (Tremblay et al., 2016). While it has been suggested to maintain refusals above 1 refusal per cow per day (Kozlowska et al., 2013), there may be a consequence on milk yield if refusals per day increase. Stefanowska et al. (2000) observed that after a milking refusal, cows had fewer eating and lying cycles when compared to cows who had a successful milking visit. Further, an increase in daily refusals on a pen-level
may limit the time the AMS is available for milking, thus limiting the availability of the AMS to cows within the pen.

Across 197 Canadian AMS herds, Matson et al. (2021) observed an average milk yield per cow of 36.7 kg/d. This is higher than the 32.0 kg/d observed across 635 North American AMS herds (Tremblay et al., 2016). This increase in average milk yield may be due to an improvement in AMS management practices between the years of data collection. Further, according to the Canadian Dairy Information Centre (CDIC), the average milk production of Canadian dairy cows in 2020 was 32.6 kg/d (CDIC, 2020). These data point to the improved milk yield that Canadian AMS herds can achieve compared to the average Canadian dairy herd, as Matson et al. (2021) observed that during 2019 and 2020, Canadian AMS herds averaged 4.1 kg/d more milk per cow that the average Canadian herd. The average milk components of Canadian AMS herds were reported to be 4.03% fat and 3.30% protein (Matson et al., 2021). According to the CDIC, the average fat and protein content of milk in Canadian herds was 3.89 and 3.26% respectively in 2020 (CDIC, 2020). Again, these data offer insight into the improvements in production that can be achieved in AMS herds compared to the average Canadian dairy herd. Overall, cows per robot, successful milkings/d, milking refusals, and milk and component yield can act as useful tools for AMS producers to benchmark their individual operations.

1.1.2 Opportunities and Challenges of AMS milking
Some of the potential benefits that the AMS offers includes an increase in milk production, improved cow comfort, an improved lifestyle for producers, and reduced labour requirements. A non-economic factor in the decision to transition to AMS is producer quality of life. In a survey study of Canadian AMS producers, it was reported that producers experienced more time flexibility, a decrease in labour-intensive work, and simpler employee management (Tse et al., 2018). Along with an improvement in quality of life, producers also reported greater job satisfaction, better working conditions, and an increase in production, profit, and efficiency (Tse et al., 2018). Interestingly, one benefit of transitioning from conventional milking systems to AMS observed by Tse et al. (2017) was that the average herd size increased by 10%. This increase in herd size may be due to a decrease in time required to milk cows daily, thus producers were able to increase herd size without increasing workload. Illness detection in AMS housed cows may be improved as the software generates cow-level data. Across 217 AMS producers, Tse et al. (2017) reported that 80% of producers perceived that illness detection was simpler because of the data provided by the AMS. These data generated by the AMS can include body weight, body temperature, udder health, and reports in milking, activity, and rumination. The AMS software will create alert and alarms if there is a major deviation in the baseline behavioural or production data of the cow. These data preceding illness diagnosis are more useful for earlier intervention if they can predict the risk of infection or detect disease more efficiently prior to clinical symptoms developing (King et al., 2018). Overall, it appears that most producers are satisfied with their decision to transition and would recommend AMS to other dairy producers (Tse et al., 2018).
While these benefits of transitioning to AMS are promising, it includes an increase in capital cost and changes management strategies to be more data based. Adapting to the information collected by the robot may prove challenging to managers transitioning to AMS. In a survey of 217 Canadian producers who transitioned to AMS, Tse et al. (2017) reported that approximately 20% of producers had difficulty in detecting changes in cow health. A potential cause for this is that AMS producers may not be spending as much time with the cows to visually detect health changes, even though theoretically they should have more time to observe cows (Tse et al., 2017). It may also be caused by producers not visually looking at the udder of a cow twice daily, as they would in a conventional system during milking, and solely relying on the information collected by the AMS for udder health.

AMS herds may also be more susceptible to the negative consequences of lameness. This is because a cow with painful feet or legs might be less willing to voluntarily visit the AMS. Lame cows have been observed to have a lower milking frequency than sound cows (Jacobs and Siegfried, 2012). Lameness frequency has been reported in AMS freestall herds to range from 15 to 26% (King et al., 2016). Matson et al. (2022) observed that clinical lameness prevalence (locomotion score ≥ 3) in AMS herds was 28.3% across 75 Ontario AMS herds. In a study of 41 commercial AMS herds in Canada, lame cows (lameness score ≥ 3) produced 1.6 kg/d less milk and had 0.3 fewer milkings/d (King et al., 2017). A reduction in the willingness of cows to visit the AMS decreases the efficiency of the system and increases labour requirements as cows must be fetched to visit the unit. Thus, AMS producers must be mindful of the lameness incidence in their herds due to the impacts on production characteristics.
1.1.3 Cow Behaviour in AMS

Milking cows with an AMS provides them the opportunity to set their own milking, feeding, and resting schedules within their herd. Milking behaviour in cows housed in AMS is dependent on the willingness of the individual to voluntarily enter the milking unit. Cows housed in an AMS can be milked more than twice daily, which may increase daily milk yield, as cows being milked greater than twice daily typically produce higher quantities of milk (Svennersten-Sjaunja and Pettersson, 2008). Antagonistic behaviours surrounding the AMS unit may also impact a cow’s willingness to be milked. Hopster et al. (2002) observed that low-ranking cows may be forced by social competition to visit the AMS at night. This behavioural change may result in an increased variability in the milking interval for low-ranking cows, which could have an adverse effect on milk production (Bach and Busto, 2005). Variability in milking interval length may cause a decrease in milk yield due to a decrease in apparent milk synthesis rate (Bach and Busto, 2005). This negative response in milk yield has been shown to decrease linearly in multiparous cows with increased variability in milking interval length (Bach and Busto, 2005).

Another factor that has been shown to affect the milking behaviour and milk yield of dairy cows in AMS is the number of robots per pen. When comparing pens with 1 or >1 AMS/pen, Tremblay et al. (2016) observed that farms with 2 or more AMS units/pen had greater milk yield than those with 1 AMS/pen. The difference may be due to fewer antagonistic interactions between dominant and subordinate cows when trying to visit the AMS unit (Siewert et al., 2018). This agrees with Tremblay et al. (2016) who suggested that a possible cause for an increase in milk yield when 2 AMS units are available per pen is that submissive cows can access one of the units when the other is being monopolized by a dominant cow.
It has been suggested that in AMS individual cows will develop their own unique daily time budgets for feeding and drinking patterns (Melin et al., 2005). Regardless of the housing system, feeding behaviour typically follows a diurnal pattern, where fewer cows are feeding and more cows are lying overnight (Wagner-Storch and Palmer 2003; Jacobs and Siegford, 2012). An increase in total daily feeding time has been observed by Hart et al. (2013) when comparing cows milked 2x/d vs. 3x/d. Milk production is largely driven by feed intake and the nutrients obtained from digestion (Johnston and DeVries, 2018), thus an increase in milk yield may promote greater feeding activity. Additionally, DeVries et al. (2003) noted that cows are motivated to feed around the time of milking, where an increase in milking frequency may also increase daily meals. Wagner-Storch and Palmer (2003) observed more consistent percentages of AMS milked cows feeding compared to parlour milked cows. The increase in feeding consistency observed in AMS housed cows may be a result of each cow developing their own unique time budget within a herd setting. The proposed individuality in feeding behaviour of AMS housed cows does not, however, outweigh the need for adequate feed bunk space. Deming et al. (2013) observed that every 10-cm increase in feed bunk space per cow was associated with a 1.7 kg/d increase in milk yield in a study of 13 commercial AMS farms. Further, Matson et al. (2021) reported that every 10-cm increase in feed bunk space per cow was associated with a 0.3 kg/d increase in milk yield in their study of 197 commercial AMS farms. These results support the importance of feed bunk space in AMS housed cows. In addition to milking frequency, milk yield, and feed bunk space, feeding frequency has been shown to alter feeding behaviour. Greater feed delivery frequency results in increasing cow consumption of the TMR throughout the day, increasing total daily feeding time (DeVries et al., 2005). Frequency of PMR delivery
was positively associated with greater milk fat synthesis in the mammary gland in Canadian AMS herds (Castro et al., 2022). This association is likely due to an increase in eating bouts, decrease in meal size, and an improved ruminal environment which favors the synthesis of de novo fatty acids (Castro et al., 2022). Therefore, cows housed in an AMS may have more consistent feeding patterns than conventionally milked cows, particularly in situations where there is adequate feed availability.

Lying time is arguably the most important part of a cows time budget, as it is when milk is produced and a greater proportion of daily rumination occurs (McWilliams et al., 2022). Lying has been shown to have priority over feeding when cows were simultaneously deprived of both (Metz, 1985). Cows deprived of lying time have fewer eating bouts and total daily eating time, resulting in slug feeding and potential digestive disorders (Pineiro et al., 2019). Additionally, depriving lying time can have negative effects on the welfare of dairy cows (Cooper et al., 2007). Average lying time in AMS housed cows has been reported to be 11.5 ± 0.9 h/d (King et al., 2016) and 11.7 ± 1.1 h/d (McWilliams et al., 2022). This is slightly higher than the 10.6 h/d reported by Solano et al. (2016), but lower than the 11.9 h/d observed by Gomez and Cook (2010), both of which were observing freestall housed cows milked in a conventional system. From these data, there does not appear to be a difference in total daily lying time between freestall housed cows milked in AMS vs. conventional systems. King et al. (2016) observed that AMS housed cows transitioned from lying to standing 9.3x/d, with an average lying bout length of 76 min. On 41 commercial AMS herds, daily lying time was positively associated with the frequency of feed push-ups (King et al., 2016). Further, King et al. (2016) reported that herds with deep-bedded stalls had lying bouts that were 11.5 min greater than herds with mattress
stalls. Therefore, management and housing factors can affect total daily lying time, lying bout length, and number of lying bouts per day in cows housed in AMS herds.

1.1.4 AMS Housing Factors

Additional housing factors that may affect the productivity of AMS herds include those that are related to lameness. As previously mentioned, an increase in lameness prevalence can have negative effects on daily visits to the AMS (King et al., 2017). In an observational study of 75 Ontario AMS herds, Matson et al. (2021) reported that feed bunk space was negatively associated with clinical lameness prevalence. Those researchers reported that for every 10-cm increase in feed bunk space per cow, there was an associated 1.7% decrease in clinical lameness prevalence (Matson et al., 2022). This relationship may be explained by research by Deming et al. (2013), who demonstrated that feed bunk space was positively associated with an increase in lying time. Thus, cows who have greater access to the feed bunk may have less idle standing time, and more lying time, reducing the risk of lameness. While increasing lying time may decrease the risk of lameness, bedding type has also been observed to be a lameness risk factor. The use of sand bedding in AMS freestalls has been associated with a 9.3% lesser clinical lameness prevalence when compared to organic-substrate bedding (i.e. straw) (Matson et al., 2022). Sand is an ideal bedding type as it is comfortable, limits bacterial growth, has a cool surface temperature, and reduces cow slippage in alleys (Bewley et al., 2017). Bedding type has also been associated with milk quality in AMS herds. Matson et al. (2022) also reported that sand bedding tended to be associated with a 27,126 cell/mL lesser SCC than herds bedding with organic substrates. These results further promote the benefits of greater feeding and lying comfort in AMS herds.
1.1.5 AMS Management Factors

The perceived maximum cows per AMS have been reported to be $\leq 60$ cows/AMS (Jacobs and Siegford, 2012). A more recent study reported that the average number of cows/AMS was $47.5 \pm 14.9$ across 197 Canadian dairy herds (Matson et al., 2021), which is similar to the 49 cows/AMS reported by King et al. (2016). It is interesting to note that while the perceived capacity of an AMS is 60 cows, the observed densities on commercial dairies in Canada are lower. As the number of cows per robot increases, milkings per day may decrease and time spent in the robot (boxtime) will increase (Tremblay et al., 2018). Matson et al. (2021) reported that for each additional 10 cows per AMS unit there was a 0.15 unit decrease in total number of milkings per cow per day. This may be the reason that in commercial settings, there are fewer than 60 cows per AMS. Although, cows with faster milk speed can be in a pen with a higher cow density per AMS without negatively impacting milk production because they can be milked more efficiently. Thus, determining the density of cows per AMS to maximize milk production should involve the relationships between boxtime, milking speed, and number of milkings/d (Tremblay et al., 2018).

AMS units can deliver programmed portions of concentrate to cows during their milking visits. Motivation for cows to visit the AMS has typically revolved around offering concentrates to cows in the milking unit. A highly palatable feed could be a strong motivator for a cow to visit the AMS and build a positive association between concentrate delivery and the AMS (Madsen et al., 2010). An additional benefit of the AMS is that the amount and type of concentrate provided can be customized to each cow, which may minimize fetching and allow for precision feeding (Bach and Cabrera, 2017). When cows were presented with the choice to be milked or to eat,
Prescott et al. (1998) observed that a cows’ motivation to be milked was weak compared to her motivation to eat. Thus, offering larger quantities of concentrate in the AMS may increase voluntary milking visits, which would theoretically increase milk production and decrease fetching (Schwanke et al., 2019). The relationship of increasing concentrate allocation to increase milk visits however is not definite. When offering either 6.3 kg/d concentrate in the AMS and 0 kg/d concentrate in the PMR or 3.1 kg of concentrate at the AMS and 3 kg/d of concentrate in the PMR, Schwanke et al. (2019) observed an increase in milk visits for cows receiving a greater quantity of concentrate in the AMS (5.9 vs. 4.6 visits/d). Although, other researchers offering different quantities of concentrate at the AMS have not reported a difference in milking frequency between treatment groups (Bach et al., 2007; Henriksen et al., 2018; Hare et al., 2018). These differences in findings may be because increasing programmed feed does not guarantee that it will be delivered, or guarantee that it is consumed (Hare et al., 2018). This is because the total programmed allocation may not be delivered, and if the concentrate is delivered it is not guaranteed that it will be consumed. Several researchers have reported that cows were consistently delivered less concentrate than was programmed and of the concentrate delivered, not all is consumed (Bach et al., 2007; Bach and Carbrera, 2017). One reason for which cows may not meet their targeted AMS concentrate intake is because they may not be able to physically consume the concentrate fast enough. It has been demonstrated that cows can reasonably consume 250-400 g concentrate/min (Kertz et al., 1981), and the average cow takes 6-8 min to be milked in an AMS (Castro et al., 2012). Thus, during a milking visit we can reasonably assume a cow will consume about 1.75 kg concentrate. Therefore, if the average cow has 3 successful milking visits/d (Matson et al., 2022) she will likely be able to consume, on
average, 5.25 kg of concentrate/d delivered through the AMS. Efficiently managing feed tables and concentrate delivery in the AMS to limit feed waste involves both the rate of consumption and the number of milking visits per day. Although, increasing the quantity of concentrate in the AMS may increase milk yield (Halachmi et al., 2005; Henriksen et al., 2018). The increase observed in milk yield may be simply caused by an increase in energy intake by cows who are consuming more concentrate in the AMS.

Designing a feeding program can be difficult in AMS herds because if an individual cows milking frequency decreases, the amount of concentrate she will be able to consume in the AMS will also decrease. Additionally, there is a substitution effect on DMI, whereby cows consuming greater quantities of concentrate in the AMS is accompanied by a decrease in PMR intake. PMR intake was reported to decrease by 1.14 kg/d for every 1 kg/d intake of concentrate in the AMS (Bach et al., 2007). This is higher than that observed by Schwanke et al. (2019) who reported a reduction of 0.63 kg/d PMR DMI for every 1 kg/d DMI increase of AMS concentrate. In a feed first forced-flow traffic system, Hare et al. (2018) observed that PMR intake decreased by 1.58 kg for every additional 1 kg increase in concentrate intake. Further, when offering either 3 kg/d or 6 kg/d (DM basis) AMS concentrate, Schwanke et al. (2022) observed that PMR intake decreased by 0.54 kg per 1 kg of AMS concentrate allocated. The differences in the rate of substitution may be due to differences in PMR energy density (Menajovsky et al., 2018). Additionally, the digestibility of the PMR may affect the substitution effect, where a more digestible PMR will have a lower substitution effect. Further, the age, production, and physiological status of the cow may also contribute to differences in the substitution effect of PMR and concentrate (Schwanke et al., 2019). Feeding management in AMS herds also revolves
around the management of the feed bunk. Offering high quantities of concentrate in the AMS and low concentrate levels in the PMR may encourage cows to selectively consume more energy-dense components of the PMR (sorting) (Hare et al., 2018). Sorting behaviour can result in cows consuming a diet that is drastically different from that which was formulated and delivered (Miller-Cushion and DeVries, 2017), which may have negative implications on milk production. Thus, feed management in the AMS involves the interactions between AMS concentrate consumption and consumption of the PMR.

1.1.6 Transition Cow Disorders in AMS Herds

As mentioned, the AMS collects cow-level production and behavioural data that can be utilized to identify health problems (King et al., 2017b). Daily rumination time has been shown to decline with health disorders including metabolic disorders (Soriani et al., 2012). When combining rumination, activity and body weight data, King et al. (2017b) detected associations between these behavioural indicators and health disorders in AMS cows, including displaced abomasum, pneumonia, SCK, and metritis. As cows can milk more frequently in an AMS this may affect her metabolic and immune status in early lactation (Jacobs and Siegford, 2012). An increase in daily milkings often leads to an increase in milk yield (Wagner-Storch and Palmer, 2003), which may also increase the risk of NEB and development of SCK (King et al., 2018). Further, a survey of Canadian AMS herds reported that AMS herds have a greater prevalence of high milk BHB than conventionally milked herds (Tatone et al., 2017). The apparent increase in hyperketonemia prevalence for cows housed in AMS herds may also increase their risk of further health disorders. Maladaptation to a NEB, resulting in hyperketonemia, is associated with various diseases, reproductive disorders, and decreases in milk yield (Raboisson et al., 2014).
Therefore, understanding the signs, symptoms and causes of transition diseases can allow for more individualized management of transition cows housed in AMS herds.

1.2 Transition Disorders in Lactating Dairy Cows

1.2.1 Adipose Tissue and Negative Energy Balance

Dairy cows over the last century have been genetically selected to produce greater quantities of milk in their lactation. These selection pressures have resulted in physiological changes that facilitate greater mobilization of adipose tissue reserves compared to their unselected counterparts (Roche et al., 2009). Since homeostatic control is not the only regulator of lipid metabolism during early lactation, increasing dietary energy intake does not prevent body lipid mobilization (Roche et al., 2009). Thus, attempts to reduce tissue mobilization in early lactation cows by feeding energy-rich diets may not always be successful in maintaining adipose tissue reserves. Circulating non-esterified fatty acids (NEFA) are mobilized from adipose tissues, where triglycerides are converted into NEFA (Drackley, 1999). Once mobilized, NEFA attaches to serum albumin to be transported to various energy requiring tissues (Stipanuk, 2000), including peripheral tissues, mammary tissue, or the liver. The mammary gland can use NEFA as a source of milk fat during early lactation (Drackley, 1999), which can act as a sink for circulating NEFA. The liver either oxidizes NEFA as an energy source through β-oxidation or re-esterifies NEFA into triglycerides (Drackley, 1999). When fatty acid mobilization is accelerated in periods of energy deficit, β-oxidation becomes less efficient and hepatic cells create ketone bodies (Herdt, 2000). This is an important process in early lactation, when cows are in a state of negative energy balance (NEB), as organs that cannot metabolize fatty acids rely
on ketone oxidation to produce energy (Stipanuk, 2000). NEFA that are re-esterified into triglycerides are released into circulation via very low-density lipoproteins (Roche et al., 2009). During periods of NEB, the hepatic re-esterification of NEFA increases, although the export rate of triglycerides does not increase (Bauchart, 1993). This can result in an accumulation of triglycerides, which is often referred to as “fatty liver”, which impairs liver function (Roche et al., 2009). Specifically, fatty liver has been reported to impair the gluconeogenic activity of the liver, lowering blood glucose and decreasing insulin secretion (Adewuyi et al., 2005). This in turn would lead to greater lipid mobilization, increased fatty acid uptake by the liver, and increased ketogenesis (Adewuyi et al., 2005); furthering the severity of the NEB. Thus, reducing the incidence and severity of NEB may result in improved liver metabolism and reduced risk of fatty liver.

NEB can be identified by elevated serum NEFA concentrations in early lactation, where values exceeding 0.7 mM after calving may indicate severe NEB (Drackley, 1999). Elevated NEFA concentrations and maladaptation to NEB can lead to increased concentrations of BHB (Drackley et al., 2001), which can result in clinical ketosis (KET) or subclinical ketosis (SCK). SCK is defined as elevated concentrations of ketone bodies without clinical symptoms (Andersson, 1988), and can be identified by measuring BHB concentrations in the blood in early lactation (Raboisson et al., 2014). In early lactation, if blood BHB concentrations are ≥1.2 mmol/L it is indicative of SCK (McArt et al., 2012) and if serum NEFA concentrations are >1.0 mmol/L it can indicate that the cow is mobilizing body fat and may be at risk for SCK (Raboisson et al., 2014). Cows with SCK can have lower milk production (McArt et al., 2012), lower reproductive performance (Walsh et al., 2007), and may be at higher risk of developing
other health disorders (Lacasse et al., 2018). Duffield et al. (2009) reported that cows with BHB concentrations ≥1.2 mmol/L in the first week of lactation were associated with increased risks of displaced abomasum and metritis, and if serum BHB was ≥1.4 mmol/L in wk 1 or 2 post-partum, cows were at a higher risk of developing KET. In severe cases of NEB, where the cow is unable to adapt to changes in energy demands, KET may develop and is characterized by blood BHB concentrations being >3.0 mmol/L (McArt et al., 2012). It is estimated that it costs over $200 CAD per cow being treated for SCK or KET, when factoring reductions in milk yield and reproductive success, and veterinary treatment (Gohary et al., 2016). In a Canadian study, an average of 30% of dairy cows were affected by SCK (Duffield et al., 1998) and 5% experience KET at least once during lactation (Kelton et al., 1998). Therefore, due to the health and economic consequences of hyperketonemia, a consequence of maladaptation to NEB, management strategies to reduce hyperketonemia prevalence have the potential to benefit the producer and cow.

One such strategy to reduce the risk of hyperketonemia in early lactation is to manage cow body condition score (BCS). BCS profile of dairy cows is similar to an inverted milk lactation curve, where BCS declines in the first 100 DIM followed by an increase in BCS into dry-off (Roche et al., 2006). It is well established that management factors including feeding rate and diet type will affect cow BCS (Roche et al., 2009). Over-feeding energy in the dry period has been well documented to have negative effects on fresh cow performance and metabolic status in the transition period (Janovick and Drackley, 2010). Roche et al. (2007) observed that greater BCS at calving was associated with a greater loss in BCS in early lactation and a lesser rate of regain in BCS. This may be due to an increase in mobilization of adipose tissue, which may
increase the concentrations of circulating NEFA, a symptom of NEB. Further, over conditioned cows who have BCS $\geq 3.5$ (based on a scale of 1 - 5, Wildman et al., 1982) at calving have been associated with an increased risk of SCK in early lactation (McArt et al., 2015). A further consideration in limiting BCS gain pre-calving is managing late lactation energy intake. For example, over supplying energy in late lactation may also contribute to an increase in BCS and subsequent lesser performance in the following lactation (Janovick and Drackley, 2010). This is especially important for first-parity animals who have been observed to have lesser ability to regain BCS, and it has been suggested that they require preferential management during late lactation (Roche et al., 2009). Therefore, consistently monitoring BCS, especially in the dry period, is an important management consideration to improve cow performance in the transition period and reduce the risk of SCK.

### 1.2.2 Sub-Acute Ruminal Acidosis

Cows in early lactation are also more susceptible to another metabolic disease called sub-acute ruminal acidosis (SARA). SARA has been linked to feed intake depression, fluctuations in daily DMI, reduced diet digestibility, gastrointestinal damage, milk fat depression, diarrhea, laminitis and liver abscesses (Plaizier et al., 2008; Abdela, 2016). Depressed rumen pH has also been linked to lower milk yield and milk fat yield (Antanaitis et al., 2019), both important production parameters for dairy cows. SARA has been characterized by the ruminal pH being between 5.2-5.8 for extended periods of time (Li et al., 2019). SARA can be caused by a multitude of factors, one of which is the transition from a high forage dry cow diet to a lower forage lactating diet after calving (DeVries et al., 2009), which does not allow rumen microbes
and papillae to acclimate to the higher levels of dietary starch (Stone, 2004). SARA can also be induced by offering large quantities of concentrates in lactation (Keunen et al., 2002), which favors the build-up of lactate-producing bacteria, thus lowering rumen pH. The drastic changes in diet composition during the transition period can take 7 to 14 d for rumen adaptation, which causes a lag in DMI in early lactation (Grant et al., 2015). The consequential shift in ruminal volatile fatty acid (VFA) production results in a decrease in acetic acid production and an increase in propionic and butyric acid production (Antanaitis et al., 2019). It is estimated that SARA affects 19 to 29% of dairy cows in early and mid-lactation (Krause and Oetzel, 2006). Further, Garrett et al. (1997) reported that SARA prevalence in early and mid-lactation were 19% and 26%, respectively. Mitigating SARA in today’s industry is challenging as increasing dietary starch is positively associated with milk yield, until rumen pH decreases below 5.8 (Maekawa et al., 2002). Additionally, early lactation cows housed in AMS may receive concentrates that are high in starch to increase daily energy intake and motivate cows to visit the AMS (Schwanke et al., 2019). Although, offering these concentrates that are high in starch in early lactation may increase the risk of developing SARA. Thus, feed ingredients which increase energy intake, with minimal effects on rumen pH, could prove beneficial to early lactation dairy cow health and production.

### 1.2.3 Feeding Strategies to Reduce NEB in AMS Herds

In addition to offering concentrates to attract cows to visit the AMS (Schwanke et al., 2019), AMS herds can offer specific formulations of concentrate to individual cows housed in a group setting based on individual needs. This is known as precision feeding, which typically targets cows based on stage of lactation or milk production, to better meet their energy
requirements. The individual variation in milk yield response to concentrate intake is sufficient
to economically justify precision feeding (André et al., 2010), thus it is a strategy that should be
implemented on AMS herds. A common feeding strategy in AMS herds is to feed a low level of
concentrates at calving, then increase the concentrate offered linearly (Kokkonen et al., 2004).
Around lactation peak, AMS feed tables typically match milk yield, as milk yield increases
concentrate allowance increases (André et al., 2010). While AMS feed management has
improved since those early studies, it has been suggested that feeding management on AMS
herds contributes to an increased prevalence of NEB compared to conventionally milked herds
(Tatone et al., 2017). This phenomenon may be supported by King et al. (2018), who observed
that milk yield relative to the amount of AMS concentrate consumed was associated with higher
BHB concentrations and was greater for cows diagnosed with SCK. Those authors concluded
that AMS concentrate allocation settings need to account for milk production of cows during
their first 3 wk of lactation (King et al., 2018). Offering a combination of 2 or more concentrates
through the AMS may maximize returns by balancing the proportions of concentrate offered
based on milk yield, stage of lactation, and body weight (Bach and Cabrera, 2017). This could be
especially beneficial to transition cows, as they are typically in a negative energy balance. This is
especially relevant to AMS herds as when conducting an observational study of the within-herd
prevalence of SCK on 795 Ontario dairy herds, Tatone et al. (2017) reported a 21% SCK
prevalence based on first test DHIA milk BHB. Interestingly, those researchers observed that
herds milking with AMS were associated with a 5% higher within-herd SCK prevalence and
multiparous cows had higher odds of SCK at the first test DHIA (Tatone et al., 2017). This
further highlights the potential problems surrounding NEB and SCK in AMS housed cows.
While increasing dietary energy through concentrate in early lactation may reduce the risk of NEB, the potential development of SARA must also be considered when formulating total rations. If total rations are formulated with NFC (non-fibre carbohydrates) exceeding 43.5%, SARA may develop in lactating dairy cows (Gozho et al., 2007). Past research has demonstrated that replacing dietary starch with dietary sugar improved rumen health, DMI, and milk production (Penner and Oba, 2009). It is plausible that offering dairy cows sugar sources in early lactation may improve energy balance without causing adverse ruminal effects. When Moore et al. (2020) supplemented liquid molasses through the AMS from 1 – 60 DIM, they observed that cows receiving molasses had fewer repeat diagnoses of SCK than control cows. An alternative consideration is to increase dietary gluconeogenic precursors, such as glycerol, which may improve transition cow adaptation to milk production (Aschenbach et al., 2010). Transition cows greatly benefit from increases in exogenous gluconeogenic precursor supply from direct provision of gluconeogenic feed additives (Aschenbach et al., 2010). Thus, glycerol supplementation may be beneficial as a potential source of energy that may enhance gluconeogenesis.

1.3 Glycerol Supplementation

1.3.1 Glycerol as a Feed Ingredient

Glycerol is a by-product of biodiesel production, and 10 L of crude glycerol is produced for every 100 L of biodiesel (Donkin, 2008). Crude glycerol contains 80 to 88% glycerol, with the remaining portions being primarily salts and methanol, which can be toxic if fed to animals (Donkin, 2008). Crude glycerol has a low economic value but can be further purified to 99%
glycerol content or higher, making it a potentially competitive livestock feed ingredient (Kholif, 2019). Additionally, several government policies are promoting the production of biodiesels, thus the supply of crude glycerol is expected to increase. Glycerol as a dietary feed ingredient for lactating dairy cows first began in the 70’s (Fisher et al., 1973) and has been demonstrated to improve metabolic health status in transition dairy cows (DeFrain et al., 2004). There have since been numerous research studies regarding the suitability of glycerol as a dietary supplement (e.g. DeFrain et al., 2004; Kass et al., 2013; Paiva et al., 2016; Van Soest et al., 2023). There are two methods in which glycerol is absorbed by dairy cows. Glycerol may either be passively absorbed from the rumen for gluconeogenesis or triglyceride synthesis (Werner-Omazic et al, 2015) or it may be rapidly fermented by microbes in the rumen to propionate and butyrate (Rémond et al., 1993). Glycerol can be included up to 10.8% DM of the diet without adverse ruminal effects, making it a safe dietary ingredient (Carvalho et al., 2011). Additionally, glycerol may have the potential to improve metabolism, feed efficiency, and can reduce ketosis symptoms (Kupcynski et al., 2020). Although, the impacts of glycerol supplementation on metabolic health status and milk production are not fully understood, and more research is required to fully understand these relationships.

1.3.2 Ruminal Fermentation of Glycerol

When glycerol is included in the diet, the rumen environment may be altered due to the fermentation of glycerol. The effects of glycerol fermentation in the rumen are, however, not fully understood. In a series of in vivo and in vitro studies, Werner-Omazic et al. (2014) reported that approximately 75% of the glycerol administered in liquid solutions can escape rumen fermentation and be available for gluconeogenesis. Whereas intraruminal glycerol has been
reported to decrease ruminal pH and decrease the acetate to propionate ratio (Kijora et al., 1998). A decrease in the acetate to propionate ratio has also been reported by Paiva et al. (2016) when crude glycerol was offered at 70, 140, or 210 g/kg of diet DM. Alternatively, when Kristensen and Raun (2007) infused glycerol into the rumen, they reported a decrease in acetate and an increase in butyrate, while propionate was not affected. When drenching cows with glycerol, DeFrain et al. (2004) reported an increased proportion of ruminal butyrate. The varying effects of glycerol intake on ruminal VFA concentrations may be due to different doses, the purity of the glycerol, and how the glycerol was consumed (drenched or fed) (Kholif, 2019). It is difficult to determine the proportions of glycerol that are absorbed vs. fermented in the rumen (Kholif, 2019), though the maximal rate of glycerol disappearance in the rumen can range from 1.2 to 2.4 g/h (Rémond et al., 1993). This is similar to results from Kristensen and Raun (2007), who reported that approximately 10% of the administered glycerol was recovered as glycerol in the portal vein and used by the liver for glucose synthesis. The alterations in rumen VFA proportions due to glycerol fermentation may affect daily feed intake. Feed intake has been reported to decrease when glycerol is included in dry cow diets (DeFrain et al., 2004) and lactating cow diets (Ezequiel et al., 2015; Paiva et al., 2016). Van Soest et al. (2023) reported that cows receiving glycerol for 21 d prepartum consumed 0.8 kg/d more DM however cows receiving glycerol for 21 d postpartum consumed 0.5 kg/d less DM. The decrease in feed intake when glycerol is fed to dairy animals has been attributed to the high energy production and satiety, due to an increase in total ruminal VFA production and an increased flow of VFA to the liver (Trabue et al., 2007). Additionally, the high energy density of glycerol can influence oxidation reactions in the liver (Kholif, 2019). The satiety signals may be explained by the hepatic oxidation theory, where
hepatic fuel oxidation causes ATP concentrations in hepatocytes to increase, which sends inhibitory signals through the vagus nerve to signal satiety (Allen et al., 2009).

The main objective when administering glycerol in the diet of dairy cows is to treat and/or prevent NEB in transition cows. Glycerol is an appropriate ingredient for improving metabolic health status, as there is evidence that it increases blood glucose levels (Kholif, 2019). If glycerol is directly absorbed in the rumen epithelium or small intestine, it will be converted into glycerol-3-phosphate and used to drive gluconeogenesis in the liver (Rojek et al., 2008). When crude glycerol (80.2%) was delivered as a drench, greater plasma glucose levels have been observed in transition dairy cows (Goff and Horst, 2001). When top dressing crude glycerol (80.2%) at 0, 0.43, or 0.86 kg/d from -14 to 21 DIM, DeFrain et al. (2004) observed that plasma glucose tended to increase in the post-partum period. Early research around delivering glycerol by oral drenching or through the concentrate in early lactation concluded that serum BHB and NEFA concentrations decreased during supplementation (Johnson, 1954; Fisher et al., 1973). More recently, when feeding pure glycerol (99%) to lactating buffalo, Saleem et al. (2018) observed a decrease in the concentrations of serum BHB and NEFA. When supplementing 250.9 g/d of a glycerol product, Van Soest et al. (2023) reported that in the first week postpartum cows receiving glycerol experienced lower serum NEFA concentrations compared to control cows. Although, there is also evidence that glycerol supplementation does not alter blood BHB and serum NEFA levels. When Omazic et al. (2013) supplemented 0.5 kg/d of both crude (88.1%) and pure glycerol (99.5%) for the first 4 weeks of lactation, they did not observe a change in plasma BHB. Similarly, Kass et al. (2013) orally drenched primiparous cows 500 mL of crude glycerol (82.6%) from 4 to 21 DIM, and they did not observe a difference in serum BHB
concentrations. Those researchers did, however, observe an interaction of treatment and time on serum NEFA concentration where serum NEFA concentrations in the treatment group were lower than in the control group from 4 to 9 DIM (Kass et al., 2013). Further, when injecting 15 mg of glucagon and orally administering 400 mL of pure glycerol (99%) simultaneously for the first 14 d postpartum, Osman et al. (2010) observed decreased plasma NEFA concentrations for both weeks. Van Soest et al. (2023) reported similar results where cows receiving 259 g DM of a glycerol product (99.9% purity) either pre- or postpartum had reduced serum NEFA concentrations compared to control cows. A decrease in circulating NEFA concentrations may indicate that the cow is mobilizing less adipose tissue, meaning they have an improved metabolic health status (Drackley, 1999). The main reasons for the inconsistency among the relationship between glycerol supplementation and metabolic health status have been attributed to the quality of the crude glycerol, the rate of glycerol fermentation in the rumen, and the quantity of glycerol that is directly absorbed in the rumen epithelium and is metabolized in the liver (Paiva et al., 2016). These results warrant further investigation into the potential benefits of supplementing glycerol to transition cows to improve their metabolic health status.

1.3.3 Glycerol and Milk Production

The most important metric of performance for dairy producers is the effect of supplementation on total milk production and milk component yield. Increasing the energy density of the diet for lactating dairy cows is expected to improve lactation performance, yet the relationship between glycerol supplementation and milk yield is inconsistent. Glycerol supplementation has been shown to increase (Kass et al., 2013; Omazic et al., 2013), decrease (Paiva et al., 2016), and not affect (Ezequiel et al., 2015) milk yield. Omazic et al. (2013)
reported that milk yield tended to increase when cows were offered pure glycerol (>99% purity) but offering crude glycerol (88.1% purity) had no effect on milk yield. Similarly, Lomander et al. (2012) reported an increase in milk yield in cows who were fed 450 g pure glycerol (99.5% purity) in the first 90 DIM. When crude glycerol (80.6% purity) was fed at 21% for 21 days to mid lactation cows, Paiva et al. (2016) reported a decrease in milk yield. This is contrary to results reported by Ezequiel et al. (2015), who when offering crude glycerol (83% purity) to mid lactation cows, observed no difference in milk production. Khalili et al. (1997) also reported no change in milk production when feeding glycerol at 3.6% of total dietary DM (purity not reported) to mid lactation cows. It is interesting to note that the increases in milk yield by Omazic et al. (2013) and Lomander et al. (2012) were observed when glycerol was supplemented in early lactation, whereas milk yield either did not change (Ezequiel et al., 2015; Khalili et al., 1997) or decreased (Paiva et al., 2016) when glycerol was supplemented in mid lactation cows. In support of this, it has been suggested that stage of lactation during glycerol supplementation may affect the response (Paiva et al., 2016). Therefore, glycerol supplementation may be more beneficial to early lactation cows, rather than mid to late lactation cows.

The impact of glycerol supplementation has also been reported to impact milk fat concentration. Ezequiel et al. (2015) observed that supplementing crude glycerol (83% purity) linearly reduced the yield of fat corrected milk. Milk fat concentration may decrease due to increases in the propionate to acetate ratio due to glycerol fermentation (Carvalho et al., 2011), as acetate is the main precursor for de novo milk fat production. Another theory for the reduction in milk fat production is if cows receiving glycerol have lower concentrations of circulating NEFA it is an indication of lower lipid mobilization and lipolysis (Ezequiel et al., 2015). This
may depress milk fat yield, as the mammary gland has been reported to act as a sink to circulating NEFA, where circulating NEFA accounts for around 40% of milk fat during early lactation (Bell, 1995). In support of this, when supplementing a pure glycerol product (99.9% purity), Van Soest et al. (2023) reported that cows receiving glycerol postpartum had less preformed fatty acid concentrations than control cows, indicating that control cows were mobilizing more adipose tissue. When supplementing pure glycerol (99.7% purity) to mid lactation cows, Bajramaj et al. (2017) observed a significant decrease in milk fat yield and milk fat content. These researchers hypothesized that the decrease in milk fat is caused by an increase in CLA formation in the rumen and a CLA-mediated decrease in mammary expression of genes associated with lipogenesis (Bajramaj et al., 2017). When replacing high-moisture corn with pure glycerol (99.5% purity) at 10.8% of the ration, Carvalho et al. (2011) observed that milk fat content was unchanged. Increased energy intake has previously been observed to decrease milk fat yield and concentration (Sutton, 1989); therefore, the lack of response observed by Carvalho et al. (2011) may be because they offered diets with the same energy density. Alternatively, Ezequiel et al. (2013) observed that milk fat content followed a quadratic curve, where it was highest at 0% and 30% crude glycerol (83% purity) inclusion, and lowest at 15% inclusion. These authors speculated that this was due to a dose response, where an animal’s metabolism switches to a higher lipid mobilization profile when dietary starch content drops below a certain threshold (Ezequiel et al., 2013). The effects of glycerol supplementation on milk fat percent and yield are, therefore, not necessarily due to glycerol itself, but an increase in total energy intake, or an alteration of ruminal VFA profiles.
1.4 Thesis Objectives and Hypotheses

The adoption of AMS may increase daily milk yields, reduce labour requirements, and improve production efficiency (Tse et al., 2017). There are still, however, management issues that need to be investigated including the management of fresh cows. It has been demonstrated that AMS herds in Ontario have an increased prevalence of within-herd SCK prevalence (Tatone et al., 2017) and the quantity of concentrate delivered to fresh cows may not be adequate to mitigate NEB (King et al., 2018). Overall, the adoption of the AMS may result in increased risk of NEB, which may be mitigated through precision feeding strategies (Bach and Cabrera, 2017). Offering a specific type and quantity of concentrate to fresh cows delivered through the AMS may alleviate the effects of NEB. Formulating the AMS concentrate with glycerol, which can be metabolized into gluconeogenic precursors, may further mitigate the effects of maladaptation to NEB as it has been suggested to reduce ketosis symptoms (Kupcynski et al., 2020). Additionally, glycerol can be included up to 10.8% DM of the ration without adverse ruminal effects (Carvalho et al., 2011), thus reducing the risk of SARA development in early lactation. Glycerol supplementation has also been linked to an increase in daily milk yield (Kass et al., 2013), although the purity, dose, method of administration, and stage of lactation may all affect the response (Paiva et al., 2016).

Therefore, the objective of this thesis research was to determine the effects of glycerol supplementation to early lactation cows, delivered through the AMS concentrate, on the metabolic health status, milking behaviour, and productivity of AMS housed cows. It was
hypothesized that supplying glycerol to early lactation cows will improve their energy balance in early lactation and increase their milking behaviour and milk yield up to mid lactation.
2 CHAPTER 2: EFFECT OF GLYCEROL SUPPLEMENTATION IN EARLY LACTATION ON METABOLIC HEALTH, MILKING ACTIVITY, AND PRODUCTION OF DAIRY COWS IN AUTOMATED MILKING SYSTEM HERDS.

2.1 INTRODUCTION

The use of automated milking systems (AMS) has grown exponentially across the dairy industry over the last 20 yr. The continued adoption of AMS has been attributed to the potential decreases in daily labor and improved quality of life, milk production, and cow health (Tse et al., 2017; 2018). Despite this growth, there are still gaps in the literature on how to effectively manage lactating cows housed in facilities with AMS. The success of an AMS depends on cows voluntarily visiting the unit to be milked multiple times per day. The motivation for a cow to be milked compared to other behaviours is intrinsically low (Prescott et al., 1998), thus, AMS units can deliver programmed quantities of concentrate to attract the cow to the unit (Madsen et al., 2010). Additionally, delivering concentrate through the AMS allows a specific quantity and type of concentrate to be delivered to an individual cow, commonly referred to as precision feeding (Bach and Cabrera, 2017). Precision feeding may be particularly beneficial to fresh cows milked with AMS, who may be at an increased risk of excessive negative energy balance compared to conventionally-milked cows. For example, Tatone et al. (2017) reported a higher within-herd SCK prevalence for cows in AMS herds in Ontario, Canada compared to conventionally milked cows, as detected through elevated milk BHB levels. Elevated ketones on these farms may be an
artifact of greater milk production (Jacobs and Siegford, 2012). King et al. (2018) demonstrated that cows on AMS farms who were diagnosed with elevated blood BHB in the first 3 wk postpartum initially had greater milk yield, but by 21 DIM that difference was gone. This may be due to an initial increase in milk production in early lactation for AMS cows that was not matched with an increase in concentrate delivered through the AMS. In support of this, King et al. (2018) also observed that those cows with higher milk yield, and associated increased energy demands, were not offered more concentrate in the AMS during that time period. This would suggest that there may be situations where inadequate AMS concentrate is supplemented in early lactation relative to the milk production needs of the cows. Additionally, cows with SCK may be less motivated to voluntarily visit the AMS than metabolically healthy cows. Milking frequency differences have previously been observed for healthy cows versus cows diagnosed with SCK in free-traffic AMS herds (King et al., 2018). Thus, improving the energy balance of fresh cows in AMS may also improve their voluntary milking visits.

Modifying the energy density of feedstuffs offered to fresh cows, to support increased energy demands and lower dry matter intake (DMI) postpartum, is a common strategy to mitigate the effects of NEB. However, energy-dense feed ingredients utilized are typically greater in starch, which may increase the risk of developing sub-acute ruminal acidosis (SARA) (Antanaitis et al., 2019). This may be especially problematic for AMS cows who consume individual meals of high-starch concentrate at the AMS (Bach and Cabrera, 2017). Thus, offering alternative energy sources, rather than rapidly fermentable starches that are less disruptive to the rumen could be beneficial. For example, Moore et al. (2020) supplemented liquid molasses for the first 60 DIM through an AMS and observed a decrease in repeat SCK diagnoses for cows.
receiving the liquid molasses supplementation. Delivering other alternative energy sources in the AMS concentrate may be another strategy to combat the effects of NEB. Glycerol is a gluconeogenic precursor that was first examined as a treatment for ketosis in the 1970’s (Fisher et al., 1973). As glycerol is a byproduct of the biofuel industry, the availability has increased leading to more utilization (Kholif, 2019). Researchers have demonstrated that pure liquid glycerol (99.5%) can be included up to 10.8% DM in lactating dairy cow rations without adverse ruminal effects (Carvalho et al., 2011). There are mixed results on the effects of glycerol supplementation on the metabolic status of fresh cows. When supplementing 0, 430, or 860 g/d of crude glycerol (80.2% purity) from -14 to 21 DIM, DeFrain et al. (2004) reported that plasma glucose tended to increase in the post-partum period, while BHB and NEFA concentrations were unchanged. When supplementing a dry glycerol powder (99.9% purity, 66% glycerol), 21 d prepartum (averaging 261 g/d DM) and for 21 d postpartum (averaging 251 g/d DM) in a factorial design, Van Soest et al. (2023) reported that during the first week postpartum, cows that received glycerol (pre- or postpartum) had lower serum NEFA concentrations compared to control cows. Further, cows that received glycerol postpartum tended to produce milk with lesser preformed fatty acid concentrations and yield, and lost the least BW from -21 to 21 DIM (Van Soest et al., 2023). These results indicate potential improvement in the metabolic health status of fresh cows receiving glycerol supplementation.

Alterations in metabolic health status when supplementing glycerol may affect the milk production of lactating cows. When offering 250 g/d of pure glycerol (>99% purity) for the first 4 wk of lactation, Werner Omazic et al. (2013) reported that milk yield tended to increase during that time period. Lomander et al. (2012) also reported an increase in milk yield when
supplementing 450 g/d glycerol (99.5% purity) for the first 90 DIM. Alternatively, Paiva et al. (2016) reported a decrease in milk yield when crude glycerol (80% purity) was fed at 210 g/kg DM of the ration. When Ezequiel et al. (2015) offered 0, 15, or 30% of ration DM as crude glycerol (83% purity) to mid lactation cows, they observed no difference in milk yield while milk fat was highest at 0 and 30% glycerol inclusion. The differences in responses to milk yield may be due to the purity of the glycerol, the stage of lactation when supplementation occurred, and the quantity and duration of supplementation.

The objective of this study was to determine the effects of dry pure glycerol supplementation blended in the AMS concentrate to fresh cows for the first 21 DIM on markers of metabolic health, milking behaviour, and productivity in commercial AMS herds. It was hypothesized that the supplementation of dry pure glycerol through the AMS concentrate would reduce the effects of NEB in early lactation cows, as evidenced by improved blood markers of metabolic status, as well as an increase in successful milkings at the AMS and an increase in daily milk yield.

### 2.2 MATERIALS AND METHODS

#### 2.2.1 Animals, Housing, and Management

This study utilized 389 cows, including 160 primiparous and 229 multiparous (3.25 ± 1.32 lactations, mean ± SD), from 5 commercial AMS dairy herds (Table 1) in southwestern Ontario (Canada). Cows were enrolled 21 d before their expected calving date and were observed up to 150 DIM in that subsequent lactation. Farm demographics, including number of
AMS units per farm, average milking herd size, average milking frequency, and average daily milk yield are presented in Table 1. Cow enrollment began May 2022 and ended November 2022, and data collection ended May 2023. The use of cows and experimental procedures complied with the guidelines of the Canadian Council on Animal Care (2009) and were approved by the University of Guelph Animal Care Committee (AUP#4493). Cows were removed from the study at the discretion of the participating producers if the animal experienced severe health disorders (e.g. lameness/leg injuries, milk fever) that may have prevented the cow from visiting the AMS for an extended period of time. Similarly, cows were excluded from the study if they experienced severe distress at calving (e.g. cesarean-section, severe lameness, and milk fever).

All cows were dried off ~60 d before their expected calving. All farms fed a single dry cow TMR, except for farm 4, which fed a close-up dry cow TMR 21 d prior to expected calving (Table 2). On all farms, cows were moved to a calving pack approximately 14 d prior to their expected calving date. Following calving, cows were moved (in 4 of the 5 farms) to a fresh cow pen for 3-21 d post calving. At one farm, fresh cows were moved into the main lactating pen immediately following calving. All farms had free-stall housing; farms 1, 3, 4, and 5 had sand bedding and farm 2 had mattress-based stalls, bedded with wood shavings. Stocking density on all the farms never exceeded 1 cow per stall in any of the dry or lactating cow pens. Cows were milked by AMS units on all farms [4 farms with Lely Industries N.V., (Maassluis, The Netherlands) units, and 1 farm with DeLaval, Tetra Laval Group, (Tumba, Södermanland, Sweden) units]; all farms’ cows had free flow traffic access to the AMS units. The AMS settings, including maximum milkings/d and optimal milk yield/milking, were recorded for each farm, and are presented in Table 3. Feed allowance tables, with minimum concentrate programmed
reported (as fed), in the AMS software are shown by farm and parity in Table 4. All herds fed a pelleted concentrate through the AMS (Table 5). Further, all herds fed single PMR rations to the lactating cows (Table 6), which were formulated to meet the specific production characteristics at each specific farm (Table 7).

2.2.2 Experimental Design

Across herds, we conducted a randomized control trial where 21 d prior to calving, cows were randomly assigned to 1 of 2 post-partum treatments within farm, controlling allocation for parity (primiparous (PP) and multiparous (MP)). Cows were programmed by the researchers onto the AMS feed tables 21 d prior to calving, thus producers were blinded to treatment assignment, on each farm with treatment assignments alternating between the two treatments based on expected calving date. Sample size and power analyses were used to calculate (as per Morris, 1999) the minimum number of replicates needed per treatment (n = 200) to detect a 7.5% level of observed mean difference between treatments for the continuous outcome variables, including milk yield, milking frequency, milk composition, and rumination time. Estimates of variation (average CV = 38%) for these variables were based on previously reported values (Moore et al., 2020). Treatments consisted of: 1) control group (CON), which were fed the standard AMS concentrate on each farm from 1-150 DIM and 2) glycerol group (GLY), which were fed a treatment AMS concentrate that consisted of the base formulation of the standard AMS concentrate on each farm plus a minimum 250 g/d (as fed) of dry glycerol powder formulated into the standard concentrate (e.g. based on 4 kg/d as-fed intake, the concentrate was formulated to include 62.5 g glycerol product/1 kg concentrate on an as fed basis) for the first 21
DIM (Table 8), after which cows received the farms’ standard AMS concentrate from 22-150 DIM. The 2 treatments were applied to individual cows upon calving and up to 21 DIM. The first 21 DIM was considered the treatment period, and from 22 to 150 DIM was considered the follow-up period. The dry glycerol powder (glycerol) that was formulated into the treatment concentrate contained 66% pure (99.9% purity) glycerol (United States Pharmacopeia grade) and 34% silica (carrier) and flavouring agents (PROBIOTECH International Inc., Saint-Hyacinthe, QC, Canada). The silica carrier and flavoring agents were not included in the standard concentrate. The addition of the glycerol to the concentrate slightly diluted the rest of the ingredients of the concentrate, thus feed tables for GLY cows were constructed to offer 250 g/d as fed more concentrate/d in the first 21 DIM to mitigate the dilution (actual = 270 ± 4 g, as fed, more concentrate delivered/d). Besides the additional 250 g/d increase in concentrate programmed in the first 21 DIM, GLY cow AMS feed tables were designed the same as the base feed table on each farm (Table 4). Calibration of the AMS feed scale was completed 1x/month at each farm on all their AMS units to ensure accurate amounts of AMS concentrate were being delivered. The daily allotment of glycerol for GLY cows was split equally by the AMS across milkings/d, as per the AMS software. There were 213 CON cows and 214 GLY cows initially enrolled in the study. Of the GLY cows, 38 cows did not meet the minimum treatment requirement of 250 g/d as-fed of glycerol in the first 21 DIM and, therefore, their data were not included in the analysis. Thus, the analysis included data from 213 CON cows and 176 GLY cows (Table 1).
2.2.3 Feed Samples and Analyses

Duplicate dry cow TMR and lactating cow PMR feed samples and singular AMS concentrate samples (base and treatment) were taken 1x per month at each farm for analyses of nutrient composition and particle size distribution (for TMR and PMR). Following collection, feed samples were frozen at -20°C for later analyses, at which point they were thawed for 24 h before analyses.

The TMR and PMR samples collected for particle size analyses were separated using a 4-screen Penn State Particle Separator (PSPS; Maulfair and Heinrichs, 2013), which separated the sample into 4 fractions based on particle size: long (>19mm), medium (8 to 19 mm), short (4 to 8 mm), and fine (<4mm). Separated portions were then oven dried at 55°C for 48 h (Table 2; Table 7).

TMR, PMR, the standard concentrate and the treatment concentrate that were collected for nutrient composition analyses were oven dried at 55°C for 48 h, then ground to pass through a 1-mm screen (Model 4 Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ, USA). Ground samples were pooled by farm and month, and sent to A&L Laboratory Services Inc. (London, ON, Canada) for analysis of ash (550°C; AOAC International, 2000, method 942.05), ADF (AOAC International, 2000, method 2002.04), CP (N x 6.25; AOAC International, 2000, method 990.03; Leco FP-628 Nitrogen Analyzer, Leco Corp., St. Joseph, MI), starch (heat-stable amylase and amylglucosidase; AOAC International, 2000, method 996.11), sugar (AOAC International, 2000, method 968.28), crude fat (using pet ether; AOAC International, 2000, method 920.39), lignin (using ADF residue and H₂SO₄), minerals (using aquaregia digestion,
inductively coupled plasma atomic emission spectroscopy), NDF with heat stable α-amylase and sodium sulfite (AOAC, 2000: method 2002.04) and calculation of net energy (using NRC, 2001 equations) (Table 2; Table 5; Table 7; Table 8).

2.2.4 Milking Characteristics, Components, and Analyses

Milking frequency, milking refusal frequency, concentrate delivered, and milk production for each milking visit from 1 to 150 DIM were recorded by the software associated with the AMS unit on each farm. In situations where cows were removed from the milking herd prior to the end of the follow-up period, only days with complete data were included in the analyses. Milk fat and protein were determined with the AMS manufacturer’s automatic sampling device on farms 1, 2, 3, and 4. Milking data were downloaded on a per milking visit basis for each cow from each farm for the duration of the trial. This data was downloaded in 2x/wk intervals and was spliced to ensure continuous data for each farm. This data was summarized on a per cow per day basis from 1 to 150 DIM.

2.2.5 Rumination, Health, and Blood Metabolite Data Collection

Rumination time was monitored continuously using an electronic rumination monitoring system (HR-TAG-LD, SCR Engineers Ltd.), as validated by Schirmann et al. (2009) on all farms. Rumination data was automatically recorded by the specific AMS software and was attached to cow identification collars. Rumination data was recorded for every cow upon parturition 24 h/d for the duration of the experiment (1 to 150 DIM). In cases where the rumination collars malfunctioned, or the cow was removed from the herd before the end of the
follow-up period, only days with complete data were included in the analysis. The data was stored in 2-h intervals and was summarized for each cow by day to calculate daily rumination minutes.

Blood samples were collected from each study cow during farm visits that occurred 2x/wk, to determine whole blood BHB, whole blood glucose, and serum NEFA concentrations. Farms were visited ±1 h on each visit to ensure consistency surrounding feeding schedules. To collect the blood sample, cows were restrained in either a headlock gate or freestall. Blood samples were collected from the coccygeal vein using 10 mL blood serum collection tubes (red top) on -7 DIM (actual -6.9 ± 0.25) (mean ± SD), 2 DIM (actual 1.9 ± 0.08), 5 DIM (actual 5.3 ± 0.09), 9 DIM (actual 8.9± 0.08 d), 12 DIM (actual 12.2 ± 0.09 d), and 15 DIM (actual 15.9±0.08 d). As validated by Kanz et al. (2015) and Wittock et al. (2013), respectively, BHB and glucose were analyzed cow-side at every sampling using a single drop of blood on a ketone test strip or glucose test strip and read with a Freestyle Precision Neo meter (Abbott Diabetes Care, Saint Laurent, QC, Canada). Glucose concentrations recorded from the meter were corrected using the equation [0.6 + (0.86 x glucometer reading)], as per Wittock et al. (2013). Following the BHB and glucose test, blood collection tubes were stored upright in a cooler until they were returned to the laboratory. The cooled blood collection tubes were centrifuged at 1,500 x g at 18°C for 15 min to separate the serum. The serum was aliquoted into duplicate 3 mL tubes (~1.5 mL in each) and frozen at -20°C until the time of analysis. Serum samples were sent to the Animal Health Laboratory, University of Guelph (Guelph, ON, Canada), to be analyzed for NEFA (reagent supplied by Randox Laboratories, Crumlin, UK) using a photometric test on the Roche Cobas 6000 c501 instrument (Roche, Basel, Switzerland). The NEFA serum analyses were conducted
on the samples taken at -7, 2, and 5 DIM relative to calving. Postpartum blood BHB was categorized as high (BHB ≥ 1.2 mmol) or normal (BHB < 1.2 mmol/L; McArt et al., 2012) in each of the five postpartum samples.

Body condition score (BCS) was recorded for each cow beginning at the day of enrollment (-21 d to calving), at calving, and every 21 d up to 63 DIM (4 post calving observations). Scores were determined using a 5-point scale, with 0.25 increments (Wildman et al., 1982). Prepartum BCS was categorized as high (≥3.5) or normal (<3.5) (Roche et al., 2009). Lameness score (LS) was recorded at the same intervals as BCS (-21, 0, 21, 42, and 63 d relative to calving). The LS were determined by using a 5-point scale as described by Flower and Weary (2006). The same observer assessed the BCS and LS of each cow at every sampling timepoint.

2.2.6 Calculations and Statistical Analyses

All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., Cary, NC). Prior to analyses, all data was assessed for normality using the UNIVARIATE procedure. Assumptions for normality were met for all variables except for daily milk refusals, which was normalized using the natural logarithm after adding +1 to each of the daily refusal values. Continuous outcome variables (AMS concentrate delivered, milking activity, milk yield, rumination activity, milk components, blood metabolites, BCS, and LS) were analyzed in linear regression models using the MIXED procedure of SAS and the categorical variable, categorized blood BHB, was tested in a logistic regression model using the GLIMMIX procedure. For all models, significance was declared at \( P \leq 0.05 \) and tendencies at \( P \leq 0.1 \). If the \( P \)-value of a
covariate (prepartum NEFA, categorized BHB, glucose, and categorized BCS) was ≤ 0.05 it was retained in the model, otherwise covariates were removed from the model.

To test the effect of treatment on AMS data (refusals, concentrate delivered, milking frequency, milk yield, rumination time, milk fat percent, milk fat yield, milk protein percent, and milk protein yield), data were summarized by cow, treatment, and day (DIM) and tested in linear regression models using the MIXED procedure of SAS. The treatment period (1-21 DIM) and the follow-up period (22-150 DIM) were analyzed separately; DIM was considered a repeated measure in each model. For all models, farm was considered a random effect and cow within farm was the subject of the repeated statement. Each of these models tested the fixed effect of treatment and DIM. These models also included the fixed effect of parity, which was retained in the model if the $P$-value was ≤ 0.05. The covariate of categorized prepartum BCS was included in the above models if the $P$-value was ≤ 0.05. Interaction terms tested included treatment × parity and treatment × DIM, which remained in the above-mentioned models if the $P$-value was ≤ 0.10. According to Schwarz’s Bayesian information criterion, covariance structure was selected for each individual model based on best fit; these included structures were compound symmetry and heterogenous first-order autoregressive.

To test the effect of treatment on serum NEFA, blood BHB, blood glucose, BCS, and LS, data were summarized by cow, treatment, and sample number and tested in linear regression models using the MIXED procedure of SAS. For these data, sample number was considered a repeated measure. Each of these models tested the fixed effect of treatment and sample. For the above models, farm was considered a random effect and cow within farm was the subject of the...
repeated statement. Covariates tested in the above models included the prepartum measures of categorized BCS, NEFA, categorized BHB, and glucose, and were retained in the model if the $P$-value was $\leq 0.05$. The fixed effects of parity were tested in each model, and remained if the $P$-value was $\leq 0.05$. Interactions remained in the model if the $P$-value was $\leq 0.10$. Interaction terms tested were treatment $\times$ parity and treatment $\times$ sample. According to Schwarz’s Bayesian information criterion, covariance structure was selected for each individual model based on best fit; these included structures were compound symmetry and heterogenous first-order autoregressive.

To determine treatment differences in the number of cows exceeding the cut off for SCK (BHB $\geq 1.2$ mmol/L) or normal (BHB $< 1.2$ mmol/L), a multivariable logistic regression analyses with a binary distribution and logit link using the GLIMMIX SAS procedure was used, where high blood BHB was treated as categorical (i.e. occurring yes or no). Treatment was considered a fixed effect in the model. This model also included the fixed effect of parity, while the categorized prepartum BCS (high vs. normal) was included as a covariate. A treatment $\times$ prepartum BCS interaction was detected, thus an odds ratio for high BHB based on prepartum BCS by treatment was calculated through the SLICE function in the GLIMMIX procedure.

2.3 RESULTS

During the first 21 DIM and from 22-150 DIM, the AMS concentrate delivery model included parity, DIM, and prepartum BCS as fixed effects (Table 9). During the treatment period (1-21 DIM), there was a treatment by DIM interaction for AMS concentrate delivery ($P<0.001$; Table 9), whereby differences were detected between treatments ($P<0.001$) from 2-14 DIM and
at 16 DIM (Figure 1). During the follow-up period (22-150 DIM), there was a treatment by DIM interaction for AMS concentrate delivery ($P=0.03$; Table 9), whereby GLY cows received more concentrate at 22 DIM and on 50% of the days between 60 and 150 DIM (Figure 1).

When accounting for the effects of parity and DIM, milking frequency (milkings/d) was greater for GLY cows compared to CON cows during the first 21 DIM ($P=0.03$; Table 9), where they had 0.1 more successful milkings per day (Figure 2). The increase in milking frequency was also present in the follow-up period (22-150 DIM; Table 9), where GLY cows milked 0.1 more times per day than CON cows ($P=0.047$; Figure 2). Cows were refused for milking at the AMS unit an average of 1.25 refusals/d from 1-21 DIM, during treatment application, and averaged 0.86 refusals/d from 22-150 DIM during the follow-up period, with no differences detected between treatment groups in either period when accounting for the effects of parity and DIM ($P=0.15$, $P=0.83$; Table 9).

GLY cows tended to produce 0.8 kg/d more milk in the 1-21 DIM treatment period than CON cows, when accounting for the effects of parity and DIM ($P=0.09$; Table 9; Figure 3). GLY cows had greater milk yield in the follow-up period (22-150 DIM; $P<0.001$; Table 9), where they yielded an average of 1.5 kg/d more milk than CON cows when accounting for the effects of parity and DIM (Figure 3). Milk fat content averaged 4.45% in the treatment period when accounting for parity and DIM, with no treatment differences detected ($P=0.30$; Table 10). Milk fat content averaged 3.75% in the follow-up period when accounting for the fixed effects of DIM ($P=0.15$; Table 10). There was a treatment by DIM interaction for milk fat content in the follow-up period ($P=0.001$; Table 10), whereby CON cows had higher milk fat content at 93, 94, 108,
Milk fat yield averaged 1.57 kg/d in the 21 d treatment period, with no treatment differences detected, when accounting for effects of parity and DIM \((P=0.84; \text{Table 10})\). During the follow-up period (22-150 DIM), milk fat yield averaged 1.68 kg/d, with no treatment differences detected when accounting for the effects of parity and DIM, and categorized prepartum BCS as a covariate \((P=0.42; \text{Table 10})\). There were no detected treatment differences in milk protein content in the treatment or follow-up periods, which averaged 3.47% and 3.11%, respectively, when accounting for the effect of DIM \((P=0.52; P=0.98; \text{Table 10})\). When accounting for parity and DIM, milk protein yield averaged 1.20 kg/d in the treatment period, with no detected difference between treatments \((P=0.47; \text{Table 10})\). GLY cows produced 0.04 kg/d more milk protein than CON cows during the follow-up period from 22-150 DIM, when accounting for the effects of parity, DIM, and prepartum BCS \((P=0.03; \text{Table 10})\).

No treatment differences in daily rumination time were detected in either the treatment \((P=0.89; 1-21 \text{ DIM})\) or follow-up \((P=0.71; 22-150 \text{ DIM})\) periods when accounting for parity and DIM (Table 9). There were no detected treatment effects \((P=0.78)\) on serum NEFA concentrations when accounting for the effects of parity, sample and prepartum NEFA \((P\leq 0.01; \text{Figure 4a})\). When accounting for the effects of parity and sample, there was a treatment by sample interaction \((P=0.03)\) for blood BHB concentrations (mmol/L) (Figure 4b), which revealed a tendency at 5 DIM for GLY cows to have a higher blood BHB concentration than CON cows \((P=0.06)\). When accounting for the effects of parity and sample, there was a tendency for a treatment by sample interaction \((P=0.09)\) for blood glucose concentrations (mmol/L)
(Figure 4c), whereby CON cows had higher blood glucose than GLY cows at 9 DIM ($P=0.03$) and at 12 DIM ($P=0.02$).

When categorizing blood BHB samples as either high ($\geq 1.2$ mmol/L) or normal ($<1.2$ mmol/L), there were 43 CON and 37 GLY cows with at least 1 incidence of high blood BHB postpartum. When categorizing prepartum BCS as either over-conditioned (BCS $\geq 3.5$) or normal (BCS $<3.5$), there were 118 CON and 91 GLY cows who were over-conditioned in the prepartum period. When further analyzed by prepartum BCS, there were 31 CON and 19 GLY cows with high prepartum BCS ($\geq 3.5$) and 12 CON and 18 GLY cows with normal prepartum BCS ($<3.5$) that had at least 1 incidence of high blood BHB postpartum (Figure 5). While accounting for the effect of parity, there was a tendency ($P=0.08$) for a treatment by categorized prepartum BCS interaction on incidence of high blood BHB. Specifically, CON cows with high prepartum BCS ($\geq 3.5$) were 3.5x more likely ($P=0.002$) to have a high BHB test compared to CON cows with a normal prepartum BCS ($<3.5$). The model also demonstrated that PP animals had a reduced risk of high BHB ($P<0.001$).

When accounting for sample day and BCS at calving, there was a treatment by sample day interaction ($P=0.05$) for postpartum BCS; mean comparison demonstrated that GLY cows had a higher BCS than CON cows at the fourth sampling (63 DIM; Figure 6). There were no treatment differences ($P=0.37$) on the average LS for cows in the postpartum period, which averaged $1.35\pm0.03$ across the 4 postpartum samples.
2.4 DISCUSSION

This is the first study, to our knowledge, to supplement dry glycerol through the AMS concentrate to fresh cows for the first 21 DIM. During the 21 d treatment period, and by design, GLY cows were delivered 270 ± 4 g/d more concentrate on an as-fed basis (249 g/d on a DM basis), reflecting the glycerol portion of the concentrate. Based on the glycerol specifications, those cows received 164 g pure glycerol on a DM basis. During the first 21 DIM, CON and GLY cows were delivered an average of 3.94 kg/d and 4.21 kg/d (as fed) of AMS concentrate, respectively. During this period, the AMS feed tables on farms 1, 2, 4, and 5 transitioned from a fixed feeding rate to a milk yield feeding rate, and farm 3 transitioned at 22 DIM. Once the production-based supplementation began, concentrate allocation was designed to increase linearly as expected daily milk yield increased, thus higher yielding cows were delivered more concentrate. The option of increasing AMS concentrate allocation early in lactation can be used to meet nutrient requirements for milk production and possibly minimize metabolic disorders at an individual cow level (King et al., 2018). The steady increase in concentrate delivered observed during the first 21 d can be attributed to the nearly 30 kg increase in daily milk yield from 1 to 21 DIM for all cows. Milk yield in the treatment period increased from <15 kg at 1 DIM to 44.5 kg and 43 kg at 21 DIM for GLY and CON cows, respectively. It is interesting to note that the commercial Ontario farms enrolled in this study all implemented production-based supplementation by 14 DIM, except for one herd, which transitioned at 22 DIM, whereas King et al. (2018) reported that all 8 commercial Ontario herds in their study supplemented for the first 21 DIM based on DIM and not milk production. Bach and Cabrera (2017) also noted that
common AMS feeding strategies involve a low level of concentrate in the first week of lactation, followed by a linear increase in early lactation, and production-based feeding beginning at 3 wk.

The average serum NEFA, blood BHB and blood glucose concentrations observed was 0.50 mmol/L, 0.63 mmol/L, and 3.13 mmol/L for CON cows and 0.48 mmol/L, 0.64 mmol/L, and 3.14 mmol/L for GLY cows in the current study. When supplementing 250.9 g/d of the same dry glycerol powder (99.9% purity) in the PMR for 21-d postpartum, Van Soest et al. (2023) reported that average serum NEFA, blood BHB, and blood glucose concentrations was 0.73 mmol/L, 0.97 mmol/L, and 2.69 mmol/L for control cows and 0.66 mmol/L, 0.88 mmol/L, and 2.69 mmol/L for glycerol cows. Comparing the blood metabolite values from the current study to those reported by Van Soest et al. (2023), regardless of treatment, cows in the current study had improved metabolic profiles as seen through decreases in serum NEFA and blood BHB and increases in blood glucose. Overall, the averages observed in the current study did not indicate a severe metabolic imbalance between treatment groups. However, in the current study GLY cows tended to have higher blood BHB at 5 DIM than CON cows and had lower blood glucose levels at 9 and 12 DIM than CON cows. Contrary to our findings, Chung et al. (2007) observed increased glucose and decreased BHB concentrations when 250 g/cow of a glycerol product was top dressed for the first 21 DIM. A potential reason for this difference may be related to no differences in milk yield observed by Chung et al. (2007), while the GLY cows in our study tended to have higher milk yield. This would have resulted in greater mammary uptake of glucose, as the mammary gland requires approximately 72 g of glucose to produce 1 kg of milk (Kronfeld, 1982), thus possibly explaining the lower blood glucose of our GLY cows. Van Soest et al. (2023) did not observe any treatment differences for postpartum blood glucose when
supplementing 251g DM of dry glycerol (99.9% purity) in the PMR for the first 21 DIM, but they did observe lower NEFA concentrations for cows receiving glycerol postpartum. Similar to our results, DeFrain et al. (2004) reported that glycerol supplementation (80.2% purity) decreased plasma glucose concentrations at 7 DIM and from 14 to 21 DIM for cows receiving 430 g/d and 860 g/d of glycerol respectively. Further, DeFrain et al. (2004) also reported a treatment by day interaction for BHB concentrations, whereby cows receiving 860 g/d of glycerol had greater BHB concentrations from 7 to 21 DIM. These previous results, as well as the current, may be explained by the fermentation patterns of glycerol in the rumen. Researchers have previously reported that glycerol supplementation can increase concentrations of ruminal butyrate (Rémont et al., 1992; Khalili et al., 1997). Further, Linke et al. (2004) reported that either feeding or drenching glycerol increased the percentage of ruminal butyrate and plasma BHB. Increases in ruminal concentrations of butyrate, induced by glycerol fermentation, may contribute to alimentary ketogenesis (i.e. conversion of absorbed butyrate to ketone bodies; Bergman, 1971). Thus, it is plausible that the ruminal fermentation of glycerol to butyrate increased blood BHB for cows supplemented with glycerol in the current study.

In the current study 20.6% of cows had at least one test of high BHB (BHB ≥ 1.2 mmol/L) in the first 21 DIM, which is indicative of SCK (McArt et al., 2012). This is similar to global averages (22.7%, Loiklung et al., 2022), but lower than previously reported in AMS herds (32.7%, King et al., 2018; 49.4%, Moore et al., 2020). Despite the incidence of high BHB being comparatively low in the current study versus other AMS studies, there was still an impact of feeding glycerol in the first 21 DIM. CON cows with high prepartum BCS (i.e. ≥ 3.5) were 3.5x more likely to test positive for SCK than CON cows with normal prepartum BCS, whereas the
risk for GLY cows was the same regardless of prepartum BCS. It is well established that cows with high BCS (i.e. over-conditioned) at calving are at an increased risk of SCK (Gillund et al., 2001). Therefore, the lack of increased risk of high BHB in over-conditioned GLY cows may be due to a protective effect from glycerol supplementation in the first 21 DIM, where glycerol reduced the need for adipose lipolysis (Karlsson et al., 2019). In support of this, cows on the GLY treatment had higher BCS at 63 DIM, compared to CON cows, suggesting that they were able to maintain condition better during the first 63 DIM. This reduced fat mobilization may be further supported by the numerical decrease in serum NEFA concentrations at 2 and 5 DIM for GLY cows compared to CON cows. Thus, glycerol supplementation in early lactation may have reduced the severity of NEB, particularly in those over-conditioned cows that are prone to that, and the consequential down stream effects.

In our study, GLY cows tended to produce 0.8 kg/d more milk than CON cows in the first 21 DIM. This increase in milk yield continued past the treatment period, where from 22-150 DIM, GLY cows yielded 1.5 kg/d more milk than CON cows. Other researchers have reported varied results when supplementing glycerol on milk production, where increases (Kass et al., 2013) decreases (Paiva et al., 2016), and no effects (Van Soest et al., 2023) have been reported. When top-dressing 450 g of glycerol (99.5% purity) for 90 DIM, Lomander et al. (2012) reported an increase in milk yield during the first 90 DIM when supplementation occurred. It is interesting to note that increases in milk yield previously reported occurred when glycerol was supplemented in early lactation (Werner Omazic et al., 2013; Kass et al., 2013; Lomander et al., 2012), whereas decreases in milk yield were observed when glycerol was supplemented to mid-lactation cows (Paiva et al., 2016). Thus, stage of lactation may affect the response to glycerol.
supplementation (Paiva et al., 2016), specifically, supplementation in early lactation may be more beneficial than supplementation in mid or late lactation. In the current study, GLY cows likely produced more milk during the 21-d treatment period due to an improvement in their metabolic health status. It has been previously suggested that a successful adaptation to the onset of lactation and subsequent NEB can provide a successful and productive lactation, whereas maladaptation can lead to impaired milk yield (Duffield et al., 2009). Further, in the first 21 DIM and from 22-150 DIM, GLY cows had a 0.1 increase in milkings/d compared to CON cows. When conducting an observational study of 75 commercial Ontario AMS herds, Matson et al. (2022) reported that every additional 0.1 milkings/d was associated with a 0.57 kg/d increase in milk yield. Thus, an improvement in the metabolic health status during the treatment period along with an increase in milking frequency for GLY cows are both likely contributing to the 0.8 kg/d and 1.5 kg/d increase in milk yield during the treatment and follow-up periods respectively.

With no detected differences in AMS refusals/d, GLY cows had 0.1 more successful milkings/d than CON cows during the 21-d treatment period. While offering concentrate through the AMS is primarily designed to act as a motivation for cows to voluntarily visit the AMS (Prescott et al., 1998), there is little scientific evidence that increasing AMS concentrate allocation increases voluntary visits. When offering either 3.5 kg/d or 5 kg/d of AMS concentrate, Halachmi et al. (2005) did not observe an increase in voluntary daily milkings. Alternatively, when offering either 3.0 kg/d DM or 6.0 kg/d DM of AMS concentrate to mid lactation PP cows, Schwanke et al. (2019) observed a 0.5 milkings/d increase in milking frequency for cows receiving more AMS concentrate. The varied responses reported in the literature suggest that the 0.27 kg/d (as fed) increase in AMS concentrate delivered in the current
study was likely not large enough to affect voluntary daily milkings, as this increase is much lower than reported in other studies (1.5 kg/d, Halachmi et al., 2005; 5 kg/d, Bach et al., 2007; 3 kg/d DM, Schwanke et al., 2019). Maintaining consistent voluntary AMS visits involves multiple factors; for example, daily AMS milking frequency has also been associated with lameness prevalence on AMS herds (Miguel-Pacheco et al., 2014; King et al., 2017). We did not observe treatment differences in LS, thus an improvement in soundness is not likely causing the increase in milking frequency observed for GLY cows. In the first 21 DIM, GLY cows tended to have an increased milk yield. In an AMS system, milking allowances are determined by expected milk yield, DIM, and parity. An increase in the expected milk yield would lower the minimum time between milkings, thus the increase in milk yield for GLY cows may have lowered the minimum time between milkings, allowing them to be milked more frequently.

Milking frequency and milking refusals followed a similar trend in the follow-up period as was seen during the first 21 DIM. From 22-150 DIM GLY cows had 3.4 milkings/d compared to CON cows with 3.3 milkings/d, with no difference in daily refusals. It is likely that the increase in successful milkings was driven by the increase in daily milk yield, where the milking intervals were shorter for higher yielding cows, thus they had the opportunity to be milked more frequently. Greater milk yield for GLY cows in the follow-up period may be driven by the observed increase in milking frequency during the first 21 DIM. Dahl et al. (2004) observed that cows being milked 6x/d vs. 3x/d in the first 21 DIM, followed by 3x/d milking for the remainder of the lactation, had higher milk yields which persisted throughout the lactation. This improvement in milking persistency has also been observed by Bar-Peled et al. (1995) who tested 6 x/d milking up to 42 DIM. Further, Hale et al. (2003) reported that 21 d of 4x/d milking
followed by 2x/d milking increased milk yield persistency compared to 2x/d milking in the first 21 DIM. Although these differences in milking frequencies are much larger than that observed in the current study (3.4 vs. 3.3 milkings/d), the potential implications remain. It is noteworthy that the proportional increase in milking frequency was similar to the increase in milk yield from 22-150 DIM; specifically, GLY cows had a 3% increase in milking frequency and a 3.5% (1.5 kg) increase in milk yield compared to CON cows. Thus, the increase in milking frequency may be contributing to the increase in milk yield in the follow-up period, when GLY cows were not being supplemented with glycerol.

GLY cows transitioned from consuming 4.87 kg/d (as fed) of the treatment concentrate at 21 DIM to consuming 0 kg/d of the glycerol concentrate and 4.58 kg as-fed/d of the standard AMS concentrate at 22 DIM. From 22-150 DIM, GLY cows continued to receive the standard AMS concentrate. At 22 DIM GLY cows were delivered less AMS concentrate than CON cows. This was likely due to the setting managing maximum single day increases for one AMS concentrate type, thus at 22 DIM when the pellet type transitioned, the quantity of standard concentrate did not reach the total programmed for the individual cow in 1 d. At 23 DIM the standard concentrate delivered to GLY cows was not restricted by the maximum daily increase setting in the AMS software. For the entire follow-up period, 22-150 DIM, all cows were on a milk yield-based feed table, whereby increases in milk yield were associated with increases in concentrate delivered. For 50% of the days from 60-150 DIM, GLY cows were delivered more AMS concentrate. As AMS concentrate delivery is based on milk yield, DIM, parity, and milking frequency of each individual cow, the increase in milk yield and milking frequency for
GLY cows compared to CON cows likely caused the interaction observed in the current study for AMS concentrate delivery.

There was no treatment difference observed for rumination time in either period of the current study. Cows averaged 520.5 min/d in the first 21 DIM and 576.5 min/d from 22-150 DIM of daily rumination. The daily rumination time observed in the current study is higher than reported in a similar study by Moore et al. (2020), who reported average daily rumination for AMS cows in the first 60 DIM of 474.8 min/d for control cows and 476.9 min/d for molasses supplemented cows. Rumination time for AMS housed cows has been reported in the past at 558 ± 41 min/d (mean ± SD) for mid lactation cows (McWilliams et al., 2022), and 492.6 ± 98.7 min/d (mean ± SD) for healthy cows (King et al., 2017). The lack of treatment differences on daily rumination time is somewhat surprising as GLY cows consumed more AMS concentrate and produced more milk, suggesting an increase in daily DMI as milk yield is largely driven by nutrients consumed (Johnston and DeVries, 2018). This may however be explained by a substitution effect (Schwanke et al., 2019), whereby the increase in AMS concentrate delivered to GLY cows could have been associated with a decrease in PMR intake. While we were unable to measure individual PMR DMI, cows being supplemented with glycerol in past research have been observed to have decreased DMI (DeFrain et al., 2004). When supplementing the same dry glycerol powder (99.9% purity) at a similar feeding rate to our own study, Van Soest et al. (2023) observed that cows receiving glycerol postpartum consumed 0.6 kg DM/d less PMR during the first 21 DIM during supplementation and 1.1 kg DM/d less PMR from 22-42 DIM after supplementation, with no effect on milk yield. Further, Ezequiel et al. (2015) reported a decrease in DMI and 3.5% fat corrected milk when crude glycerol (83% purity) was
supplemented at 0, 15, and 30% of ration DM. Although it was not possible to measure
individual feed intake on these commercial farms, given there were no treatment differences in
daily rumination time, it suggests that PMR intake likely did not differ between treatments, and
the increase in milk yield was potentially driven by the increase in AMS concentrate intake.

Except for milk protein yield in the follow-up period, there were no treatment differences
detected in milk composition in the current study. Researchers have previously observed
decreases in milk fat percent and yield when cows were supplemented with glycerol from -14 to
21 DIM (DeFrain et al., 2004) and beginning at 114 ± 29 DIM (Ezequiel et al., 2015). When
supplementing crude glycerol (83% purity), Ezequiel et al. (2015) observed a quadratic effect
where milk fat content was highest at 0% and 30% glycerol inclusion, and lowest at 15%
glycerol inclusion. Further, Van Soest et al. (2023) reported that cows who did not receive
glycerol supplementation in the first 21 DIM had the highest concentration and yield of
preformed fatty acids, suggesting that they mobilized more adipose tissue. Excessive adipose
tissue mobilization results in elevated concentrations of circulating NEFA, which can be
transferred to milk as a source of preformed fatty acids (Hostens et al., 2012). A decrease in
adipose tissue mobilization for GLY cows, as indicated by decreased BCS loss and numerically
lower NEFA concentrations in the current study, would suggest that GLY cows should have
lower milk fat concentration in the early part of lactation. While there was no significant
difference in milk fat concentrations, there was a numerical 0.1% decrease in milk fat percent for
GLY cows in both the treatment (1 – 21 DIM) and follow-up (22 – 150 DIM) periods. Despite
the concentration of milk fat being slightly lower, milk fat yield was likely maintained due to the
increases in daily milk yield. There were no treatment differences in milk protein content in the
current study. This is consistent with previous research, as varied results in milk protein response to glycerol supplementation have been documented. Harzia et al. (2013) reported decreases in milk protein concentration with up to 3 kg/d of dietary crude glycerol (82.6% purity), whereas Kass et al. (2013) observed no difference in milk protein content when drenching 573 g/d of crude glycerol (82.6%). Further, Ezequiel et al. (2015) did not observe differences in milk protein content or yield when supplementing crude glycerol (83% purity) at 0, 15, and 30 % ration DM. When supplementing 0, 200, or 400 g/d of crude glycerol (80-85% purity), Boyd et al. (2013) observed an increase in milk protein content for cows receiving 400 g/d. With no observed change in milk protein content, the increase in milk protein yield observed (+ 0.04 kg/d) in the follow-up period can likely be attributed to the greater daily milk yield of the GLY cows.

2.5 CONCLUSION

The supplementation of dry pure glycerol through the AMS concentrate for the first 21 DIM appeared to have some positive impacts on indicators of energy metabolism and on maintenance of BCS in dairy cows. Specifically, supplementation reduced the risk of elevated BHB in over-conditioned cows, and also resulted in less BCS loss to 63 DIM. Postpartum supplementation of pure glycerol resulted in greater milking frequency and a tendency for increased milk yield during the treatment period (1 to 21 DIM) and greater milking frequency and milk yield during the follow-up period (22-150 DIM). Overall, these results indicate that pure glycerol supplementation in AMS concentrate can be used to improve the metabolic health
status of early lactation cows, as well as their milking behaviour and milk yield through to mid lactation.

2.6 ACKNOWLEDGEMENTS

Thank you to the farm owners at staff who assisted throughout the duration of the study (Southern Ontario, Canada). Thank you to Sarah Bruner, Jess Brasier, Maddy McLennan, Michelle Brower, and Kris Lutz for their help during farm sample collection and sample processing (Guelph, ON, Canada). Funding and research support were received from Priobiotech International, the Natural Sciences and Engineering Research Council of Canada, and Highly Qualified Personnel from the Ontario Ministry of Agriculture, Food and Rural Affairs. The authors have not stated any conflicts of interest.
### Table 1. Number of automated milking systems, cows enrolled, and average milk yield and frequency by parity and treatment by farm.

<table>
<thead>
<tr>
<th></th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of AMS per farm</td>
<td>4</td>
<td>3</td>
<td>11</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Average lactating herd size</td>
<td>140</td>
<td>130</td>
<td>450</td>
<td>140</td>
<td>370</td>
</tr>
<tr>
<td>Total number of cows enrolled</td>
<td>72</td>
<td>85</td>
<td>115</td>
<td>89</td>
<td>28</td>
</tr>
<tr>
<td>Number of PP CON cows</td>
<td>21</td>
<td>15</td>
<td>29</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Number of MP CON cows</td>
<td>28</td>
<td>27</td>
<td>28</td>
<td>30</td>
<td>17</td>
</tr>
<tr>
<td>Number of PP GLY cows</td>
<td>13</td>
<td>16</td>
<td>28</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Number of MP GLY cows</td>
<td>10</td>
<td>27</td>
<td>30</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>Milk yield PP (kg/d)</td>
<td>35.8</td>
<td>37.2</td>
<td>39.3</td>
<td>34.3</td>
<td>-</td>
</tr>
<tr>
<td>Milk yield MP (kg/d)</td>
<td>48.1</td>
<td>48.7</td>
<td>52.0</td>
<td>52.6</td>
<td>39.0</td>
</tr>
<tr>
<td>Milking frequency PP (milking/d)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.2</td>
<td>3.3</td>
<td>-</td>
</tr>
<tr>
<td>Milking frequency MP (milking/d)</td>
<td>3.3</td>
<td>3.6</td>
<td>3.4</td>
<td>3.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

1. Average daily milk yield and milk frequency from 0 to 150 DIM.
2. Parity = primiparous (PP) or multiparous (MP)
3. Treatments consisted of 1) Control= standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dried glycerol (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22-150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.
4. Only multiparous cows were enrolled at Farm 5.
5. The milk yield (kg/d) presented is from all cows enrolled in the study from 1-150 DIM.
Table 2. Nutrient composition\(^1\) (mean ± SD) of the dry cow total mixed rations (TMR) fed by farm.

<table>
<thead>
<tr>
<th>Item, % DM</th>
<th>Dry Cow TMR on each farm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1(^3)</td>
</tr>
<tr>
<td>DM %</td>
<td>52.4 ± 5.41</td>
</tr>
<tr>
<td>CP</td>
<td>13.3 ± 1.11</td>
</tr>
<tr>
<td>ADF</td>
<td>28.9 ± 5.35</td>
</tr>
<tr>
<td>NDF</td>
<td>41.9 ± 6.73</td>
</tr>
<tr>
<td>NDFD48</td>
<td>51.6 ± 2.67</td>
</tr>
<tr>
<td>TDN</td>
<td>66.4 ± 4.17</td>
</tr>
<tr>
<td>Lignin</td>
<td>4.8 ± 1.50</td>
</tr>
<tr>
<td>Starch</td>
<td>15.7 ± 4.11</td>
</tr>
<tr>
<td>Sugar</td>
<td>2.3 ± 1.03</td>
</tr>
<tr>
<td>Fat</td>
<td>3.4 ± 0.80</td>
</tr>
<tr>
<td>Ash</td>
<td>8.9 ± 0.96</td>
</tr>
<tr>
<td>Ca</td>
<td>1.2 ± 0.25</td>
</tr>
<tr>
<td>P</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td>K</td>
<td>1.5 ± 0.20</td>
</tr>
<tr>
<td>Na</td>
<td>0.5 ± 0.13</td>
</tr>
<tr>
<td>Mg</td>
<td>0.5 ± 0.16</td>
</tr>
<tr>
<td>NE(_L), Mcal/kg of DM(^2)</td>
<td>1.51 ± 0.10</td>
</tr>
</tbody>
</table>

\(^1\)Values were obtained from chemical analysis of TMR samples.

\(^2\)NE\(_L\) is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.

\(^3\)Farm 1 dry cow TMR particle distribution (% of DM): 35±10% long (>19mm), 31±6.8% medium (8 to 19mm), 16±2.0% short (4 to 8mm), 17±2.1% fine (<4mm).
Farm 2 dry cow TMR particle distribution (% of DM): 19±5.5% long (>19mm), 42±3.5% medium (8 to 19mm), 17±1.1% short (4 to 8mm), and 21±3.8% fine (<4mm).

Farm 3 dry cow TMR particle distribution (% of DM): 15±5.1% long (>19mm), 42±2.4% medium (8 to 19mm), 18±1.9% short (4 to 8mm), and 25±3.3% fine (<4mm).

Farm 4 dry cow TMR particle distribution (% of DM): 25±5.1% long (>19mm), 41±2.4% medium (8 to 19mm), 15±1.5% short (4 to 8mm), and 19±5.6% fine (<4mm).

Farm 5 dry cow TMR particle distribution (% of DM): 16±3.5% long (>19mm), 43±0.6% medium (8 to 19mm), 16±1.4% short (4 to 8mm), and 25±3.2% fine (<4mm).
Table 3. Milk settings (maximum number of milkings/d and optimal milk yield per milking) by farm and parity over the first 150 DIM for all study cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIM range</td>
<td>0-30</td>
<td>30-150</td>
<td>0-50</td>
<td>50-150</td>
<td></td>
</tr>
<tr>
<td>Max. milkings (milkings/d)</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Minimum yield/milking (kg/milking)</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIM range</td>
<td>0-30</td>
<td>30-150</td>
<td>0-50</td>
<td>50-150</td>
<td>0-60</td>
</tr>
<tr>
<td>Max. milkings (milkings/d)</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Minimum yield/milking (kg/milking)</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

¹Only multiparous cows were enrolled at Farm 5.
Table 4. Milking feed tables\(^1\) (automated milking system concentrate programmed during milking) by farm and parity over the first 150 DIM, treatments\(^2\) consisted of control and glycerol cows and were applied over the first 21 DIM.

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primiparous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIM range</td>
<td>0-8 9-150</td>
<td>0-8 9-150</td>
<td>0-21 22-150</td>
<td>0-14 15-150</td>
<td>-</td>
</tr>
<tr>
<td>Concentrate (kg/d as fed)</td>
<td>2.8 2.5</td>
<td>3.5 4.5</td>
<td>4 4.5</td>
<td>2.5 4.5</td>
<td>-</td>
</tr>
<tr>
<td>Milk yield table (Y/N)(^4)</td>
<td>N Y</td>
<td>N Y</td>
<td>N Y</td>
<td>N Y</td>
<td>-</td>
</tr>
<tr>
<td><strong>Multiparous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIM range</td>
<td>0-8 9-150</td>
<td>0-12 13-150</td>
<td>0-21 21-150</td>
<td>0-14 15-150</td>
<td>0-12 13-150</td>
</tr>
<tr>
<td>Concentrate (kg/d as fed)</td>
<td>2.8 3.0</td>
<td>3.5 4.5</td>
<td>4 4.5</td>
<td>2.5 4.0</td>
<td>5.5 6.0</td>
</tr>
<tr>
<td>Milk yield table (Y/N)(^4)</td>
<td>N Y</td>
<td>N Y</td>
<td>N Y</td>
<td>N Y</td>
<td>N Y</td>
</tr>
</tbody>
</table>

\(^1\)Glycerol cows were programmed to receive 250 g/d (as fed) more concentrate over the first 21 DIM to ensure they received the minimum glycerol intake of 250 g/hd/day (as-fed).

\(^2\)Treatments consisted of 1) Control= standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dried glycerol (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22-150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.

\(^3\)Only multiparous cows were enrolled at Farm 5.

\(^4\)Milk yield tables create concentrate allowances based on expected milk yield per cow. If milk yield table is N) then the concentrate allocation is fixed. If milk yield table is Y) then the minimum concentrate allowance is posted in the table, and the concentrate allocation may increase based on the individual cows’ milk yield.
Table 5. Nutrient composition¹ (mean ± SD) of the automated milking system (AMS) pellets fed to control cows (CON) in the first 21 DIM then all cows (control and glycerol cows², CON + GLY) until 150 DIM by farm during milking.

<table>
<thead>
<tr>
<th>Item, % DM</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM %</td>
<td>90.4 ± 0.68</td>
<td>90.9 ± 0.71</td>
<td>91.3 ± 0.56</td>
<td>91.0 ± 0.80</td>
<td>93.4 ± 0.65</td>
</tr>
<tr>
<td>CP</td>
<td>19.3 ± 0.60</td>
<td>20.7 ± 0.42</td>
<td>20.3 ± 0.43</td>
<td>17.8 ± 0.87</td>
<td>20.4 ± 0.34</td>
</tr>
<tr>
<td>ADF</td>
<td>14.0 ± 0.46</td>
<td>10.3 ± 0.50</td>
<td>12.5 ± 0.05</td>
<td>12.7 ± 0.53</td>
<td>13.5 ± 1.25</td>
</tr>
<tr>
<td>NDF</td>
<td>29.2 ± 0.97</td>
<td>23.5 ± 0.38</td>
<td>24.1 ± 0.66</td>
<td>26.0 ± 1.06</td>
<td>33.6 ± 1.22</td>
</tr>
<tr>
<td>TDN</td>
<td>78.2 ± 0.36</td>
<td>80.9 ± 0.38</td>
<td>79.1 ± 0.04</td>
<td>78.9 ± 0.41</td>
<td>78.4 ± 0.97</td>
</tr>
<tr>
<td>Starch</td>
<td>22.1 ± 0.48</td>
<td>24.7 ± 0.33</td>
<td>24.9 ± 1.06</td>
<td>26.0 ± 0.87</td>
<td>17.1 ± 0.72</td>
</tr>
<tr>
<td>Sugar</td>
<td>9.9 ± 0.33</td>
<td>10.4 ± 0.41</td>
<td>7.6 ± 0.37</td>
<td>9.6 ± 0.52</td>
<td>7.8 ± 0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>3.7 ± 0.84</td>
<td>3.8 ± 0.22</td>
<td>8.3 ± 0.51</td>
<td>2.7 ± 0.62</td>
<td>3.6 ± 0.00</td>
</tr>
<tr>
<td>Ash</td>
<td>7.0 ± 0.23</td>
<td>7.5 ± 0.13</td>
<td>5.5 ± 0.52</td>
<td>7.5 ± 0.15</td>
<td>7.3 ± 0.19</td>
</tr>
<tr>
<td>Ca</td>
<td>1.3 ± 0.05</td>
<td>1.4 ± 0.01</td>
<td>0.6 ± 0.11</td>
<td>1.2 ± 0.15</td>
<td>1.3 ± 0.16</td>
</tr>
<tr>
<td>P</td>
<td>0.9 ± 0.05</td>
<td>0.7 ± 0.01</td>
<td>0.6 ± 0.04</td>
<td>0.7 ± 0.06</td>
<td>0.9 ± 0.04</td>
</tr>
<tr>
<td>K</td>
<td>1.1 ± 0.03</td>
<td>1.1 ± 0.01</td>
<td>1.0 ± 0.05</td>
<td>1.1 ± 0.07</td>
<td>1.2 ± 0.08</td>
</tr>
<tr>
<td>Na</td>
<td>0.2 ± 0.03</td>
<td>0.6 ± 0.11</td>
<td>0.3 ± 0.07</td>
<td>0.6 ± 0.05</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>Mg</td>
<td>0.4 ± 0.02</td>
<td>0.4 ± 0.03</td>
<td>0.3 ± 0.06</td>
<td>0.4 ± 0.02</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>NEₐ, Mcal/kg of DM³</td>
<td>1.81 ± 0.01</td>
<td>1.87 ± 0.01</td>
<td>1.83 ± 0.01</td>
<td>1.82 ± 0.01</td>
<td>1.81 ± 0.02</td>
</tr>
</tbody>
</table>

¹Values were obtained from chemical analysis of AMS pellet samples.
²Treatments consisted of 1) Control= standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dried glycerol (targeting 250 g cow/d intake as fed) for 21 DIM then standard AMS pellet from 22-150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.
³NEₐ is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.
Table 6. Ingredient breakdown (％ of DM) of the lactating cow partial mixed ration fed to all cows by farm.

<table>
<thead>
<tr>
<th>Item</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>44.7</td>
<td>34.6</td>
<td>43.7</td>
<td>50.4</td>
<td>48.6</td>
</tr>
<tr>
<td>Haylage</td>
<td>33.5</td>
<td>42.2</td>
<td>29.3</td>
<td>8.6</td>
<td>25.9</td>
</tr>
<tr>
<td>Barley silage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.8</td>
<td>-</td>
</tr>
<tr>
<td>Oat silage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.4</td>
</tr>
<tr>
<td>Dry corn</td>
<td>-</td>
<td>10.4</td>
<td>8.0</td>
<td>12.2</td>
<td>-</td>
</tr>
<tr>
<td>HM corn</td>
<td>8.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.1</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>3.7</td>
<td>3.2</td>
<td>7.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Canola meal</td>
<td>-</td>
<td>3.3</td>
<td>-</td>
<td>5.9</td>
<td>11.4</td>
</tr>
<tr>
<td>Dried corn distillers</td>
<td>-</td>
<td>3.0</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supplement</td>
<td>7.8</td>
<td>2.5</td>
<td>6.5</td>
<td>7.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Dry hay</td>
<td>1.8</td>
<td>-</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Straw</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
<td>-</td>
<td>4.7</td>
</tr>
</tbody>
</table>
Table 7. Nutrient composition\(^1\) (mean ± SD) of the lactating cow partial mixed ration (PMR) fed to all cows by farm.

<table>
<thead>
<tr>
<th>Item, % DM</th>
<th>Farm 1(^3)</th>
<th>Farm 2(^4)</th>
<th>Farm 3(^5)</th>
<th>Farm 4(^6)</th>
<th>Farm 5(^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM %</td>
<td>46.5 ± 2.52</td>
<td>52.8 ± 5.12</td>
<td>52.3 ± 0.70</td>
<td>48.0 ± 2.42</td>
<td>49.3 ± 1.65</td>
</tr>
<tr>
<td>CP</td>
<td>12.9 ± 0.18</td>
<td>14.8 ± 0.52</td>
<td>14.7 ± 1.02</td>
<td>16.3 ± 0.37</td>
<td>16.4 ± 1.16</td>
</tr>
<tr>
<td>ADF</td>
<td>24.1 ± 1.91</td>
<td>20.9 ± 1.18</td>
<td>22.2 ± 2.68</td>
<td>22.7 ± 0.02</td>
<td>23.2 ± 1.99</td>
</tr>
<tr>
<td>NDF</td>
<td>34.6 ± 0.85</td>
<td>30.4 ± 1.23</td>
<td>31.2 ± 2.35</td>
<td>33.1 ± 1.61</td>
<td>33.0 ± 2.32</td>
</tr>
<tr>
<td>NDFD48</td>
<td>61.6 ± 5.12</td>
<td>61.6 ± 6.08</td>
<td>61.6 ± 8.11</td>
<td>63.1 ± 4.45</td>
<td>65.1 ± 1.73</td>
</tr>
<tr>
<td>TDN</td>
<td>70.1 ± 0.69</td>
<td>72.6 ± 0.92</td>
<td>71.6 ± 2.09</td>
<td>71.3 ± 0.50</td>
<td>70.8 ± 1.55</td>
</tr>
<tr>
<td>Lignin</td>
<td>3.4 ± 0.28</td>
<td>4.0 ± 0.76</td>
<td>3.9 ± 0.94</td>
<td>3.3 ± 0.10</td>
<td>3.3 ± 0.54</td>
</tr>
<tr>
<td>Starch</td>
<td>22.0 ± 1.88</td>
<td>22.1 ± 1.47</td>
<td>21.0 ± 1.42</td>
<td>21.8 ± 1.04</td>
<td>18.6 ± 1.27</td>
</tr>
<tr>
<td>Fat</td>
<td>2.9 ± 0.49</td>
<td>4.7 ± 0.33</td>
<td>3.7 ± 0.22</td>
<td>3.4 ± 0.20</td>
<td>4.2 ± 0.08</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.5 ± 0.62</td>
<td>2.4 ± 0.34</td>
<td>1.8 ± 0.32</td>
<td>1.4 ± 0.36</td>
<td>3.1 ± 1.95</td>
</tr>
<tr>
<td>Ash</td>
<td>8.6 ± 0.64</td>
<td>6.5 ± 0.17</td>
<td>7.9 ± 0.46</td>
<td>6.7 ± 0.46</td>
<td>7.6 ± 0.06</td>
</tr>
<tr>
<td>Ca</td>
<td>1.3 ± 0.53</td>
<td>1.0 ± 0.03</td>
<td>1.2 ± 0.09</td>
<td>0.9 ± 0.09</td>
<td>1.0 ± 0.11</td>
</tr>
<tr>
<td>P</td>
<td>0.3 ± 0.02</td>
<td>0.3 ± 0.01</td>
<td>0.4 ± 0.02</td>
<td>0.4 ± 0.03</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>K</td>
<td>1.7 ± 0.04</td>
<td>1.5 ± 0.06</td>
<td>1.9 ± 0.08</td>
<td>1.6 ± 0.15</td>
<td>1.7 ± 0.08</td>
</tr>
<tr>
<td>Na</td>
<td>0.4 ± 0.08</td>
<td>0.4 ± 0.03</td>
<td>0.7 ± 0.13</td>
<td>0.5 ± 0.03</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>Mg</td>
<td>0.4 ± 0.22</td>
<td>0.4 ± 0.02</td>
<td>0.4 ± 0.05</td>
<td>0.3 ± 0.01</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>NE(_L), Mcal/kg of DM(^2)</td>
<td>1.61 ± 0.02</td>
<td>1.67 ± 0.02</td>
<td>1.64 ± 0.05</td>
<td>1.64 ± 0.01</td>
<td>1.62 ± 0.04</td>
</tr>
</tbody>
</table>

\(^1\)Values were obtained from chemical analysis of PMR samples.
\(^2\)NE\(_L\) is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.
\(^3\)Farm 1 lactating cow PMR particle distribution (% of DM): 18±4.9% long (>19mm), 46±4.0% medium (8 to 19mm), 16±1.7% short (4 to 8mm), and 19±2.2% fine (<4mm).
Farm 2 lactating cow PMR particle distribution (% of DM): 15±5.9% long (>19mm), 42±3.5% medium (8 to 19mm), 17±1.1% short (4 to 8mm), and 21±3.8% fine (<4mm).

Farm 3 lactating cow PMR particle distribution (% of DM): 10±1.4% long (>19mm), 45±2.9% medium (8 to 19mm), 17±1.9% short (4 to 8mm), and 28±2.2% fine (<4mm).

Farm 4 lactating cow PMR particle distribution (% of DM): 18±3.1% long (>19mm), 45±3.0% medium (8 to 19mm), 14±1.2% short (4 to 8mm), and 22±3.4% fine (<4mm).

Farm 5 lactating cow PMR particle distribution (% of DM): 14±3.5% long (>19mm), 46±3.7% medium (8 to 19mm), 14±1.4% short (4 to 8mm), and 25±3.1% fine (<4mm).
Table 8. Nutrient composition\(^1\) (mean ± SD) of the automated milking system (AMS) pellet formulated with dried glycerol delivered through the AMS to cows for the first 21 DIM fed to glycerol\(^2\) cows on each farm.

<table>
<thead>
<tr>
<th>AMS Glycerol Pellet</th>
<th>Farm 1</th>
<th>Farm 2</th>
<th>Farm 3</th>
<th>Farm 4</th>
<th>Farm 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM %</td>
<td>93.7 ± 0.60</td>
<td>91.9 ± 0.83</td>
<td>91.0 ± 0.87</td>
<td>91.4 ± 0.88</td>
<td>92.3 ± 0.15</td>
</tr>
<tr>
<td>CP</td>
<td>18.4 ± 0.25</td>
<td>21.5 ± 0.66</td>
<td>18.6 ± 0.50</td>
<td>17.7 ± 1.02</td>
<td>24.6 ± 0.40</td>
</tr>
<tr>
<td>ADF</td>
<td>13.6 ± 1.11</td>
<td>10.5 ± 0.16</td>
<td>13.6 ± 0.16</td>
<td>12.8 ± 0.60</td>
<td>15.8 ± 0.62</td>
</tr>
<tr>
<td>NDF</td>
<td>27.5 ± 2.81</td>
<td>22.5 ± 1.01</td>
<td>22.2 ± 0.02</td>
<td>23.9 ± 0.78</td>
<td>27.2 ± 1.83</td>
</tr>
<tr>
<td>TDN</td>
<td>78.3 ± 0.87</td>
<td>80.7 ± 0.13</td>
<td>78.4 ± 0.12</td>
<td>78.9 ± 0.46</td>
<td>76.6 ± 0.30</td>
</tr>
<tr>
<td>Starch</td>
<td>23.1 ± 2.31</td>
<td>22.0 ± 2.70</td>
<td>25.5 ± 0.54</td>
<td>23.1 ± 1.45</td>
<td>13.4 ± 0.86</td>
</tr>
<tr>
<td>Sugar</td>
<td>10.2 ± 1.29</td>
<td>10.5 ± 0.62</td>
<td>7.2 ± 0.66</td>
<td>9.2 ± 0.69</td>
<td>9.2 ± 0.08</td>
</tr>
<tr>
<td>Fat</td>
<td>3.0 ± 0.55</td>
<td>3.6 ± 0.59</td>
<td>6.0 ± 0.23</td>
<td>2.4 ± 0.24</td>
<td>2.8 ± 0.47</td>
</tr>
<tr>
<td>Ash</td>
<td>9.5 ± 0.36</td>
<td>8.5 ± 1.70</td>
<td>7.4 ± 0.21</td>
<td>8.9 ± 0.60</td>
<td>9.9 ± 0.66</td>
</tr>
<tr>
<td>Ca</td>
<td>1.5 ± 0.07</td>
<td>1.2 ± 0.25</td>
<td>0.7 ± 0.01</td>
<td>1.2 ± 0.09</td>
<td>1.8 ± 0.14</td>
</tr>
<tr>
<td>P</td>
<td>0.8 ± 0.04</td>
<td>0.7 ± 0.01</td>
<td>0.6 ± 0.01</td>
<td>0.6 ± 0.01</td>
<td>0.7 ± 0.02</td>
</tr>
<tr>
<td>K</td>
<td>1.0 ± 0.07</td>
<td>1.2 ± 0.03</td>
<td>1.0 ± 0.02</td>
<td>1.0 ± 0.06</td>
<td>1.2 ± 0.06</td>
</tr>
<tr>
<td>Na</td>
<td>0.2 ± 0.03</td>
<td>0.6 ± 0.06</td>
<td>0.4 ± 0.01</td>
<td>0.5 ± 0.10</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>Mg</td>
<td>0.4 ± 0.03</td>
<td>0.4 ± 0.01</td>
<td>0.3 ± 0.01</td>
<td>0.4 ± 0.01</td>
<td>0.7 ± 0.01</td>
</tr>
<tr>
<td>NE(_L), Mcal/kg of DM(^2)</td>
<td>1.81 ± 0.02</td>
<td>1.87 ± 0.01</td>
<td>1.81 ± 0.01</td>
<td>1.82 ± 0.01</td>
<td>1.76 ± 0.01</td>
</tr>
</tbody>
</table>
Table 9. Effect of dried glycerol supplemented through the automated milking system (AMS) pellet for the first 21 DIM on the milking activity, concentrate intake, yield, and rumination time of dairy cows milked in AMS. Treatment\(^1\) was applied from 1-21 DIM then all cows received the base pellet from 22-150 DIM.

<table>
<thead>
<tr>
<th>Treatments(^1)</th>
<th>SE</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>GLY</td>
</tr>
<tr>
<td>1-21 DIM(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate delivered, kg/d(^3)</td>
<td>3.94</td>
<td>4.21</td>
</tr>
<tr>
<td>Milking frequency, #/d</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>AMS refusals(^4), #/d</td>
<td>1.11</td>
<td>1.38</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>34.8</td>
<td>35.6</td>
</tr>
<tr>
<td>Rumination time, min/d</td>
<td>520.2</td>
<td>520.9</td>
</tr>
<tr>
<td>22-150 DIM(^5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentrate delivered, kg/d(^3)</td>
<td>4.77</td>
<td>4.95</td>
</tr>
<tr>
<td>Milking frequency, #/d</td>
<td>3.3</td>
<td>3.4</td>
</tr>
<tr>
<td>AMS refusals(^4), #/d</td>
<td>0.85</td>
<td>0.87</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>43.4</td>
<td>44.9</td>
</tr>
<tr>
<td>Rumination time, min/d</td>
<td>577.4</td>
<td>575.6</td>
</tr>
</tbody>
</table>

\(^1\)Treatments consisted of 1) Control= standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dried glycerol (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22-150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.

\(^2\)Control (CON) cows received the base pellet and glycerol cows (GLY) received the base pellet formulated with dried glycerol.

\(^3\)There is a significant treatment*DIM interaction for concentrate intake 1-21 DIM (P<0.001) and 22-150 DIM (P=0.03).

\(^4\)AMS refusals were log-transformed for normality.

\(^5\)All cows received the standard AMS pellet from 22-150 DIM.
Table 10. Effect of dried glycerol supplemented through the automated milking system (AMS) pellet for the first 21 DIM on the milk components of dairy cows milked in AMS. Treatment was applied from 1-21 DIM then all cows received the base pellet from 22-150 DIM.

<table>
<thead>
<tr>
<th>Treatments²</th>
<th>SE</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Parity</td>
</tr>
<tr>
<td>CON</td>
<td>GLY</td>
<td></td>
</tr>
<tr>
<td>1-21 DIM³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Milk fat yield, kg/d</td>
<td>1.58</td>
<td>1.57</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.47</td>
<td>3.48</td>
</tr>
<tr>
<td>Milk protein yield, kg/d</td>
<td>1.20</td>
<td>1.21</td>
</tr>
<tr>
<td>22-150 DIM⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk fat⁵, %</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>Milk fat yield, kg/d</td>
<td>1.67</td>
<td>1.70</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.11</td>
<td>3.11</td>
</tr>
<tr>
<td>Milk protein yield, kg/d</td>
<td>1.37</td>
<td>1.41</td>
</tr>
</tbody>
</table>

¹Milk component analysis was available for farm 1-4.
²Treatments consisted of 1) Control= standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dried glycerol (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22-150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet from 22-150 DIM.
³Treatments were applied from 1-21 DIM.
⁴All cows received the standard AMS pellet from 22-150 DIM.
⁵A significant treatment*DIM interaction (P= was observed for milk fat % from 22-150 DIM.
**Figure 1.** Daily concentrate delivered (kg/d; mean ± SE) by treatment for 1-150 DIM. All cows had free flow access to the automated milking system (AMS) with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet + dried glycerol (minimum 250 g/hd/d as fed intake). Treatments were administered over 21 DIM beginning the day of calving, then from DIM 22-150 all cows received the control standard AMS pellet. Grey dashed line indicates when treatment period ended.
Figure 2. Successful milkings per day (mean ± SE) by treatment for 1-150 DIM. All cows had free flow access to the automated milking system (AMS) with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet + dried glycerol (minimum 250 g/hd/d as fed intake). Treatments were administered over 21 DIM beginning the day of calving, then from DIM 22-150 all cows received the control standard AMS pellet. Grey dashed line indicates when treatment period ended.
Figure 3. Daily milk yield (kg/d; (mean ± SE)) by treatment for 1-150 DIM. All cows had free flow access to the automated milking system (AMS) with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet + dried glycerol (minimum 250 g/hd/d as fed intake). Treatments were administered over 21 DIM beginning the day of calving, then from DIM 22-150 all cows received the control standard AMS pellet. Grey dashed line indicates when treatment period ended.
Figure 4. Metabolic biomarkers (mmol/L; mean ± SE)) by treatment and sampling day for a) serum NEFA b) blood BHB, and c) blood glucose. All cows had free flow access to the automated milking system (AMS) with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet + dried glycerol (minimum 250 g/hd/d as fed intake). Treatments were administered over 21 DIM beginning the day of calving, then from DIM 22-150 all cows received the control standard AMS pellet. Grey dashed line indicates the beginning of the treatment period.
Figure 5. Incidence of cows within treatment being diagnosed with BHB≥1.2 mmol/L based on their pre-partum BCS (≥3.5 or <3.5). Five BHB blood tests were taken: 1) test 1 occurring between 1-4 DIM, 2) test 2 at 3-6 DIM, 3) test 3 at 7-10 DIM, 4) test 4 at 10-13 DIM, and 5) test 5 occurring between 13-17 DIM. All cows had free flow access to the automated milking system (AMS) with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet + dried glycerol (minimum 250 g/hd/d as fed intake). Treatments were administered over 21 DIM beginning the day of calving, then from DIM 22-150 all cows received the control standard AMS pellet.
Figure 6. BCS (mean ± SE) of cows, by treatment, from 0 to 63 DIM. Five BCS were taken: 1) test 1 at 0±3 DIM, 2) test 2 at 21±3 DIM, 3) test 3 at 42±3 DIM, and 4) test 4 occurring at 63±3 DIM. All cows had free flow access to the automated milking system (AMS) with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet + dried glycerol (minimum 250 g/hd/d as fed intake). Treatments were administered over 21 DIM beginning the day of calving, then from DIM 22-150 all cows received the control base pellet.
3 CHAPTER 4: GENERAL DISCUSSION

3.1 Important Findings

The overall objective of my thesis was to determine the effects of glycerol supplementation to early lactation cows, delivered through the AMS concentrate, on the metabolic health status, milking behaviour, and productivity of AMS housed cows. To address that objective, and with the help of a research team, I recruited 5 commercial AMS herds in southwestern Ontario to participate in a study described in Chapter 2. On these herds, cows were allocated to 1 of 2 treatment groups: 1) control group (CON) received the standard AMS concentrate from 1-150 DIM, and 2) glycerol group (GLY) received a treatment AMS concentrate from 1-21 DIM and then received the standard AMS concentrate from 22-150 DIM. The treatment AMS concentrate contained 77 kg glycerol product/tonne of pellet on farms 1, 2, 3 and 4 and contained 60 kg glycerol product/tonne on farm 5. The treatment period for all cows was from 1 – 21 DIM and the follow-up period was from 22 – 150 DIM. In each herd, AMS units were equipped to deliver 2 different types of AMS concentrate, thus, treatments could be applied simultaneously at a cow level within pen. We began feeding the treatment pellet in May 2022 and ended feeding in December 2022, with follow-up period ending in May 2023.

In Chapter 2 we aimed to supplement early lactation cows with 250 as-fed g/d of a glycerol product, which was achieved by altering the AMS feed tables for GLY cows to offer 250 more as-fed g/d of the AMS concentrate. The formulation and concentration of the treatment concentrate was determined on each farm by the average feeding rate in the first 21 DIM on each
farm and using the base concentrate formulation from each farm (i.e. based on 4 as-fed kg/d, 62.5 kg of the glycerol product was included per tonne of the treatment pellet). During the first treatment period (1-21 DIM) GLY cows were delivered 270 ± 4 as-fed g/d more AMS concentrate (249 g/d DM) than CON cows. During the follow-up period (22-150 DIM) there was a treatment by DIM interaction whereby GLY cows were delivered more concentrate on 50% of the days from 60 – 150 DIM. This interaction was likely caused by the increase in milk yield for GLY cows, as during that period all cows were fed in the AMS using yield-based feed tables. Glycerol supplementation during the treatment period (1-21 DIM) improved milking visit behaviour, whereby GLY cows had 3.3 milkings/d and CON cows had 3.2 milkings/d. GLY cows tended to produce more milk during that treatment period, yielding 35.6 kg/d compared to 34.8 kg/d for CON cows. During the follow-up period (22-150 DIM), GLY cows continued to have more daily milkings at 3.4 milkings/d compared to 3.3 milkings/d for CON cows and produced 1.5 kg/d more milk. The greater milking frequency observed in the treatment and follow-up period was likely driven by the AMS milk allowance settings, which are based on expected milk yield, with an expected minimum yield before a cow may be milked. Thus, the increase in milk yield observed for GLY cows during both periods increased their daily milking allowances and reduced the interval between milkings. Matson et al. (2022) reported that every additional 0.1 milkings/d in AMS herds was associated with a 0.57 kg/d increase in milk yield. Our data yielded similar results, where in the follow-up period we observed an increase in milk yield of 1.5 kg/d with 0.1 more milkings/d for GLY cows. It is estimated that at a 250 g/hd/d feeding rate costs $1/d, thus during the entire treatment period it cost $21/cow. Based on government of Canada milk prices in Ontario (CDC, 2023), each litre of milk is worth $0.83, the
increased revenue from GLY cows was $118.7, and thus the GLY cows would have profited $97.7 more than CON cows. It is worth noting that this was across 150 DIM, and typical lactations last ~305 d, therefore GLY cows may have become even more profitable as the lactation progressed, assuming that the difference between groups held.

The effect of glycerol supplementation on milk yield has had varied results in past literature. Werner-Omazic et al. (2013) and Kass et al. (2013) reported increases in milk yield when glycerol was supplemented to early-lactation cows, whereas Van Soest et al. (2023) reported no effect on milk yield when glycerol was supplemented both pre- and postpartum. The improvement in milk yield in our study may be due to an improvement in the metabolic health of GLY cows during the treatment period, coupled with the increase in milking frequency during both the treatment and follow-up periods. There was a numerical decrease in serum NEFA concentrations at 2 and 5 DIM for GLY cows, which is an indicator of decreased adipose tissue mobilization (Drackley, 1999). This, coupled with the higher BCS for GLY cows at 63 DIM, are both suggestive indicators that GLY cows had an improved energy balance throughout early lactation. Overall, the increase in milk yield observed for GLY cows is likely due to a combination of an improved energy balance throughout early lactation and an increase in milking frequency observed in the treatment and follow-up periods.

It is interesting to note that when classifying cows as either high ≥ 1.2 mmol/L or normal < 1.2 mmol/L BHB, we observed a treatment by prepartum BCS interaction, whereby CON cows with high prepartum BCS were 3.5x more likely to have a high BHB test than CON cows with a normal prepartum BCS. For GLY cows however there was no difference in the risk of
developing high BHB based on prepartum BCS category. This may indicate a protective effect of glycerol for over-conditioned prepartum cows. The apparent decreased risk of developing high BHB for over-conditioned GLY cows may have also contributed to the increase in milk yield observed, as high BHB has been associated with decreases in milk yield (Duffield et al., 2009).

In Chapter 2, we hypothesized that the supplementation of glycerol through the AMS would improve milking behaviour, increase milk yield, and improve the metabolic health status of those supplemented with glycerol. This hypothesis was based on previous research by Madsen et al. (2010) who observed that supplementing highly-palatable feeds increases milking frequency in the AMS. Further, Lomander et al. (2012) and Kass et al. (2013) both reported increases in milk yield when cows were supplemented with glycerol in early lactation. Lastly, Van Soest et al. (2023) and Chung et al. (2007) both reported improvements in the metabolic health status of lactating cows when supplemented with glycerol. Overall, our study yielded similar results to our hypothesis, as GLY cows had greater daily milking frequency and milk yield, and when controlling for prepartum BCS, over-conditioned GLY cows were at no more risk of developing high BHB, whereas over-conditioned CON cows were. Interestingly, the blood BHB and glucose concentrations displayed a treatment by time interaction, where CON cows appeared to have improved metabolic markers compared to GLY cows. This was similar to results reported by DeFrain et al. (2004), who observed a treatment by time interaction where glycerol supplemented cows had decreased glucose and increased BHB concentrations. This may be due to the fermentation of glycerol in the rumen, where an increase in ruminal production of butyrate may have caused alimentary ketogenesis (Bergman, 1970). Researchers have previously reported that glycerol supplementation can increase concentrations of ruminal butyrate (Rémond
et al., 1992; Khalili et al., 1997). Thus, the increase in BHB concentrations and decrease in glucose may have been due to the fermentation of glycerol, and not indicating a decline in their metabolic health.

3.2 Limitations and Future Research

One limitation with our study is the method in which the AMS allocates concentrates. During the 21-d treatment period, 4 of the herds transitioned from DIM based AMS supplementation to yield-based AMS supplementation feed table. This individualizes the amount of concentrate that is delivered to a cow based on her milk yield, which makes treatment application difficult as the daily quantity of AMS concentrate delivered is variable. Concentrate allocation is also dependent on the cow visiting the AMS multiple times per day, as their daily allocation is evenly distributed throughout milkings. Challenges with this type of targeted supplementation are not unique to the current study. In a similar study where early lactation cows (1-60 DIM) were supplemented liquid molasses through the AMS, Moore et al. (2020) targeted 1 kg/d for MP cows and 0.88 kg/d for PP cows. In that study, liquid molasses was delivered at 87% and 90% of the targeted feeding rate for MP and PP cows, respectively.

The difficulty in achieving the targeted supplementation of a treatment through the AMS may be due to a variety of issues; these include milkings/d, yield/d, illness, and feed table design. Of the original 214 GLY cows recruited, 38 were excluded from our analysis (see Chapter 2) as they did not meet the minimum of 250 as fed g/d intake averaged across the 21-d treatment period. The predominant issue in the group of excluded cows was that an error was made on the feed table for MP cows on farm 1. The treatment pellet allocation was not increased sufficiently;
as such, some cows were not being offered enough concentrate in the first 21 DIM. The feed
tables for treatment cows on all farms were created along with the technical support staff from
the corresponding AMS companies, so it was surprising that we had made this error. When
analyzing this group of excluded cows independently, besides the lower feed intake in the first
21 DIM, there were otherwise no indications that they were abnormal.

Another limitation of the Chapter 2 study is that we were unable to measure individual
DMI of AMS concentrate or PMR. The AMS concentrate data utilized in this study is the as fed
quantity of concentrate delivered per day, not consumed. Current AMS systems do not offer a
method to determine the actual concentrate consumed/d. As such, some of the cows in the study
may have consumed more, or less, of the concentrate that was programmed to be delivered. If
GLY cows did not consume all the concentrate delivered during a milking visit, it is possible that
a CON cow consumed treatment pellet that was leftover, which could have impacted the results.
At a research farm, this could be mitigated by installing a vacuum or dumping system and weigh
scale to ensure that all the feed bin in the AMS is completely empty between cows. As this study
was conducted on commercial herds, this option was not feasible. Further, monitoring individual
cow PMR intake would have allowed a comparison of the energy utilization of cows on either
treatment. It would have been interesting to monitor daily DMI as in a recent study, glycerol
supplementation was shown to decrease total DMI (including both PMR and AMS concentrate)
(Van Soest et al., 2023). Additionally, it is well established that changes in AMS concentrate
provision are often coupled with changes in PMR consumption (Schwanke et al., 2019). As the
GLY cows in the current study had increased milk yield, it would have been interesting to
determine whether they also had a proportional increase in DMI.
Another potential limitation to our work is that GLY cows were offered more AMS concentrate in the first 21 DIM as the glycerol product was added on top of the base concentrate formulation. We determined that this was the best approach in formulating the treatment pellet as we avoided substituting other pellet ingredients for the glycerol product. Thus, when we programmed more treatment concentrate from the AMS to be delivered to GLY cows they theoretically were receiving the same quantity and concentration of ingredients from the base concentrate in addition to the glycerol. One other method of supplementation that could be explored would be top-dressing the glycerol product through the AMS. A similar approach had been attempted by one of the farms in our study, where they supplemented palm-fat to high producing cows through the AMS. If the ingredient is palatable, then it is an approach that may offer more consistent intakes as the feeding rates will be much lower for a top-dress product.

Lastly, we measured serum NEFA concentrations on the first (actual 1.9 ± 0.08 DIM) (mean ± SD) and second (actual 5.3 ± 0.09 DIM) blood sample post-partum. As cows did not begin receiving glycerol until calving, they had only begun consuming the treatment pellet when we started blood sampling. In future work, it may be beneficial to extend the NEFA sampling up to 14 DIM to ensure that cows have been receiving the glycerol for multiple days before sampling. When supplementing glycerol pre- or postpartum in a factorial design, Van Soest et al. (2023) measured serum NEFA concentrations at 3 and 7 d relative to parturition, which could have given the postpartum treatment more time to affect the cow. By testing serum NEFA so early in the treatment period, we may have missed some of the effects of glycerol supplementation on serum NEFA concentrations.
Future work should also explore the effects of glycerol supplementation on the effects of reproduction and overall health. My Chapter 2 study included a total of 389 cows, which relative to other studies focusing on reproduction (Abdelli et al., 2017; Pineiro et al., 2019), is a relatively small sample size. In an observational study measuring health outcomes in commercial AMS herds (i.e. SCK, displaced abomasum, mastitis), King et al. (2018) calculated a minimum sample size of 600 lactating cows. Based on our findings in Chapter 2, supplementing glycerol to fresh cows has the potential improve reproduction metrics (days to first breeding, first service conception, days open) and reduce the incidence of clinical diseases, including displaced abomasum, metritis, and SCK due to the potential improvements in animal metabolism (Duffield et al., 2009).

3.3 Implications

Overall, the results of my thesis research indicate that supplementing glycerol through the AMS concentrate to early lactation dairy cows may be an effective way to improve the metabolic health status of early lactation cows, as well as improve their milking behaviour and yield until mid lactation. The dairy nutrition industry may benefit from this research by implementing glycerol supplementation into early lactation cow rations. The benefits observed in the current study where GLY cows had improved milking behaviour and milk yield are key metrics to dairy cow performance in AMS and are common topics of discussion with producers. Based on the estimated feed cost and revenue from increased milk yield, producers may gain an additional $97.7 in the first 150 DIM for lactating cows. As AMS systems may be equipped to offer more than one type of concentrate, capitalizing on key stages of production, such as early lactation, by
offering different types of concentrate is a feasible opportunity. This is an exciting opportunity for AMS producers to see improvements in early lactation cow performance, which could translate into more yield and better farm profitability.
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