Effects of dietary glycerin inclusion at 0, 5, 10, and 15 percent of dry matter on energy metabolism and nutrient balance in finishing beef steers

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ABSTRACT: Expansion of the biodiesel industry has increased the glycerin (GLY) supply. Glycerin is an energy-dense feed that can be used in ruminant species; however, the energy value of GLY is not known. Therefore, the effects of GLY inclusion at 0, 5, 10, and 15% on energy balance in finishing cattle diets were evaluated in 8 steers (BW = 503 kg) using a replicated Latin square design. Data were analyzed with the fixed effects of dietary treatment and period, and the random effects of square and steer within square were included in the model. Contrast statements were used to separate linear and quadratic effects of GLY inclusion. Glycerin replaced dry-rolled corn (DRC) at 0, 5, 10, and 15% of dietary DM. Dry matter intake decreased linearly (P = 0.02) as GLY increased in the diet. As a proportion of GE intake, fecal energy loss tended to decrease linearly (P < 0.07), and DE also tended to increase linearly (P = 0.07) as dietary level of GLY increased. Urinary energy loss was not different (P > 0.31) as GLY increased in the diet. Methane energy loss as a proportion of GE as GLY increased in the diet. Methane energy loss tended to respond quadratically (P = 0.10), decreasing from 0 to 10% GLY inclusion and increasing thereafter. As a proportion of GE intake, ME tended to respond quadratically (P = 0.10), increasing from 0 to 10% GLY and then decreasing. As a proportion of GE intake, heat production increased linearly (P = 0.02) as GLY increased in the diet. Additionally, as a proportion of GE intake, retained energy (RE) tended to respond quadratically (P = 0.07), increasing from 0 to 10% GLY inclusion and decreasing thereafter. As a proportion of N intake, urinary and fecal N excretion increased linearly (P < 0.04) as GLY increased in the diet. Furthermore, grams of N retained and N retained as a percent of N intake both decreased linearly (P < 0.02) as GLY increased in the diet. Total DM digestibility tended (P < 0.10) to respond quadratically, increasing at a decreasing rate from 0 to 5% GLY inclusion. Overall, RE tended to decrease as GLY increased in the diet in conjunction with a decrease in N retention, which could indicate an increased metabolic cost to the animal associated with feeding GLY. Based on RE, the feeding value of GLY in high-concentrate diets is greater than DRC at 5 and 10% of DM but less at 15% of DM.

Key words: beef cattle, energy metabolism, glycerin


INTRODUCTION

Glycerin (GLY) is the byproduct of biodiesel production resulting from the formation of methyl esters of fatty acids from triglycerides. It has been evaluated as a feedstock in many species including pigs (Lammers et al., 2008b), poultry (Dozier et al., 2008; Lammers et al., 2008a), sheep (Gunn et al., 2010a,b), dairy cows (Donkin et al., 2009), growing cattle (Hales et al., 2013a), and finishing cattle (Parsons et al., 2009). The use of GLY in ruminant diets is not new; it has been used to treat ketosis (Fisher et al., 1973) and prevent lactation-induced ketosis in dairy cows (Goff and Horst, 2001; DeFrain et al., 2004). Glycerin is an appealing byproduct in feedlot diets because it is primarily converted to propionate in the rumen, thus acting as a precursor for glucose...
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16-d diet adaptation and 5 d of fecal and urine collection was added to continuous culture fermenters in place of 15% of DM on energy metabolism, enteric methane production, and increase retained energy (RE) in the animal. Therefore, our hypothesis was that replacing dry-rolled corn (DRC) with GLY would increase RE and decrease methane production in feedlot cattle. Our objective was to evaluate the effects of feeding GLY at 0, 5, 10, and 15% of DM on energy metabolism, enteric methane production, and nutrient balance (specifically fiber) in finishing beef steers.

MATERIALS AND METHODS

All animal use protocols were approved by the U.S. Meat Animal Research Center Animal Care and Use Committee. Steers (n = 8; 503 ± 23.39 kg of initial BW) were the U.S. Meat Animal Research Center (MARC) I composite breed made up of one-fourth Limousin, one-fourth Charolais, one-fourth Braunvieh, one-eighth Angus, and one-eighth Hereford. The dietary treatments (presented in Table 1) consisted of a DRC-based diet. Glycerin replaced DRC at 0 (GLY-0), 5 (GLY-5), 10 (GLY-10), and 15% (GLY-15) of dietary DM. During diet adaptation, cattle were housed in 4 partially covered concrete pens (2 steers/pen). During collections, cattle were moved to a metabolism facility where they were housed in individual stalls (87 by 214 cm) equipped with automatic, individual water cups. Before the start of the experiment, cattle were adapted to close human contact in the barn for at least 6 wk. During this time, steers were trained to wear fecal bags and urine harnesses. After adaptation to the collection facility, the steers were stratified by BW and assigned to 1 of 2 Latin square replicates. Each of the 4 periods in the Latin square consisted of an initial 16-d diet adaptation and 5 d of fecal and urine collections, resulting in a total of 84 d for the experiment.

Cattle were fed once daily at 0800 h throughout the experiment and had ad libitum access to feed. Steers had access to fresh water at all times. During the collection periods, orts were weighed daily 24 h after feeding the day before and a subsample was saved for later determination of DM content and GE determination. Orts, feces, and urine were collected after 24 h of measurement. For example, if an animal was fed on d 1 at 0800 h, then on d 2, orts would be collected at 0800 h. Additionally, the fecal bag would be changed and urine would be collected at 0800 h. Diet samples and orts were collected daily during each collection period. Urine was collected into a polypropylene jug under vacuum where each jug contained 100 mL of 3.7 N HCl to prevent ammonia losses. Feces were collected in a canvas bag attached to a harness. Three percent aliquots of urine and feces were collected daily, thoroughly mixed, and pooled within steer and stored at –17°C for later laboratory analyses.

Composited diet, orts, and fecal samples were dried for 48 h in a forced-air oven at 55°C. Samples were then ground through a Wiley Mill (Arthur Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. Gross energy was measured via bomb calorimetry for dried diets, orts, feces, and urine. Neutral detergent fiber content was measured on dried diets and fecal samples by placing the sample in an individual Ankom fiber bag (F57 Filter Bags; Ankom Technology, Macedon, NY) that was heat sealed. The NDF analysis was performed with an Ankom 200 Fiber Analyzer (Ankom Technology) following the procedures of Van

Table 1. Composition (DM basis) of diets based on dry-rolled corn (DRC) with glycerin (GLY) replacing DRC at 0, 5, 10, and 15%

<table>
<thead>
<tr>
<th>Ingredient, % of DM</th>
<th>GLY-0</th>
<th>GLY-5</th>
<th>GLY-10</th>
<th>GLY-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRC</td>
<td>80.02</td>
<td>75.02</td>
<td>70.02</td>
<td>65.02</td>
</tr>
<tr>
<td>GLY</td>
<td>0.00</td>
<td>5.00</td>
<td>10.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Urea</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Molasses</td>
<td>2.56</td>
<td>2.56</td>
<td>2.56</td>
<td>2.56</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>5.25</td>
<td>5.25</td>
<td>5.25</td>
<td>5.25</td>
</tr>
<tr>
<td>Beef trace mineral2</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin A, D, and E premix2</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Rumensin 802</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.29</td>
<td>1.29</td>
<td>1.29</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Analysis of composition

<table>
<thead>
<tr>
<th>Item</th>
<th>GLY-0</th>
<th>GLY-5</th>
<th>GLY-10</th>
<th>GLY-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>14.68</td>
<td>12.97</td>
<td>12.81</td>
<td>12.67</td>
</tr>
<tr>
<td>NDF, %</td>
<td>12.53</td>
<td>11.59</td>
<td>12.48</td>
<td>11.98</td>
</tr>
<tr>
<td>Ether extract,%</td>
<td>3.67</td>
<td>3.47</td>
<td>3.26</td>
<td>3.06</td>
</tr>
<tr>
<td>Ash, %</td>
<td>4.90</td>
<td>4.75</td>
<td>5.10</td>
<td>5.75</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>P, %</td>
<td>0.32</td>
<td>0.30</td>
<td>0.28</td>
<td>0.27</td>
</tr>
</tbody>
</table>

1Diets were based on DRC with 0, 5, 10, and 15% GLY on a DM basis. Glycerin replaced DRC at 0 (GLY-0), 5 (GLY-5), 10 (GLY-10), and 15% (GLY-15) of dietary DM.
2Rumensin (Elanco Animal Health, Greenfield, IN) and vitamins and minerals to exceed NRC (2000) requirements were incorporated into a commercial supplement premix. The beef trace mineral and vitamin A, D, and E premix were the same across dietary treatments.
tive (1 g/100 mL of NDF solution) were added to the
solution during the analysis. The bags containing the
residual were then dried for 24 h at 100°C in a
forced-air oven for quantification of the NDF residue.
Ether extract was quantified by refluxing ether over
samples in Soxhlet tubes for 18 h. Diet and fecal N
were quantified at a commercial laboratory (Servi-
tech Labs, Hastings, NE) using the Kjeldahl method.
The GLY was sent to a commercial laboratory for
analyses of 0.84% CP, 0.21% fat, 2.32% Na, 0.50%
methanol, 82.70% GLY, and <0.01% ethanol.

Treatment diets were mixed in 50-kg batches 3 times
per week in a small mixer and stored in 400-L feed wag-
ons. Before mixing, each ingredient was weighed on a
top loading scale. Ingredients were added to the mixer
and each diet was allowed to mix for approximately 10
min. Cleanout of the mixer unit was performed to ensure
that no cross-contamination of diets occurred.

Following each collection period, O2, CO2, and
CH4 gases were measured by indirect calorimetry using
8 portable respiration head boxes for 6 h. At least 3
air turnovers were allowed before the gas measurements
were determined. The portable box was 0.76 by 0.76 by
1.78 m and had an aluminum frame, which was covered
with 5-mm clear acrylic sheets. The box was equipped
with a 0.76-by-117-cm opening with a vinyl hood used
for attaching around the animal’s neck to provide a seal
between the box and animal. The animal’s daily diet al-
lotment was placed in each box, and the cattle consumed
>75% of the feed offered. Gas exchange was determined
by pulling air through the box across a temperature-com-
ensated dry test meter to determine airflow leaving the
box. Real-time air temperature and humidity were deter-
mined. Proportional samples of background air entering
the box and air exhausted from the box were collected
in polyethylene-aluminum-Mylar laminate gas bags to
form a composite air sample for the collection period for
each individual box. Gas samples were analyzed for O2,
CO2, and CH4 according to Nienaber and Maddy (1985).
Measurements from the 6-h period were adjusted to daily
measurements by multiplying by 4. Heat production was
calculated using the Brouwer equation (Brouwer, 1965).
Before gas measurements were collected, each head box
was calibrated for O2 consumed and CO2 produced by
burning absolute ethanol with alcohol lamps. Recoveries
ranged from 98 to 101% in all head boxes.

All data were analyzed as a Latin square design
using the Mixed procedure of SAS (SAS Inst. Inc.,
Cary, NC). The model included the fixed effects of
period and dietary treatment and the random effects of
square and steer within square. Contrast statements
were used to test the linear and quadratic effects of
GLY concentration in the diet. Effects were consid-
ered significant at P-value of ≤0.05, with tendencies
declared at P-values between 0.05 and 0.10.

RESULTS

The formulated and analyzed diet compositions
are presented in Table 1. The DM composition of the
diets ranged from 88.47 to 89.82%. The diets were not
formulated to be isonitrogenous, with the CP content
varying by 5.7% across the diets. As expected, the
NDF content decreased as GLY replaced DRC in the
diets. The ether extract decreased with inclusion of
GLY in the diets and ranged from 3.67 to 3.06% of
DM. Calcium and P were similar across all diets, and
a 2:1 Ca:P ratio was maintained.

Dry matter intake decreased linearly as GLY in-
creased in the diet from 0 to 15% (P = 0.02; Table 2).
Gross energy intake was not different across treatments
as GLY was included in the diet (P > 0.20). Fecal en-
ergy excretion tended to decrease linearly (P = 0.07).
Megacalories of DE did not differ across diets (P >
0.14); however, as a proportion of GE, fecal energy
excretion tended to decrease linearly (P = 0.07).
Methane energy respired did not differ across treatments (P
> 0.38) whereas, when expressed as a proportion of GE, 
intake, it tended to increase linearly as GLY increased in the diet (P = 0.07).

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Gross energy intake was not different across treatments
as GLY was included in the diet (P > 0.20). Fecal en-
ergy excretion tended to decrease linearly (P = 0.07).
Megacalories of DE did not differ across diets (P >
0.14); however, as a proportion of GE, DE tended to
increase linearly as GLY increased in the diet (P = 0.07).

There were no differences in urinary energy excretion
when expressed in megacalories (P > 0.63) or as a pro-
portion of GE (P > 0.31). Methane energy respired did
not differ across treatments (P > 0.38) whereas, when
expressed as a proportion of GE intake, it tended to in-
crease quadratically increasing from 10 to 15% GLY in-
clusion (P = 0.10). As GLY increased in the diet, ME in
megacalories did not differ across treatment (P > 0.14);
yet as a proportion of GE intake, ME tended to in-
crease quadratically from 0 to 5% GLY inclusion (P = 0.10).

The relationship between ME and GLY concentration
was described by the equation

\[
\text{ME} = f(\text{GLY}) = (-0.0302 \pm 0.0183)\text{GLY}^2 + (0.3682 \\
\pm 0.2883)\text{GLY} + (24.0568 \pm 0.89892); R^2 = 0.05.
\]

Solving for the first derivative indicated the opti-
mum concentration of GLY for maximum ME con-
centration of the diet is 6.1% of DM.

Heat production (Table 2) as total megacalories
did not differ across treatment as GLY increased from
0 to 15% inclusion (P > 0.36); but, when expressed
as a proportion of GE intake, it responded quadra-
tically (P = 0.02), increasing from 10 to 15% GLY in-
clusion. Megacalories of RE were not different as GLY
increased in the diet (P > 0.11). As a proportion of GE
intake, RE tended to decrease as GLY increased in
the diet (P = 0.07). The relationship between RE and GLY
concentration was described by the equation

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\[ RE = f(\text{GLY}) = (–0.0345 \pm 0.02476)\text{GLY}^2 + (0.3802 \pm 0.1942)\text{GLY} + (6.2592 \pm 0.7215); \quad R^2 = 0.05. \]

Solving for the first derivative indicated the optimum concentration of GLY for maximum RE is 5.5% of DM.

Nitrogen balance is presented in Table 3. Nitrogen intake decreased linearly as GLY increased in the diet \((P < 0.01)\). Grams of N excreted in the urine were not different across treatments \((P > 0.62)\); however, grams of N excreted in the feces decreased linearly as GLY was increased in the diet from 0 to 15% \((P < 0.01)\). There were no differences in total grams of N excreted across treatments \((P > 0.63)\).

As a proportion of total N excretion, urine excretion tended to increase linearly \((P = 0.08)\) whereas fecal excretion tended to decrease linearly \((P = 0.08)\) as GLY was increased in the diet. When expressed as a proportion of total N intake, urinary and fecal N excretion both increased linearly \((P = 0.04\) for urine and \(P = 0.03\) for feces).

Apparent grams of N digested decreased linearly as GLY was increased in the diet from 0 to 15% \((P < 0.01)\). Additionally, apparent N digestibility as a proportion of N intake decreased linearly \((P = 0.02)\) as GLY increased in the diet.

Grams of N retained decreased linearly as GLY increased in the diet \((P < 0.01)\). Furthermore, N retained as a proportion of N intake also decreased linearly as GLY increased in the diet \((P = 0.02)\).

Dry matter digestibility (Table 4) tended to respond quadratically, increasing from 0 to 5% GLY inclusion \((P = 0.10)\) and decreasing thereafter. Organic matter intake and fecal OM excretion both decreased linearly as GLY increased in the diet \((P < 0.01)\). There was no difference in OM digestibility as GLY increased in the diet \((P > 0.01)\). Intake of NDF decreased linearly as GLY increased from 0 to 15% of the diet. Likewise, fecal excretion of NDF decreased linearly when GLY concentration increased in the diet \((P < 0.01)\). There was no difference across treatments for grams of NDF digested \((P > 0.38)\) or NDF digestibility as a proportion of NDF intake \((P > 0.39)\).

**DISCUSSION**

The similarity in CP for the GLY-5, GLY-10, and GLY-15 diets was unexpected when replacing DRC with GLY. Our expectation was that CP would decrease linearly as GLY was included in the diet. The reason for the discrepancy is not understood. Additionally, the reason for the increase in NDF from 5 to 10% GLY is not known. We expected all diets containing GLY to have less NDF than the control diet and for NDF to also decrease linearly as GLY increased.

Jung and Batal (2011) also evaluated chemical characteristics of GLY samples from several production facilities and reported the ash content was 4.35%. Therefore, GLY had greater ash content than the DRC.

### Table 2. Daily influence of feeding dry-rolled corn-based diets with 0, 5, 10, and 15% glycerin (GLY) on the partitioning of energy in finishing steers fed at ad libitum intake

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>Linear P-value</th>
<th>Quadratic P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, g</td>
<td>GLY-0</td>
<td>8,111</td>
<td>343.9</td>
<td>0.02</td>
</tr>
<tr>
<td>GE intake, Mcal</td>
<td>GLY-5</td>
<td>8,010</td>
<td>36.83</td>
<td>1.594</td>
</tr>
<tr>
<td>Fecal energy, Mcal</td>
<td>GLY-10</td>
<td>7,954</td>
<td>32.90</td>
<td>0.556</td>
</tr>
<tr>
<td>Fecal energy, % of GE</td>
<td>GLY-15</td>
<td>6,875</td>
<td>24.95</td>
<td>1.377</td>
</tr>
<tr>
<td>DE, Mcal</td>
<td>GLY-0</td>
<td>25.64</td>
<td>24.93</td>
<td>1.377</td>
</tr>
<tr>
<td>DE, % of GE</td>
<td>GLY-5</td>
<td>71.70</td>
<td>70.55</td>
<td>1.368</td>
</tr>
<tr>
<td>Urinary energy, Mcal</td>
<td>GLY-10</td>
<td>74.89</td>
<td>75.35</td>
<td>0.556</td>
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<tr>
<td>Urinary energy, % of GE</td>
<td>GLY-15</td>
<td>75.35</td>
<td>28.30</td>
<td>1.380</td>
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<tr>
<td>Methane energy, Mcal</td>
<td>GLY-0</td>
<td>1.40</td>
<td>1.52</td>
<td>0.522</td>
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<tr>
<td>Methane energy, % of GE</td>
<td>GLY-5</td>
<td>3.94</td>
<td>4.81</td>
<td>1.399</td>
</tr>
<tr>
<td>ME, Mcal</td>
<td>GLY-10</td>
<td>23.58</td>
<td>22.71</td>
<td>1.399</td>
</tr>
<tr>
<td>ME:DE</td>
<td>GLY-15</td>
<td>91.81</td>
<td>90.32</td>
<td>1.234</td>
</tr>
<tr>
<td>ME, % of GE</td>
<td>GLY-0</td>
<td>65.87</td>
<td>67.90</td>
<td>2.050</td>
</tr>
<tr>
<td>Heat production, Mcal</td>
<td>GLY-5</td>
<td>18.00</td>
<td>18.26</td>
<td>0.537</td>
</tr>
<tr>
<td>Heat production, % of GE</td>
<td>GLY-10</td>
<td>50.64</td>
<td>57.63</td>
<td>2.446</td>
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<tr>
<td>Retained energy, Mcal</td>
<td>GLY-15</td>
<td>5.58</td>
<td>4.44</td>
<td>1.258</td>
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<tr>
<td>Retained energy, % of GE</td>
<td>GLY-0</td>
<td>15.22</td>
<td>10.27</td>
<td>3.561</td>
</tr>
</tbody>
</table>

1 Diets were based on dry-rolled corn with 0, 5, 10, and 15 GLY on a DM basis. Glycerin replaced DRC at 0 (GLY-0), 5 (GLY-5), 10 (GLY-10), and 15% (GLY-15) of dietary DM.

2 Pooled standard error of the least squares means \((n = 8)\).

3 Observed significance levels for treatment comparisons.
it replaced in the diet making the increase in ash as GLY increased realistic.

During our experiment, DMI was consistently less than 2% of BW during the experimental period. The cattle were allowed ad libitum access to feed. The low DMI numbers observed are thought to be a result of conducting the experiment during the hot summer months and the fact that the cattle were close to finished at the start of the experiment. A linear decrease in DMI was observed when finishing heifers were fed steam-flaked corn-based diets with 0, 2, 4, 8, 12, or 16% GLY (Parsons et al., 2009). In contrast, Hales et al. (2013a) reported no difference in DMI of steers fed in Calan gates (American Calan Inc. Northwood, New Hampshire) when GLY was included at 0, 2.5, 5, 7.5, and 10% of DM in steam-flaked corn-based growing diets. The difference in the effects of GLY on DMI could be related to the Na content of the GLY compared to DRC. Elevated Na concentrations in a cattle diet are thought to have inhibitory effects on DMI as well as reduce water intake, decrease growth, and cause mild digestive disturbances (NRC, 2000). It is unclear, however, if Na in the diet is responsible for disruptions in intake or if perhaps the accompanying anion causes the intake reductions. Cattle fed NaHCO₃ have been shown to have higher intakes than cattle fed NaCl with equivalent levels of Na (Leibholz et al., 1980). The concentration of Cl and other minerals in the GLY is unknown. The maximum tolerable Na consumption for ruminants below which feed intake is not altered is 1 g/kg of BW (NRC, 2005), which is much greater than the intakes observed in this experiment (approximately 0.005, 0.021, 0.040, and 0.051 g Na/kg BW for GLY-0, GLY-5, GLY-10, and GLY-15, respectively). Furthermore, conversion of glycerol to propionate by ruminal microbes (Bergner et al., 1995) may lead to an observed decrease in DMI. Infusion of propionate into the portal vein (Anil and Forbes, 1980) or into the rumen (Oba and Allen, 2003) has been shown to reduce intake in sheep and cattle. It is possible that glycerol is directly absorbed from the rumen or lower gastrointestinal tract (Rémond et al., 1993). If glycerol is metabolized to glycerol 3-phosphate in the liver and enters the glycolytic pathway, there could be a reduction in intake due to greater oxidation of acetyl CoA through the tricarboxylic acid cycle (Allen et al., 2009). However, if the majority of glycerol reaching the liver enters gluconeogenesis via glycerol 3-phosphate, there would likely be no effect on intake (Allen et al., 2009). Whatever the reason, the decrease in DMI as GLY concentration is increased in cattle diets seems consistent throughout the literature.

Although the steers in the current experiment had a linear decrease in DMI as GLY increased in the diet, the GE consumed was not different across treatments. This could imply that DMI was potentially regulated by the energy density of the diet. Literature data has established a correlation between dietary energy concentration and DMI. When cattle are consuming energy-dense high-concentrate diets, DMI is thought to be

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**Table 3.** Influence of feeding dry-rolled corn-based diets with 0, 5, 10, and 15% glycerin (GLY) on N balance in finishing steers fed at ad libitum intake

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
<th>SEM²</th>
<th>Linear P-value³</th>
<th>Quadratic P-value³</th>
</tr>
</thead>
<tbody>
<tr>
<td>N intake, g/d</td>
<td>GLY-0</td>
<td>GLY-5</td>
<td>GLY-10</td>
<td>GLY-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>190.7</td>
<td>166.4</td>
<td>163.6</td>
<td>139.6</td>
<td>7.77</td>
<td>&lt;0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Feces</td>
<td>72.3</td>
<td>80.5</td>
<td>78.3</td>
<td>78.5</td>
<td>11.01</td>
<td>0.65</td>
<td>0.62</td>
</tr>
<tr>
<td>Total</td>
<td>131.4</td>
<td>132.2</td>
<td>131.3</td>
<td>125.7</td>
<td>12.96</td>
<td>0.63</td>
<td>0.70</td>
</tr>
<tr>
<td>N excretion, % of total N excretion</td>
<td>GLY-0</td>
<td>GLY-5</td>
<td>GLY-10</td>
<td>GLY-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>53.6</td>
<td>59.9</td>
<td>58.9</td>
<td>61.7</td>
<td>2.88</td>
<td>0.08</td>
<td>0.54</td>
</tr>
<tr>
<td>Feces</td>
<td>46.4</td>
<td>40.1</td>
<td>41.1</td>
<td>38.3</td>
<td>2.88</td>
<td>0.08</td>
<td>0.54</td>
</tr>
<tr>
<td>N excretion, % of N intake</td>
<td>38.4</td>
<td>48.2</td>
<td>48.2</td>
<td>62.4</td>
<td>8.63</td>
<td>0.04</td>
<td>0.78</td>
</tr>
<tr>
<td>Apparent N digested Grams per day</td>
<td>131.6</td>
<td>114.6</td>
<td>110.7</td>
<td>92.3</td>
<td>6.09</td>
<td>&lt;0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>Feces</td>
<td>31.0</td>
<td>31.2</td>
<td>32.3</td>
<td>34.3</td>
<td>1.35</td>
<td>0.03</td>
<td>0.38</td>
</tr>
<tr>
<td>N retained Grams per day</td>
<td>59.3</td>
<td>34.2</td>
<td>32.4</td>
<td>13.8</td>
<td>12.98</td>
<td>&lt;0.01</td>
<td>0.76</td>
</tr>
<tr>
<td>Feces</td>
<td>30.6</td>
<td>20.7</td>
<td>19.6</td>
<td>3.4</td>
<td>9.65</td>
<td>0.02</td>
<td>0.69</td>
</tr>
</tbody>
</table>

¹Diets were based on dry-rolled corn with 0, 5, 10, and 15% GLY on a DM basis. Glycerin replaced DRC at 0 (GLY-0), 5 (GLY-5), 10 (GLY-10), and 15% (GLY-15) of dietary DM.

²Pooled standard error of the least squares means (n = 8).

³Observed significance levels for treatment comparisons.
controlled by the animal’s energetic demands and metabolic factors (NRC, 2000). Additionally, the decrease in fecal energy loss indicates that GLY is more digestible than the DRC it replaced in the diet. Likewise, the decrease in fecal energy loss could be associated with the decrease in DMI and resulting changes in rate of passage. Plausibly, if DMI was decreased, the rate of passage would also be decreased, which would increase digestibility. The tendency for a quadratic increase in DM digestibility and OM digestibility as GLY increases in the diet supports a decreased rate of passage. In addition, the decreasing loss of fecal energy as GLY increased in the diet resulted in greater DE as a proportion of GE intake. The lack of differences in urinary energy losses are not surprising, as it appears most of the benefit from feeding GLY is the increased digestibility and decreased fecal energy loss as previously discussed.

Previous research has demonstrated that shifting ruminal VFA production toward propionate decreases methane production because the metabolic pathway leading to propionate is a hydrogen sink (Boadi et al., 2004). In the current experiment, we hypothesized that because GLY would be primarily converted to propionate within the rumen and decrease the acetate-to-propionate ratio, that methane energy respired would decrease linearly as GLY concentration increased. In contrast with our hypothesis, Avila-Stagno et al. (2013) included glycerol in forage-based ruminant diets using a rumen simulation technique system. The diets included brome hay and corn silage with glycerol at 0, 5, 10, and 15% of dietary DM. The increased methane production between the 10 and 15% GLY inclusion could potentially be attributed to a decreased rate of passage and increased digestibility in conjunction with increased methane production.

The ME-to-DE ratio observed in the current experiment was not expected based on the NRC (2000). According to the NRC (2000), the ratio of ME to DE is 80% but can vary according to intake, age of animal, and feed source. In the current experiment, the ratio of ME to DE ranged from 91 to 94%. The lack of consistency between the NRC (2000) and the current dataset could be caused by many factors such as the method of data collection, which was respiration calorimetry in the current experiment and serial slaughter in past experiments. Quantification of ME in serial slaughter experiments would be achieved by using an equation to estimate CH$_4$ production, and in the current experiment CH$_4$ was directly measured. Dietary factors such as level of dietary fat may also help explain why the ratio of ME to DE is greater in the current experiment than reported by the NRC (2000). Previous literature has reported that feeding supplemental fat can decrease enteric CH$_4$ production by 3.8 to 5.6% for each 1% of supplemental fat in the diet (Beauchemin et al., 2008). Generally, added dietary fat decreases CH$_4$ production through biohydrogenation of unsaturated fatty acid precursors contained in the diet.

### Table 4. Influence of feeding dry-rolled corn-based diets with 0, 5, 10, and 15% glycerin (GLY) on DM digestibility, NDF, and OM balance in finishing steers fed at ad libitum intake

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>Linear P-value</th>
<th>Quadratic P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter digestibility, %</td>
<td>GLY-0</td>
<td>73.3</td>
<td>1.40</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>75.9</td>
<td>0.41</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>75.1</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>75.1</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>OM Intake, g/d</td>
<td>GLY-0</td>
<td>7,133.7</td>
<td>323.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>7,547.5</td>
<td>149.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>6,476.5</td>
<td>131.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>6,476.5</td>
<td>109.1</td>
<td>0.80</td>
</tr>
<tr>
<td>Fecal excretion, g/d</td>
<td>GLY-0</td>
<td>1,955.3</td>
<td>276.19</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>1,708.5</td>
<td>114.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>1,493.3</td>
<td>18.06</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>1,493.3</td>
<td>18.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Digested, g/d</td>
<td>GLY-0</td>
<td>5,758.4</td>
<td>74.8</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>5,938.4</td>
<td>77.8</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>5,839.0</td>
<td>77.4</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>4,983.2</td>
<td>76.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Digestibility, % of intake</td>
<td>GLY-0</td>
<td>74.8</td>
<td>1.32</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>77.8</td>
<td>0.41</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>77.4</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>76.4</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>NDF Intake, g/d</td>
<td>GLY-0</td>
<td>1,015.2</td>
<td>43.37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>977.9</td>
<td>83.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>492.0</td>
<td>413.3</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>423.3</td>
<td>38.50</td>
<td>0.38</td>
</tr>
<tr>
<td>Fecal excretion, g/d</td>
<td>GLY-0</td>
<td>551.4</td>
<td>24.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>486.5</td>
<td>413.3</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>505.8</td>
<td>423.3</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>423.3</td>
<td>38.50</td>
<td>0.38</td>
</tr>
<tr>
<td>Digested, g/d</td>
<td>GLY-0</td>
<td>463.8</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>445.8</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>505.8</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>423.3</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td>Digestibility, % of intake</td>
<td>GLY-0</td>
<td>45.9</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GLY-5</td>
<td>47.3</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GLY-10</td>
<td>50.1</td>
<td>3.18</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GLY-15</td>
<td>45.9</td>
<td>3.18</td>
<td>0.85</td>
</tr>
</tbody>
</table>

1 Diets were based on dry-rolled corn with 0, 5, 10, and 15% GLY on a DM basis. Glycerin replaced DRC at 0 (GLY-0), 5 (GLY-5), 10 (GLY-10), and 15% (GLY-15) of dietary DM.

2 Pooled standard error of the least squares means ($n = 8$).

3 Observed significance levels for treatment comparisons.
Additionally, the increased heat production could indicate an increase in ruminal fermentation as GLY concentration increased from 0 to 15% in the diet. The inclusion of GLY caused a quadratic response in retained energy (RE) yet a linear decrease in retained N. We hypothesize that at the 5 and 10% level of inclusion, GLY was beneficial from an energy standpoint as evidenced by a quadratic response in DE and DM digestibility. At 15% GLY inclusion, it appears that microbial protein synthesis was impeded and overall microbial N, which could indicate more postgastric fermentation. Furthermore, the increase urinary N loss could be indicative of a less microbial protein synthesis and the use of urea in the diets. Kijora et al. (1998) showed that 15N-urea incorporation into bacterial protein was reduced by feeding glycerol to cattle. Additionally, reductions in ruminal isobutyrate and isovalerate concentrations indicated that feeding glycerol at greater than 10% of DM reduced ruminal AA metabolism (Kijora et al., 1998). Hales et al. (2013b) reported that microbial efficiency responded quadratically, increasing from 0 to 5% GLY inclusion and then decreasing from 5 to 10% GLY. Conversely, Shin et al. (2012) noted that utilization of dietary N by ruminal microbes was increased as GLY concentration increased from 0 to 15% in the diet and they observed a reduction in ammonia-N in ruminal fluid across sources of roughage including cottonseed hulls and corn silage by feeding GLY in lactating cow diets. The differing responses to GLY reported by Kijora et al. (1998), Hales et al. (2013b), and Shin et al. (2012) are most likely because of the differing diet types: concentrate based (Kijora et al., 1998) or forage based (Hales et al., 2013b; Shin et al., 2012).

Interestingly, the inclusion of GLY caused a quadratic response in RE yet a linear decrease in retained N. We hypothesize that at the 5 and 10% level of inclusion, GLY was beneficial from an energy standpoint as evidenced by a quadratic response in DE and DM digestibility. At 15% GLY inclusion, it appears that microbial protein synthesis was impeded and overall ruminal fermentation was reduced, thus explaining the decreased RE observed for 15% GLY. Therefore, the addition of degradable intake protein sources may enhance GLY in high-concentrate finishing diets.

The tendency for a quadratic response in DM digestibility as GLY increased in the diet is similar to animal performance measurements published in the literature. Hales et al. (2013a) reported that ADG responded quadratically to increased GLY; ADG increased from 0 to 7.5% GLY and decreased from 7.5 to 10% GLY in growing cattle diets. If these results were presumably related to differences in diet digestibility, the ADG data reported by Hales et al. (2013a) would be similar to the effect of GLY on DM digestibility in the current experiment. In contrast, Shin et al. (2012) noted that feeding GLY had no effect on apparent digestibility of DM in lactating dairy cows fed high forage diets that were cottonseed hull or corn-silage based.

The linear decrease in OM intake and fecal excretion were expected, as GLY contains less OM than DRC and DMI decreased linearly as GLY increased in the whole animal as evidenced by the increased losses of N in urine and feces as a proportion of N intake. Potentially, the animals have an increased loss of microbial N, which could indicate more postgastric fermentation. Furthermore, the increase urinary N loss could be indicative of a less microbial protein synthesis and the use of urea in the diets. Kijora et al. (1998) showed that 15N-urea incorporation into bacterial protein was reduced by feeding glycerol to cattle. Additionally, reductions in ruminal isobutyrate and isovalerate concentrations indicated that feeding glycerol at greater than 10% of DM reduced ruminal AA metabolism (Kijora et al., 1998). Hales et al. (2013b) reported that microbial efficiency responded quadratically, increasing from 0 to 5% GLY inclusion and then decreasing from 5 to 10% GLY. Conversely, Shin et al. (2012) noted that utilization of dietary N by ruminal microbes was increased as GLY concentration increased from 0 to 15% in the diet and they observed a reduction in ammonia-N in ruminal fluid across sources of roughage including cottonseed hulls and corn silage by feeding GLY in lactating cow diets. The differing responses to GLY reported by Kijora et al. (1998), Hales et al. (2013b), and Shin et al. (2012) are most likely because of the differing diet types: concentrate based (Kijora et al., 1998) or forage based (Hales et al., 2013b; Shin et al., 2012).
diet. Hales et al. (2013b) also reported a linear decrease in fecal OM output as GLY increased in the diet. The tendency for a linear decrease in apparent OM digested in the current experiment is not in agreement with Hales et al. (2013b), who noted a linear increase in apparent OM digestibility. The primary difference between the current experiment and the one of Hales et al. (2013b) is the amount of concentrate grain in the diets.

The decrease in NDF as GLY increased in the diet was expected, because GLY replaced DRC, which contains a higher concentration of NDF than GLY. Likewise, DMI decreased linearly and thus NDF intake responded in a similar manner. Furthermore, the linear decrease in fecal NDF excretion is plausible, because NDF intake decreased with increased GLY concentration of the diet. The lack of difference in NDF digested was expected, as Hales et al. (2013b) reported that ruminal digestibility of NDF was not different as GLY increased in a steam-flaked corn-based diet from 0 to 10%. These results are not consistent with research evaluating GLY in diets fed to lactating cows. Shin et al. (2012) reported that digestibility of NDF decreased linearly as GLY increased from 0 to 10% in the diet. Furthermore, Donkin et al. (2009) reported that total-tract digestibility of NDF tended to be less in lactating cows fed diets including 5, 10, or 15% GLY compared to the 0% GLY control. These data would suggest that feeding GLY in high-forage diets depresses ruminal fiber digestibility. The lack of difference in NDF digested in the current experiment may mean that depression of fiber digestibility depends on the type of diet, (i.e., forage vs. concentrate based). Apparently, fiber digestibility is not reduced in high-concentrate feedlot diets, likely because of the difference in microbial populations and less ruminal cellulolytic activity when feeding concentrate-based diets.

Including GLY in feedlot diets decreased DMI and fecal energy loss, with no effect on urinary energy loss. Methane respired as a proportion of GE intake decreased from 0 to 10% GLY and increased thereafter. Furthermore, inclusion of GLY increased heat production whereas RE increased from 0 to 10% GLY and decreased thereafter. As a proportion of N intake, urinary and fecal N excretion increased as GLY increased in the diet. Furthermore, grams of N retained and N retained as a percent of N intake both decreased as GLY increased in the diet. The fact that DMI and fecal energy loss decreased yet total heat production increased and N retained decreased indicates that GLY is of high metabolic cost to ruminant animals on a high-concentrate diet. Based on RE, the feeding value of GLY in high-concentrate diets is greater than DRC at 5 and 10% of DM but less at 15% of DM. Therefore, when considering using GLY as a replacement for DRC, producers should be cautious as the feeding value diminishes as inclusion increases above 10% of DM.

**LITERATURE CITED**


