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

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ABSTRACT

The objective of this study was to quantify the effects of supplementing early-lactation cows with a dry pure glycerol product, delivered through the automated milking system (AMS) concentrate, in the first 21 d in milk (DIM) on metabolic markers, milking behavior, and milk production. In 5 commercial AMS dairy herds, 389 dairy cows were randomly assigned, controlling for parity, 21 d before expected calving to 1 of 2 treatments, within farm: (1) control group (CON) receiving the standard AMS pellet (n = 213) from 1 to 150 DIM, or (2) glycerol group (GLY) receiving the treatment AMS pellet (n = 176) formulated to deliver 250 as fed g/d of glycerol product from 1 to 21 DIM (mean actual = 249 g/d dry matter [DM]), then they received the standard AMS pellet from 22 to 150 DIM. Across all farms, cows were fed partial mixed rations (PMR) that were similar in ingredient and nutrient composition. One prepartum blood sample and 5 postpartum blood samples were collected from each cow to determine serum nonesterified fatty acids (NEFA), blood β-hydroxy butyrate (BHB), and blood glucose concentrations. Cow body condition score (BCS) was recorded every 21 d from −21 to 63 DIM. Data were collected and analyzed for the treatment period (1 to 21 DIM) and a follow-up period (22 to 150 DIM). There was no detected treatment effect on serum NEFA concentrations in the first week of lactation. There was a treatment by time interaction for blood BHB and blood glucose, where GLY cows tended to have increased BHB concentrations at 5 DIM and had decreased glucose concentrations at 9 and 12 DIM. There was an interaction of BCS with treatment on the incidence of BHB ≥1.2 mmol/L, whereby over-conditioned CON cows (BCS ≥3.5) were 3.5x more likely to have a high BHB test than CON cows with normal prepartum BCS. During the treatment period GLY cows had 0.1 ± 0.05 more successful milkings/d, were delivered 0.27 ± 0.05 DM kg/d more AMS concentrate and tended to yield 0.8 ± 0.47 kg/d more milk. During the follow-up period GLY cows had 0.1 ± 0.04 more successful milkings/d, were delivered 0.18 ± 0.06 DM kg/d more AMS concentrate, and yielded 1.5 ± 0.53 kg/d more milk than CON cows. Glycerol supplementation allowed cows to maintain better BCS, as GLY cows lost less BCS from calving to 63 DIM than CON cows. Overall, the results of this study demonstrate that supplementing pure glycerol through the AMS concentrate for the first 21 DIM can reduce BCS loss in early lactation, improve milking behavior, and increase milk yield to mid lactation.

Key Words: glycerol, transition cow, robotic milking, metabolic biomarkers, milk yield

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 with other behaviors 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.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.
milked with AMS, who may be at an increased risk of excessive negative energy balance compared with conventionally-milked cows. For example, Tatone et al. (2017) reported a higher within-herd subclinical ketosis (SCK) prevalence for cows in AMS herds in Ontario, Canada compared with conventionally milked cows, as detected through elevated milk BHB levels. Elevated ketone bodies on these farms may be an artifact of greater milk production (Jacobs and Siegford, 2012). King et al. (2018b) 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. (2018b) 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 as compared with cows diagnosed with SCK in free-traffic AMS herds (King et al., 2018a). 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, the 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 (Kupczyński et al., 2020) that was first examined as a treatment for ketosis in the 1950s (Johnson, 1954) and used primarily as such until the 1970s (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, DeFrein et al. (2004) reported that plasma glucose tended to increase in the postpartum 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 with 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 behavior, and productivity in commercial AMS herds. It was hypothesized that the supplementation of dry pure glycerol through the AMS concentrate would result in improved blood biomarkers of metabolic status in early lactation cows, as well as result in an increase in successful milkings at the AMS and an increase in daily milk yield during treatment and further into lactation.
MATERIALS AND METHODS

Animals, housing, and management

This study utilized data from 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 number of cows per AMS, average lactating herd size, total number of cows enrolled, number of PP CON cows, number of MP CON cows, number of PP GLY cows, number of MP GLY cows, milk yield PP (kg/d), milk yield MP (kg/d), milking frequency PP (milkings/d), and milking frequency MP (milkings/d) 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., caesarean-section). 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 before expected calving (Table 2). On all farms, cows were moved to a calving pack approximately 14 d before their expected calving date. Following calving, cows were moved (in 3 of the 5 farms) to a fresh cow pen for 3–21 d post calving in a free-stall pen behind the AMS units with free access to the AMS units. At one farm, fresh cows were moved into the main lactating pen immediately following calving with free access to the AMS units. There was an early lactation AMS unit at one farm where cows were milked up to 30 DIM, before being moved into the main lactating groups. 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).

Experimental Design

Across herds, we conducted a randomized complete block design trial where 21 d before calving, cows were randomly assigned to 1 of 2 postpartum 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 before calving, thus producers were blinded to treatment assignment, on each farm with treatment assignments alternated between the 2 treatments based on expected

Table 1. Number of automated milking systems, cows enrolled, and average (±SD) milk yield1 and frequency1 by parity2 and treatment3 by farm

<table>
<thead>
<tr>
<th>Farm</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>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 number of cows per AMS</td>
<td>35</td>
<td>44</td>
<td>41</td>
<td>35</td>
<td>53</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 ± 6.03</td>
<td>37.2 ± 6.41</td>
<td>39.3 ± 7.40</td>
<td>34.3 ± 7.21</td>
<td>—</td>
</tr>
<tr>
<td>Milk yield MP (kg/d)</td>
<td>48.1 ± 10.24</td>
<td>48.7 ± 9.21</td>
<td>52.0 ± 8.55</td>
<td>52.6 ± 10.23</td>
<td>39.0 ± 11.97</td>
</tr>
<tr>
<td>Milking frequency PP (milkings/d)</td>
<td>3.5 ± 0.83</td>
<td>3.5 ± 0.80</td>
<td>3.2 ± 0.77</td>
<td>3.3 ± 0.65</td>
<td>—</td>
</tr>
<tr>
<td>Milking frequency MP (milkings/d)</td>
<td>3.3 ± 0.88</td>
<td>3.6 ± 0.90</td>
<td>3.4 ± 0.88</td>
<td>3.6 ± 0.97</td>
<td>2.5 ± 0.92</td>
</tr>
</tbody>
</table>

1Average (±SD) daily milk yield and milking frequency from 0 to 150 DIM during the trial for all enrolled cows.
2Parity = primiparous (PP) or multiparous (MP).
3Treatments consisted of 1) Control = standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dry glycerol powder (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22 to 150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.
4Only multiparous cows were enrolled at Farm 5.
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 to 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 to 150 DIM. The 2 treatments were applied to individual cows upon calving and up to 21 DIM. The first 21 DIM was consid-

Table 2. Nutrient composition1 (mean ± SD) of the dry cow total mixed rations (TMR) fed by farm

<table>
<thead>
<tr>
<th>Farm TMR</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>52.4 ± 5.41</td>
<td>61.6 ± 5.19</td>
<td>53.2 ± 2.61</td>
<td>69.1 ± 8.31</td>
<td>55.1 ± 5.18</td>
</tr>
<tr>
<td>CP, % of DM</td>
<td>13.3 ± 1.11</td>
<td>13.0 ± 0.63</td>
<td>13.1 ± 2.46</td>
<td>10.8 ± 1.50</td>
<td>13.1 ± 0.90</td>
</tr>
<tr>
<td>ADF, % of DM</td>
<td>28.5 ± 5.35</td>
<td>34.2 ± 3.38</td>
<td>31.7 ± 3.29</td>
<td>35.2 ± 3.70</td>
<td>29.6 ± 0.91</td>
</tr>
<tr>
<td>NDF, % of DM</td>
<td>41.9 ± 6.73</td>
<td>49.7 ± 3.59</td>
<td>47.7 ± 4.25</td>
<td>52.2 ± 5.56</td>
<td>44.0 ± 0.64</td>
</tr>
<tr>
<td>NDFD48, % of DM</td>
<td>51.6 ± 2.07</td>
<td>51.8 ± 5.53</td>
<td>55.8 ± 2.21</td>
<td>50.1 ± 4.55</td>
<td>59.1 ± 7.77</td>
</tr>
<tr>
<td>TDN, % of DM</td>
<td>66.4 ± 4.17</td>
<td>62.2 ± 2.63</td>
<td>64.2 ± 2.56</td>
<td>61.5 ± 2.88</td>
<td>65.8 ± 0.70</td>
</tr>
<tr>
<td>Lignin, % of DM</td>
<td>4.8 ± 1.50</td>
<td>6.4 ± 1.04</td>
<td>5.8 ± 0.54</td>
<td>6.2 ± 1.06</td>
<td>4.6 ± 0.02</td>
</tr>
<tr>
<td>Starch, % of DM</td>
<td>15.7 ± 4.11</td>
<td>13.2 ± 1.82</td>
<td>13.7 ± 2.37</td>
<td>13.2 ± 4.15</td>
<td>15.3 ± 1.85</td>
</tr>
<tr>
<td>Sugar, % of DM</td>
<td>2.3 ± 1.03</td>
<td>1.8 ± 0.24</td>
<td>1.8 ± 0.94</td>
<td>1.6 ± 0.62</td>
<td>2.2 ± 0.07</td>
</tr>
<tr>
<td>Fat, % of DM</td>
<td>3.4 ± 0.80</td>
<td>3.3 ± 0.27</td>
<td>3.0 ± 0.33</td>
<td>3.0 ± 0.21</td>
<td>4.0 ± 0.04</td>
</tr>
<tr>
<td>Ash, % of DM</td>
<td>8.9 ± 0.96</td>
<td>6.3 ± 1.07</td>
<td>8.4 ± 1.01</td>
<td>5.6 ± 0.49</td>
<td>6.6 ± 0.11</td>
</tr>
<tr>
<td>Ca, % of DM</td>
<td>1.2 ± 0.25</td>
<td>0.9 ± 0.22</td>
<td>1.4 ± 0.36</td>
<td>0.6 ± 0.06</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>P, % of DM</td>
<td>0.4 ± 0.06</td>
<td>0.3 ± 0.12</td>
<td>0.4 ± 0.02</td>
<td>0.3 ± 0.04</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>K, % of DM</td>
<td>1.5 ± 0.20</td>
<td>1.2 ± 0.12</td>
<td>1.4 ± 0.21</td>
<td>1.3 ± 0.13</td>
<td>1.6 ± 0.01</td>
</tr>
<tr>
<td>Na, % of DM</td>
<td>0.5 ± 0.13</td>
<td>0.1 ± 0.05</td>
<td>0.1 ± 0.02</td>
<td>0.1 ± 0.01</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>Mg, % of DM</td>
<td>0.5 ± 0.16</td>
<td>0.5 ± 0.17</td>
<td>0.4 ± 0.06</td>
<td>0.3 ± 0.02</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>NEL, Mcal/kg of DM2</td>
<td>1.51 ± 0.10</td>
<td>1.41 ± 0.06</td>
<td>1.46 ± 0.06</td>
<td>1.39 ± 0.07</td>
<td>1.50 ± 0.01</td>
</tr>
</tbody>
</table>

1Values were obtained from chemical analysis of pooled TMR samples.
2NEL is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.

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>Farm</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</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>0–30</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>7</td>
<td>8</td>
<td>7</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>0–30</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>7</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

1Only multiparous cows were enrolled at Farm 5.
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Table 4. Milking feed tables1 (automated milking system concentrate programmed during milking) by farm and parity over the first 150 DIM, treatments2 consisted of control and glycerol cows and were applied over the first 21 DIM

<table>
<thead>
<tr>
<th>Farm</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIM range</td>
<td>0–8</td>
<td>9–150</td>
<td>0–8</td>
<td>9–150</td>
<td>0–21</td>
</tr>
<tr>
<td>Concentrate (kg/d as fed)</td>
<td>2.8</td>
<td>2.5</td>
<td>3.5</td>
<td>4.5</td>
<td>4</td>
</tr>
<tr>
<td>Milk yield table (Y/N)4</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>DIM range</td>
<td>0–8</td>
<td>9–150</td>
<td>0–12</td>
<td>13–150</td>
<td>0–21</td>
</tr>
<tr>
<td>Concentrate (kg/d as fed)</td>
<td>2.8</td>
<td>3.0</td>
<td>3.5</td>
<td>4.5</td>
<td>4</td>
</tr>
<tr>
<td>Milk yield table (Y/N)4</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>

1Glycerol 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).
2Treatments consisted of 1) Control = standard AMS pellet for 150 DIM or 2) Glycerol = standard AMS pellet formulated with dry glycerol powder (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22 to 150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.
3Only multiparous cows were enrolled at Farm 5.
4Milk 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 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>Base AMS Pellet</th>
<th>Item</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>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>
<td></td>
</tr>
<tr>
<td>CP, % of DM</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>
<td></td>
</tr>
<tr>
<td>ADF, % of DM</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>
<td></td>
</tr>
<tr>
<td>NDF, % of DM</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>
<td></td>
</tr>
<tr>
<td>TDN, % of DM</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>
<td></td>
</tr>
<tr>
<td>Starch, % of DM</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>
<td></td>
</tr>
<tr>
<td>Sugar, % of DM</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>
<td></td>
</tr>
<tr>
<td>Fat, % of DM</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>
<td></td>
</tr>
<tr>
<td>Ash, % of DM</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>
<td></td>
</tr>
<tr>
<td>Ca, % of DM</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>
<td></td>
</tr>
<tr>
<td>P, % of DM</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>
<td></td>
</tr>
<tr>
<td>K, % of DM</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>
<td></td>
</tr>
<tr>
<td>Na, % of DM</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>
<td></td>
</tr>
<tr>
<td>Mg, % of DM</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>
<td></td>
</tr>
<tr>
<td>NEL, 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>
<td></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 dry glycerol powder (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22 to 150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet.
3NEL is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.
rate 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 an average of 250 g/d as-fed intake of glycerol across the first 21 DIM (primarily due to a programming error in treatment AMS feed table set-up on farm 1, where the programmed daily intake was not sufficiently increased to account for the glycerol in the treatment pellet) 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).

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; Heinrichs, 2013; 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 (<4 mm). Separated

Table 6. Ingredient breakdown (% of DM) of the lactating cow partial mixed ration fed to all cows by farm

<table>
<thead>
<tr>
<th>Lactating cow PMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Corn silage</td>
</tr>
<tr>
<td>Alfalfa haylage</td>
</tr>
<tr>
<td>Barley silage</td>
</tr>
<tr>
<td>Oat silage</td>
</tr>
<tr>
<td>Dry hay</td>
</tr>
<tr>
<td>Straw</td>
</tr>
<tr>
<td>Dry corn</td>
</tr>
<tr>
<td>High moisture corn</td>
</tr>
<tr>
<td>Soybean meal</td>
</tr>
<tr>
<td>Canola meal</td>
</tr>
<tr>
<td>Dried corn distillers</td>
</tr>
<tr>
<td>Supplement</td>
</tr>
</tbody>
</table>

Table 7. Nutrient composition (mean ± SD) of the lactating cow partial mixed ration (PMR) fed to all cows by farm

<table>
<thead>
<tr>
<th>Lactating cow PMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm</td>
</tr>
<tr>
<td>DM %</td>
</tr>
<tr>
<td>CP, % of DM</td>
</tr>
<tr>
<td>ADF, % of DM</td>
</tr>
<tr>
<td>NDF, % of DM</td>
</tr>
<tr>
<td>NDFD48, % of DM</td>
</tr>
<tr>
<td>TDN, % of DM</td>
</tr>
<tr>
<td>Lignin, % of DM</td>
</tr>
<tr>
<td>Starch, % of DM</td>
</tr>
<tr>
<td>Fat, % of DM</td>
</tr>
<tr>
<td>Sugar, % of DM</td>
</tr>
<tr>
<td>Ash, % of DM</td>
</tr>
<tr>
<td>Ca, % of DM</td>
</tr>
<tr>
<td>P, % of DM</td>
</tr>
<tr>
<td>K, % of DM</td>
</tr>
<tr>
<td>Na, % of DM</td>
</tr>
<tr>
<td>Mg, % of DM</td>
</tr>
<tr>
<td>NE₃, Mcal/kg of DM²</td>
</tr>
</tbody>
</table>

¹Values were obtained from chemical analysis of PMR samples.
²NEL is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.
³Farm 1 lactating cow PMR particle distribution (% of DM): 18 ± 4.9% long (>19 mm), 46 ± 4.0% medium (8 to 19 mm), 16 ± 1.7% short (4 to 8 mm), and 19 ± 2.2% fine (<4 mm).
⁴Farm 2 lactating cow PMR particle distribution (% of DM): 15 ± 5.9% long (>19 mm), 42 ± 3.5% medium (8 to 19 mm), 17 ± 1.1% short (4 to 8 mm), and 21 ± 3.8% fine (<4 mm).
⁵Farm 3 lactating cow PMR particle distribution (% of DM): 10 ± 1.4% long (>19 mm), 45 ± 2.9% medium (8 to 19 mm), 17 ± 1.9% short (4 to 8 mm), and 28 ± 2.2% fine (<4 mm).
⁶Farm 4 lactating cow PMR particle distribution (% of DM): 18 ± 3.1% long (>19 mm), 45 ± 3.0% medium (8 to 19 mm), 14 ± 1.2% short (4 to 8 mm), and 22 ± 3.4% fine (<4 mm).
⁷Farm 5 lactating cow PMR particle distribution (% of DM): 14 ± 3.5% long (>19 mm), 46 ± 3.7% medium (8 to 19 mm), 14 ± 1.4% short (4 to 8 mm), and 25 ± 3.1% fine (<4 mm).
portions were then oven-dried at 55°C for 48 h (Table 2; Table 7). The 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 amyloglucosidase; AOAC International, 2000, method 966.11), sugar (AOAC International, 2000, method 968.28), crude fat (using pet ether; AOAC International, 2000, method 920.39), lignin (using ADF residue and $\text{H}_2\text{SO}_4$), 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).

Milking Characteristics, Components, and Analyses

Milking frequency, milking refusal frequency, concentrate delivered, and milk production for each milking visit from 1 to 150 DIM (excluding the day of calving with colostrum milkings) were recorded by the software associated with the AMS unit on each farm. In situations where cows were removed from the milking herd before the end of the follow-up period, only days with complete data were included in the analyses. Milk fat and protein content were determined at each milking across the study, on farms 1, 2, 3, and 4, with the AMS manufacturer’s automatic milk component detection device (MQC-C sensor, Lely Industries N.V., (Maassluis, The Netherlands), as validated by Fadul-Pacheco et al. (2018). 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 merged to ensure continuous data for each farm. Milk components (fat and protein) were adjusted based on milk weights per milking. This data was summarized on a per cow per day basis from 1 to 150 DIM. Energy-corrected milk (ECM) was calculated for each cow as follows: ECM (kg/d) = \[0.327 \times \text{milk yield (kg/d)} + 12.95 \times \text{fat yield (kg/d)} + 7.2 \times \text{protein yield (kg/d)}\] (Tyrrell and Reid, 1965).

Rumination, Health, and Blood Metabolite Data Collection

Rumination time was monitored continuously on all farms using an electronic rumination monitoring system (HR-TAG-LD, SCR Engineers Ltd.), as validated by Schirrmann et al. (2009). 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 merged to ensure continuous data for each farm. Milk components (fat and protein) were adjusted based on milk weights per milking. This data was summarized on a per cow per day basis from 1 to 150 DIM. Energy-corrected milk (ECM) was calculated for each cow as follows: ECM (kg/d) = \[0.327 \times \text{milk yield (kg/d)} + 12.95 \times \text{fat yield (kg/d)} + 7.2 \times \text{protein yield (kg/d)}\] (Tyrrell and Reid, 1965).

### Table 8. Nutrient composition (mean ± SD) of the automated milking system pellet formulated with dry glycerol powder delivered through the AMS to cows for the first 21 DIM fed to glycerol cows on each farm

<table>
<thead>
<tr>
<th>AMS Glycerol Pellet</th>
<th>Farm</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM %</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<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, % of DM</td>
<td>18.4 ± 0.25</td>
<td>21.5 ± 0.66</td>
<td>21.6 ± 0.50</td>
<td>17.7 ± 1.02</td>
</tr>
<tr>
<td>ADF, % of DM</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>
</tr>
<tr>
<td>NDF, % of DM</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>
</tr>
<tr>
<td>TTN, % of DM</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>
</tr>
<tr>
<td>Starch, % of DM</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>
</tr>
<tr>
<td>Sugar, % of DM</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>
</tr>
<tr>
<td>Fat, % of DM</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>
</tr>
<tr>
<td>Ash, % of DM</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>
</tr>
<tr>
<td>Ca, % of DM</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>
</tr>
<tr>
<td>P, % of DM</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>
</tr>
<tr>
<td>K, % of DM</td>
<td>1.0 ± 0.07</td>
<td>1.2 ± 0.05</td>
<td>1.0 ± 0.02</td>
<td>1.0 ± 0.06</td>
</tr>
<tr>
<td>Na, % of DM</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>
</tr>
<tr>
<td>Mg, % of DM</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>
</tr>
<tr>
<td>Net, Mcal/kg of DM</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>
</tr>
</tbody>
</table>

1Values were obtained from the chemical analysis of AMS glycerol pellet samples.  
2$\text{NEL}$ is the estimated energy needed for lactation and was calculated based on NRC (2001) equations.
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 vessels 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 Wittrock 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 Wittrock 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 5 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 (3 post-calving observations). Scores were determined using a 5-point scale, with 0.25 increments (Wildman et al., 1982). Change in BCS was calculated for each cows as the BCS at each of the 3 post-calving observations minus their calving BCS. 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 time point.

Statistical Analyses

All statistical analyses were conducted using SAS 9.4 software (SAS Institute Inc., Cary, NC). Before analyses, all data were screened for normality by visually assessing the data plots and using the Shapiro-Wilk test (with \( P > 0.05 \) indicating normality) in the UNIVARIATE procedure in SAS. 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, BCS change, 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, fixed-effect significance was declared at \( P \leq 0.05 \) and tendencies at 0.05 < \( P \leq 0.1 \). If the \( P \)-value of a covariate (prepartum NEFA, categorized BHB, glucose, and categorized prepartum BCS) was ≤0.05 it was retained in the model, otherwise covariates were removed from the model. In situations of multiple mean comparisons, the differences were investigated using the PDIFF procedure in the LSMEANS statement, with a Tukey-Kramer adjustment. The fit of all models were examined by visual evaluation of the homoscedasticity and normality of the residuals.

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. Data derived from the AMS was collected from 1 to 150 DIM, whereby 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. 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 heterogeneous first-order autoregressive. Each of these models tested the fixed effect of treatment, DIM, and parity. The covariate of categorized prepartum BCS (high or normal) as well as month of calving were included in the above models if the \( P \)-value was ≤0.05. Two- and 3-way interaction terms of the fixed effects, and retained covariates, were tested and remained in the above-mentioned models if the \( P \)-value was ≤0.10.

To test the effect of treatment on serum NEFA, blood BHB, blood glucose, BCS, BCS change, and LS, data
were summarized by cow, treatment, and sample number (i.e., post-calving sample taken on different days depending on measure) 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, sample, and parity. 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 prepartum BCS (high or normal), month of calving, NEFA, categorized BHB, and glucose, and were retained in the model if the P-value was ≤0.05. Two- and 3-way interactions of the fixed effects, and retained covariates, were tested and remained in the model 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 determine treatment differences in the number of cows exceeding the cut off for SCK high (BHB ≥1.2 mmol/L in any 1 of the 5 postpartum samples) or normal (BHB <1.2 mmol/L for all 5 postpartum samples), 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 × prepartum BCS interaction was detected; an odds ratio for high BHB based on prepartum BCS by treatment was calculated through the SLICE function (sliced by treatment) in the GLIMMIX procedure.

RESULTS

During the first 21 DIM and from 22 to 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 to 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).

Milking frequency (milking/d) was greater for GLY cows compared with 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 to 21 DIM, during treatment application, and averaged 0.86 refusals/d from 22 to 150 DIM during the follow-up period, with no differences detected between treatment groups in either period (Table 9).

When comparing milk yield, GLY cows tended to produce 0.8 kg/d more milk in the 1–21 DIM treatment period than CON cows (P = 0.09; Table 9; Figure 3). GLY cows also tended to have greater ECM yield (41.0 vs 40.3 kg/d; SE = 0.35; P = 0.1) from 1 to 21 DIM. In the follow-up period (22–150 DIM), GLY cows had greater milk yield (P = 0.046; Table 9), where they yielded an average of 1.5 kg/d more milk than CON cows (Figure 3). GLY cows also had greater ECM yield (47.3 vs 46.2 kg/d; SE = 0.52; P = 0.05) from 22 to 150 DIM. Milk fat content averaged 4.45% in the treatment period with no treatment differences detected (P = 0.30; Table 10). In the follow-up period (22–150 DIM), milk fat content averaged 3.75% (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, 109, 111–114, 129, 130, 134–136 and 138 DIM (data not shown). Milk fat yield averaged 1.57 kg/d in the 21 d treatment period, with no treatment differences detected (P = 0.84; Table 10). During the follow-up period (22–150 DIM), milk fat yield averaged 1.68 kg/d, with no treatment differences detected (P = 0.42; 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 (P = 0.52; P = 0.98; Table 10). Milk protein yield averaged 1.20 kg/d in the treatment period, with no detected difference between treatments (P = 0.47; Table 10). During the follow-up period (22–150 DIM), GLY cows produced 0.04 kg/d more milk protein than CON cows (P = 0.03; Table 10).

No treatment differences in daily rumination time were detected in either the treatment (P = 0.39; 1–21 DIM) or follow-up (P = 0.71; 22–150 DIM) periods (Table 10). There were no detected treatment effects (P = 0.78) on serum NEFA concentrations (P ≤ 0.01; Figure 4a). A treatment by sample interaction (P = 0.03) was detected 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). A tendency was observed 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 (≥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. There were 118 CON and 91 GLY cows who were over-conditioned in the preparum period, when categorizing prepartum BCS as either over-conditioned (BCS ≥3.5) or normal (BCS <3.5). When further analyzed by prepartum BCS, there were 31 CON and 19 GLY cows with high prepartum BCS (≥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). 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 (≥3.5) were more likely (odds ratio [OR] = 3.52, 95% CI = 1.57 to 7.88; P = 0.002) to have a high BHB test compared with CON cows with a normal prepartum BCS (<3.5). The model also demonstrated that PP animals had a reduced risk of high BHB (OR = 0.29, 95% CI = 0.16 to 0.54; P < 0.001).

Average BCS by treatment are shown in Figure 6. All cows lost BCS from calving (P < 0.001), with mean losses (in BCS units) of −0.35 ± 0.024 at 21 DIM, −0.46 ± 0.028 at 42 DIM, and −0.59 ± 0.030 at 63 DIM for CON cows and −0.28 ± 0.026 at 21 DIM, −0.44 ± 0.030 at 42 DIM, and −0.50 ± 0.032 at 63 DIM for GLY cows. A treatment by sample day interaction (P = 0.05) was detected for BCS change; whereby GLY cows had less (P = 0.049) BCS loss since calving than CON cows at the fourth sampling (63 DIM; Figure 6). Reflective of that, a treatment by sample day interaction (P = 0.05) was detected for mean postpartum BCS; whereby GLY cows had a higher BCS (P = 0.039) 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 ± 0.03 across the 4 postpartum samples.

**DISCUSSION**

This is the first study, to our knowledge, to supplement dry pure 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 ± 0.04 g/d more concentrate on a DM basis, reflecting the glycerol portion of the concentrate. Based on the glycerol specifications, those cows received 180 g pure glycerol on a DM basis. During those first 21 DIM, CON and GLY cows were delivered an average of 3.59 kg/d DM (3.94 kg/d as fed) and 3.86 kg/d DM (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 from 21 DIM onward. 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., 2018b). The steady increase in concentrate delivered

### Table 9

<table>
<thead>
<tr>
<th>Treatments1</th>
<th>P-values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CON</td>
</tr>
<tr>
<td>1–21 DIM</td>
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<tr>
<td>Concentrate delivered, kg/d DM3</td>
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<td>Milking frequency, #/d</td>
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<tr>
<td>22–150 DIM</td>
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<tr>
<td>Concentrate delivered, kg/d DM3</td>
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<tr>
<td>Milking frequency, #/d</td>
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<td>AMS refusals4, #/d</td>
<td>0.85</td>
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<td>Milk yield, kg/d</td>
<td>43.4</td>
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<tr>
<td>Rumination time, min/d</td>
<td>577</td>
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</table>

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

2DIM is a repeated measure over time with 1 observation/cow/day.

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

4AMS refusals were log-transformed for normality.
observed in the current study 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. Related to 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. (2018b) 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 251 g/d DM 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 in early lactation, 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 as the average serum concentrations observed were not significantly different. 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. Similarly, when substituting ground corn for crude glycerine (76.2% glycerol), Zacaroni et al. (2022) observed a decrease in plasma glucose. 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 251 g/d 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 crude 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 crude 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 their BHB concentrations steadily increase 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 (Rémond et al., 1993; Khalili et al., 1997; Zacaroni et al., 2022). 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 blood 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., 2018a; 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 related to less BCS loss since calving, compared with CON cows, suggesting that they were able to maintain condition better during the first 63 DIM. 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 downstream 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 to 150 DIM, GLY cows yielded 1.5

**Figure 2.** Successful milkings per day (mean ± SE) by treatment for 1–150 DIM. All cows had free flow access to the AMS with control (CON) cows received only a standard AMS pellet; Glycerol (GLY) cows received a standard AMS pellet that included dry glycerol powder (minimum 250 g/cow/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. From DIM 1–21 GLY cows had more successful milkings than CON cows (P = 0.027). From DIM 22–150 GLY cows had more successful milkings than CON cows (P = 0.047).
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

**Table 10.** Effect of dry glycerol supplemented through the automated milking system pellet for the first 21 DIM on the milk components of dairy cows milked in AMS. Treatment was applied from 1 to 21 DIM then all cows received the base pellet from 22 to 150 DIM.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>P-values</th>
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<tr>
<td>CON GLY SE Treatment Parity DIM</td>
<td></td>
</tr>
<tr>
<td><strong>1–21 DIM</strong></td>
<td></td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>4.5 4.4 0.07 0.30 0.019 &lt;0.001</td>
</tr>
<tr>
<td>Milk fat yield, kg/d</td>
<td>1.58 1.57 0.04 0.84 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.47 3.48 0.02 0.52 0.6 &lt;0.001</td>
</tr>
<tr>
<td>Milk protein yield, kg/d</td>
<td>1.20 1.21 0.01 0.47 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td><strong>22–150 DIM</strong></td>
<td></td>
</tr>
<tr>
<td>Milk fat, %</td>
<td>3.8 3.7 0.05 0.15 — &lt;0.001</td>
</tr>
<tr>
<td>Milk fat yield, kg/d</td>
<td>1.67 1.70 0.03 0.42 &lt;0.001 &lt;0.001</td>
</tr>
<tr>
<td>Milk protein, %</td>
<td>3.11 3.11 0.01 0.98 0.74 &lt;0.001</td>
</tr>
<tr>
<td>Milk protein yield, kg/d</td>
<td>1.37 1.41 0.01 0.03 &lt;0.001 &lt;0.001</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 dry glycerol powder (targeting 250 g/cow/d intake as fed) for 21 DIM then standard AMS pellet from 22 to 150 DIM. The treatment was administered over the first 21 DIM, then all cows received the base AMS pellet from 22 to 150 DIM.
3DIM is a repeated measure over time with 1 observation/cow/day.
4A significant treatment*DIM interaction (P = 0.001) was observed for milk fat % from 22 to 150 DIM.
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 may have 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 to 150 DIM, GLY cows had a 0.1 increase in milkings/d compared with 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 indicators of 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 (DM) 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 (4.2 kg/d DM, 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 did not likely contribute to 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 observed during the first 21 DIM. From 22 to 150 DIM GLY cows had 3.4 milkings/d compared with CON cows with 3.3 milkings/d, with no difference in daily refusals. The increase in successful milkings may have been 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 6x/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 with 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 to 150 DIM; specifically, GLY cows had a 3% increase in milking frequency and a 3.5% (1.5 kg) increase in milk yield compared with 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 4.53 kg/d DM (4.87 kg/d as fed) of the treatment concentrate at 21 DIM to consuming 0 kg/d of the treatment concentrate and 4.28 kg/d DM (4.58 kg/d as-fed) of the standard AMS concentrate at 22 DIM. From 22 to 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 programme 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 to 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 with CON cows likely caused the varied AMS concentrate delivery by treatment over time as observed in the current study.

There was no treatment difference observed for rumination time in either period of the current study. Cows averaged 520 min/d in the first 21 DIM and 577 min/d from 22 to 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 475 min/d for control cows and 477 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 493 ± 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 to 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. Unfortunately, given that this study was carried out on commercial farms, and it was not possible to measure individual feed intake, we were unable to verify if the observed effects of glycerol supplementation in the first 21 DIM were related to total daily DMI.

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 from calving to 63 DIM 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 percentage point 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 detected treatment differences in milk protein content in the current study. Previous research has demonstrated varied results in respect to milk protein response to glycerol supplementation. 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.

There are some notable limitations to the current study. One was the allocation of the AMS concentrate, as through the 21-d treatment period, 4 of the 5 herds transitioned to yield-based supplementation feed tables, which individualized the amount of concentrate that was programmed to be delivered. This makes treatment application difficult as the daily quantity of AMS concentrate...
delivered is variable based on the individual’s daily milk yield and daily milking frequency. Another limitation was that 38 GLY cows were excluded from analysis, as they did not meet the minimum 250 g/d as fed intake, averaged across the 21-d treatment period. This was predominantly caused by an error in the feed tables at farm 1. The feed tables for the PP and MP treatment cows on that farm were not sufficiently increased; as such, some cows were not offered enough concentrate in the first 21 DIM. Further, as this study was conducted on commercial herds, we were unable to measure individual DMI of the consumed AMS concentrate. Monitoring individual daily DMI would have allowed a comparison of the energy consumption and utilization of cows on either treatment, as well as determine whether there was a substitution effect (of PMR for the AMS concentrate) when supplementing cows with glycerol.

CONCLUSIONS

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 from calving 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 behavior and milk yield through to mid lactation.

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