Effects of increasing concentrations of glycerol in concentrate diets on nutrient digestibility, methane emissions, growth, fatty acid profiles, and carcass traits of lambs

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ABSTRACT: Two experiments were conducted to evaluate the effects of increasing concentrations of glycerol in concentrate diets on total tract digestibility, methane (CH₄) emissions, growth, fatty acid profiles, and carcass traits of lambs. In both experiments, the control diet contained 57% barley grain, 14.5% wheat dried distillers grain with solubles (WDDGS), 13% sunflower hulls, 6.5% beet pulp, 6.3% alfalfa, and 3% mineral-vitamin mix. Increasing concentrations (7, 14, and 21% dietary DM) of glycerol in the dietary DM were replaced for barley grain. As glycerol was added, alfalfa meal and WD-DGS were increased to maintain similar concentrations of CP and NDF among diets. In Exp.1, nutrient digestibility and CH₄ emissions from 12 ram lambs were measured in a replicated 4 × 4 Latin square experiment. In Exp. 2, lamb performance was evaluated in 60 weaned lambs that were blocked by BW and randomly assigned to 1 of the 4 dietary treatments and fed to slaughter weight. In Exp. 1, nutrient digestibility and CH₄ emissions were not altered (P = 0.15) by inclusion of glycerol in the diets. In Exp.2, increasing glycerol in the diet linearly decreased DMI (P < 0.01) and tended (P = 0.06) to reduce ADG, resulting in a linearly decreased final BW. Feed efficiency was not affected by glycerol inclusion in the diets. Carcass traits and total SFA or total MUFA proportions of subcutaneous fat were not affected (P = 0.77) by inclusion of glycerol, but PUFA were linearly decreased (P < 0.01). Proportions of 16:0, 10t-18:1, linoleic acid (18:2 n-6) and the n-6/n-3 ratio were linearly reduced (P < 0.01). The proportion of 18:0 (stearic acid), 9c-18:1 (oleic acid), linearly increased (P < 0.01) by glycerol. When included up to 21% of diet DM, glycerol did not affect nutrient digestibility or CH₄ emissions of lambs fed barley based finishing diets. Glycerol may improve backfat fatty acid profiles by increasing 18:0 and 9c-18:1 and reducing 10t-18:1 and the n-6/n-3 ratio.

Key words: biodiesel co-products, digestibility, finishing lambs, methane, trans fatty acids

INTRODUCTION

Biodiesel production has increased in recent years leading to increased stocks of glycerine, a once valuable co-product that now many consider a waste stream with disposal costs (Yazdani and Gonzalez, 2007). The inclusion of glycerol (the main component of crude glycerine) as a major component of the diet has been reported in beef cattle (Mach et al., 2009; Parsons et al., 2009), and inclusions of 10 to 20% in diet DM have been used without negatively affecting lamb performance (Gunn et al., 2010a). Three different fates have been reported for glycerol when entering the rumen: 1) passage to the lower gut, 2) absorption through the rumen wall and conversion to glucose in the liver, or 3) fermentation to propionate resulting in increases in blood glucose in cattle (Krehbiel, 2008). Pathways to propionate production are known to act as a hydrogen sink and would therefore reduce CH₄ emissions (Boadi et al., 2004). Lee et al. (2011) reported reduced CH₄ emissions by including glycerol in in vitro incubations of corn grain and alfalfa hay;
MATERIALS AND METHODS

All experiments were conducted at the Agriculture and Agri-Food Canada Research Centre in Lethbridge, Alberta between December 2010 and March 2011 with all lambs being cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Experimental Procedures

Dietary Treatments. Composition of the diets are reported in Table 1 with treatment diets being formulated by substituting glycerol (99.5% pure, Sigma–Aldrich, St. Louis, MO) for barley grain and beet pulp to achieve concentrations of 7, 14, and 21% glycerol (DM basis). Dietary concentrations of wheat dried distillers grains with solubles (WDDGS), and alfalfa meal were increased with increasing glycerol to maintain similar concentrations of CP and NDF in the DM. These concentrations were selected according to the results of Musselman et al. (2008) and Gunn et al. (2010b) who reported negative impacts on lambs DMI when replacing crude glycerin for corn at concentrations above 15% dietary DM. All diets were ground and pelleted to 6.35 mm.

Digestibility and CH₄ Emissions Study (Exp. 1)

Animals and Experimental Design. A replicated 4 × 4 Latin square experiment using 12 Canadian Arcott ram lambs (initial BW 34.5 ± 3.4 kg) was used to assess the impact of the 4 dietary treatments (3 lambs per treatment) on nutrient digestibility and CH₄ emissions over four 21-d periods. Lambs were grouped by BW and randomly assigned to a diet. The first 16 d of each period were used to adapt the lambs to the diet by providing them free access to feed and water in individual indoor pens (0.97 × 2.82 m) bedded with straw. Feed was delivered at 1000 h daily during the adaptation period. For determination of daily DMI, refusals were collected and weighed daily before feeding. On d 17, the lambs were moved to a controlled environment building and located in individual metabolism crates (0.112 × 0.92 m) located in 4 identical chambers (volume = 63.5 m³; Model C1330; Conviron, Winnipeg, MB, Canada) for measurements total tract digestibility (McKeown et al., 2010) and CH₄ emissions. Each block of 3 lambs was housed in the same chamber during the trial. All lambs within a chamber received the same diet in each period. Immediately before feeding on d 17, the lambs were fitted with strap-on canvas fecal collection bags. Feed offered was restricted to 90% of the intake determined over the previous week and water was freely available. Feces samples and CH₄ measurement data were collected for 4 consecutive 24-h days starting at 1200 h on d 17 and finishing at 1200 h on d 21. On the last day of each period, the lambs were removed from the chambers

Table 1. Ingredients and chemical composition of the diets containing increasing amounts of glycerol

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Glycerol, % of dietary DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dry rolled barley</td>
<td>57.0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0</td>
</tr>
<tr>
<td>Wheat DDGS¹</td>
<td>14.5</td>
</tr>
<tr>
<td>Sunflower hulls</td>
<td>13</td>
</tr>
<tr>
<td>Beet pulp dehydrated</td>
<td>6.5</td>
</tr>
<tr>
<td>Alfalfa meal</td>
<td>6.3</td>
</tr>
<tr>
<td>Canola oil</td>
<td>0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.1</td>
</tr>
<tr>
<td>Mineral premix²</td>
<td>1.2</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>0.4</td>
</tr>
<tr>
<td>ADE vitamin mix³</td>
<td>0.02</td>
</tr>
</tbody>
</table>

¹DDGS = dry distillers grains with solubles.
²Containing 92.6% NaCl; 4.97% Dynamate; 0.9% ZnSO₄; 0.83% MnSO₄; 0.13% CuSO₄; 0.1% ethylenediamine dihydroiodide, 80% preparation; 0.005% CoSO₄; 0.4% canola oil (as carrier of CoSO₄); and 0.0014% Na₂SeO₃. No ionophores were included in the diet.
³Containing vitamin A (10 000 000 IU/kg); vitamin D (1 000 000 IU/kg); and vitamin E (10 000 IU/kg).
and transported to their individual pens in the barn for adaptation to the next diet. Interruptions to the chamber flux measurements occurred daily from 1006 to 1036 h to feed and water the lambs and collect feces samples.

**Methane Concentration Measurements and Sampling in the Chambers.** Chamber operation and measurements have been described in detail by McGinn et al. (2004, 2006) and Beauchemin and McGinn (2005). Briefly, the chambers were vented using individual fresh air intake and exhaust ducts (diameter 30.5 cm) with dedicated fans on each duct. Before calibration, flows were adjusted to generate a positive pressure (2 Pa or less) in each chamber. Fresh air intake flow (approximately 0.28 m$^3$/s) was fed into a recycling fan unit and air entered the chamber through 3 full-width floor vents. The loop of recycled air passed through filters and a temperature controller to maintain air in the chamber at 15°C. Circulation within the chamber ensured that air leaving through the exhaust duct (approximately 0.22 m$^3$/s) was representative of the entire air volume of the chamber which was exchanged every 5 min. Air was pumped sequentially from each duct of each chamber at 1 L/min (TD3LS7; Brailsford and Company, Rye, NY) and passed through an infrared gas analyzer (Model Ultramat 5E; Siemens, Karlsruhe, Germany) via solenoids that were controlled by a datalogger (CR23X; Campbell scientific, Logan, UT). Air was dried using a Nafion dryer (MD-110; Perma Pure, Tom Rivers, NJ) and magnesium perchlorate to a dew point of below −50°C. To compensate for the volume in the sampling line, data were ignored for the first 3 min after switching the air stream. Afterward, CH$_4$ concentration was recorded each min on the data logger. Each chamber was sampled for 7 to 8 min with a cycle among the 4 chambers lasting 30 min. Each morning the analyzer was calibrated using reference gases, N$_2$ gas for the 0 and primary standard CH$_4$ for the span. The difference between the intake and exhaust flow of CH$_4$ was used to calculate the amount of CH$_4$ produced by the lambs in each chamber according to the method described by Beauchemin and McGinn (2005). The emissions from the chambers were calibrated by releasing a known amount of CH$_4$ in each chamber and calculating the mass balance of incoming and outgoing amounts of CH$_4$ (McGinn et al., 2006). Cumulative daily CH$_4$ emissions were calculated for 4 d each period. The daily CH$_4$ flux was expressed per unit of DMI and digested DM for the 3 lambs in each chamber (i.e., chamber was the experimental unit). The daily CH$_4$ flux (13.3 Mcal/kg) determined for each chamber was also expressed as a proportion of GE intake and DE intake of the 3 lambs in each chamber.

**Sample Collection.** Total tract digestibility of nutrients was determined during the 4-d collection phase in each period. The canvas fecal collection bags were lined with a pre-weighed plastic bag, which was changed daily between 1006 and 1036 h. Feces collected each day were weighed, mixed thoroughly by hand, and duplicate subsamples representing 10% of daily fecal production from each lamb were retained. Samples from each lamb were combined within each period and stored at −20°C until analyzed for DM, CP, NDF, ADF, and GE.

**Growth Study (Exp. 2)**

**Animals and Experimental Design.** Sixty Canadian Arcott weaned lambs (23.03 ± 3.6 kg) were stratified by BW and randomly assigned to 1 of the 4 experimental diets. Lambs were adapted to the experimental diets for 2 wk before the beginning of data collection. Lambs were housed in individual pens (0.97 × 2.82 m) bedded with straw, fed at 0900 h daily, and weighed weekly. All lambs had ad libitum access to feed and water throughout the study. Feed deliveries were recorded daily. Refusals were collected daily and weighed weekly for determination of weekly DMI. Daily DMI by each lamb was estimated by summing the weekly intake and dividing by the number of days of the week. The ADG was determined by dividing BW gain (initial full BW – final full BW) by the number of days in the study. Feed conversion was calculated as the ratio between ADG and DMI (g of BW gain/g of DMI).

**Slaughter and Sample Collection.** Lambs were slaughtered at a BW of ≥45 kg, in 2 lots at a commercial abattoir (Sunterra Meats Ltd., Innisfail, AB, Canada). Data from 2 lambs were removed from the study due to health complications. Within 5 min of exsanguination, a fat sample (2 to 3 g) from the base of the tail was collected from each lamb. The samples were kept on ice and transported to the laboratory where they were snap-frozen in liquid nitrogen and stored at −80°C until analyzed for fatty acids profiles. Hot carcass weights were recorded and grade rule (GR; body wall thickness) was determined from the total tissue depth of the carcass between the 12th and 13th rib at 11 cm from the carcass midline.

**Chemical Analyses**

The DM concentration of the composited feed samples was determined at 135°C for 2 h (method 930.15; AOAC, 1995) followed by hot weighing, and OM was determined by ashing the samples at 550°C for 5 h (method 942.05; AOAC, 1995). To determine CP (N × 6.25), feed samples were ground to a fine powder using a ball grinder (Mixer Mill MM200; Retsch Inc., Newtown, PA). Nitrogen was quantified by flash combustion with gas chromatography and thermal conductivity detection (Nitrogen Analyzer 1500 series; Carlo Erba Instruments, Milan, Italy). Neutral detergent fiber was determined according to Van Soest et al. (1991) using heat stable α-amylase and sodium sulfite. Acid detergent fiber was
Lipid Extraction and Determination

Lipids were extracted from adipose tissue based on the method of Hara and Radin (1978). Unless otherwise stated, chemicals were purchased from Sigma-Aldrich Inc. (Oakville, ON, Canada). Briefly, samples (1 g of adipose tissue) were homogenized in 15 mL of GC grade 2-propanol using a tissue homogenizer set at 10,000 rpm (PRO Scientific Inc., Oxford, CT). GC-grade hexane (10 mL) was added to the mixture before a second homogenization for 90 s. Samples were allowed to settle and lipids were collected from the upper hexane phase. Hexane extracts were evaporated under N₂ and lipids were stored at −80°C before methylation. Fatty acids were methylated and determined as described by He et al. (2012).

Statistical Analyses

Digestibility and CH₄ Emissions Study (Exp. 1). Data on nutrient intakes and digestibility were analyzed using the mixed procedure (SAS Inst. Inc., Cary, NC). Means were compared using the least squares mean linear hypothesis. The model included the fixed effects of treatment (diet), day and treatment by day interactions and the random effects of period (n = 4), chamber (group), and lamb nested within treatment as random effects with day of sampling within each period treated as repeated measure. The individual animal (n = 12) was the experimental unit for intake and nutrient digestibility because these data were obtained from individual lambs with separate access to feed. For CH₄ emissions, the model did not include the random effect of lamb as the chamber, (n = 4) representing data for 3 lambs was the experimental unit. The minimum values of Akaike’s Information Criterion (AIC) were used to select the covariance structure among compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive, unstructured, and banded for each parameter.

Growth Study (Exp. 2). For the DMI, ADG, and feed efficiency initial and final BW, HCW, grading rule, and fatty acid composition means (n = 15 per treatment) were compared using the least squares mean linear hypothesis test with treatment included as a fixed term (Chaves et al., 2008). For both Exp. 1 and 2, significance was declared at a P < 0.05. Only when effect of treatment (diet) was significant (P < 0.05), orthogonal polynomial contrasts were used to determine linear and quadratic responses to the concentration of glycerol incorporation (0, 7, 14, and 21% of dietary DM) and 0% glycerol vs. glycerol addition for all variables. Otherwise, contrasts were not reported. None of the quadratic contrasts were significant and are thus not reported.

RESULTS

Digestion and CH₄ Emissions Study

Dry matter, ADF, and GE intakes were not affected (P = 0.12) by glycerol addition in the digestibility study (Table 2). Intakes of NDF and CP tended to be lower in the 21% glycerol treated lambs (P = 0.10 and 0.06, respectively), likely because of numerically reduced intakes. Nutrient (CP, NDF, ADF, and DE) digestibility was not affected (P = 0.16) by inclusion of glycerol in the diets.

Methane emissions corrected for DMI or digested DM were not affected (P = 0.23) by the inclusion of glycerol in the diets (Table 3). When expressed as percentage of GE or DE intake, CH₄ emissions were numerically lower in the greater glycerol treated lambs (14 and 21%) than the control and 7% treated lambs.

Growth Study

The inclusion of glycerol in the diets linearly reduced DMI (P = 0.01) and tended to reduce ADG (P = 0.06) and final BW (P = 0.07). Feed efficiency was not affected by the treatments (P = 0.76). Hot carcass weight, dressing,

Table 2. Effects of increasing concentrations of glycerol in the diet on DMI and total tract nutrient digestibility of lambs housed in chambers (Exp. 1; n = 12)¹

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>SEM</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DM</td>
<td>1324</td>
<td>1362</td>
<td>1354</td>
<td>1213</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>CP</td>
<td>215</td>
<td>222</td>
<td>212</td>
<td>195</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>NDF</td>
<td>351</td>
<td>354</td>
<td>327</td>
<td>316</td>
<td>0.13</td>
<td>0.06</td>
</tr>
<tr>
<td>ADF</td>
<td>191</td>
<td>209</td>
<td>205</td>
<td>194</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>GE, Meal/d</td>
<td>5.8</td>
<td>6.2</td>
<td>6.1</td>
<td>5.5</td>
<td>0.38</td>
<td>0.16</td>
</tr>
<tr>
<td>Digestibility, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>70.3</td>
<td>71.3</td>
<td>72.1</td>
<td>73.1</td>
<td>1.69</td>
<td>0.55</td>
</tr>
<tr>
<td>CP</td>
<td>68.0</td>
<td>68.8</td>
<td>67.2</td>
<td>69.8</td>
<td>1.79</td>
<td>0.62</td>
</tr>
<tr>
<td>NDF</td>
<td>39.2</td>
<td>38.9</td>
<td>35.5</td>
<td>41.6</td>
<td>2.48</td>
<td>0.22</td>
</tr>
<tr>
<td>ADF</td>
<td>26.1</td>
<td>34.1</td>
<td>29.6</td>
<td>32.3</td>
<td>2.58</td>
<td>0.16</td>
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<tr>
<td>DE</td>
<td>63.3</td>
<td>72.3</td>
<td>72.3</td>
<td>74.3</td>
<td>4.40</td>
<td>0.23</td>
</tr>
</tbody>
</table>

¹Values determined for 4 d during which the animals were in the chambers.
and grade rule were not affected by glycerol inclusion in the diets (Table 4).

Glycerol did not modify ($P = 0.85$) the proportion of total SFA in subcutaneous tail fat (Table 5), but there was a linear reduction ($P < 0.01$) in the concentrations of palmitic acid (16:0) and a linear increase ($P < 0.01$) in the concentrations of stearic acid (18:0). Glycerol inclusion in the diets linearly increased ($P < 0.01$) the proportion of oleic acid (9c,11t-CLA and reduced ($P < 0.01$) the proportion of 10t,18c:1 but did not affect ($P = 0.77$) the proportion of total MUFA. Linoleic acid (18:2 n-6) and the total proportion of PUFA declined linearly ($P < 0.01$) with increasing concentrations of glycerol in the diet. Conjugated linoleic acids (9c,11t-CLA and 10t,12t-CLA) were not affected ($P = 0.41$) by the diets. Total trans fatty acid proportions were linearly decreased ($P < 0.01$). The ratio of PUFA/SFA tended to decrease ($P = 0.08$) and n-6/n-3 ratio linearly declined ($P < 0.01$) by increasing glycerol inclusion in the diets.

**DISCUSSION**

**Digestibility and CH$_4$ Emissions**

Lack of effect of glycerol inclusion in the diet of lambs on nutrient intakes and digestibility is consistent with prior literature. Rémond et al. (1993) found no difference in fermented OM when glycerol was added to a starch substrate in continuous culture conditions, but demonstrated a slight increase in digestibility when the substrate was cellulose. No differences in nutrient digestibility were reported when crude glycerin replaced alfalfa in cattle diets (Schröder and Südekum, 1999) or wheat grain under in vitro conditions (Krueger et al., 2010). In contrast, Wang et al. (2009) report increased DM digestibility with glycerol inclusion in cattle forage diets at concentrations of 0 to 3.3% of DM and Avila et al. (2011) reported linear increases in IVDMD when glycerol was included at concentrations of 0 to 21% DM as replacement of barley grain in 50% barley grain-50% barley silage based feedlot cattle diets. Based on this evidence, it is most likely that glycerol may enhance digestibility in forage diets, but may have marginal impact on digestibility of highly fermentable feedlot diets.

The lack of effect of glycerol inclusion in the diet on CH$_4$ emissions was unexpected. The emissions measured in our study are low in all treatments if compared with previous reports from sheep. Methane emissions of 11.5 to 25.7 g/kg DMI were reported for forage diets fed to sheep (Waghorn et al., 2002) and emissions of 14.9 to 15.3 g/kg DMI were reported from grazing lambs (Ullah et al., 1997). When expressed as % of GE loss, values reported here are less than those suggested by IPCC (2006) for growing lambs on pasture (4.5% of GE) and more comparable with those of finishing feedlot cattle (3% ± 1%). Beauchemin and McGinn (2005) reported 2.81 and 4.03% of GE losses and emissions of 9.2 and 13.1 g CH$_4$/kg DMI from corn and barley based finishing cattle diets. Sheep have been reported to lose 4.6 and 3.6% of GE as CH$_4$ when grazing pastures with 75% and 81% DM digestibility, respectively (Lassey et al., 1997; Judd et al., 1999). The low CH$_4$ emission values in this study may be attributable in part to the low NDF content of the diets and the use of pelleted diets. Greater NDF concentrations in feed resulted in greater CH$_4$ emissions and pelleted alfalfa produced 24% less CH$_4$ compared with fresh cut alfalfa in rams (Waghorn et al., 2002). Ad-
ditionally, the ground pelleted diets might have contributed to a high fractional outflow rate which has been reported to have an important negative correlation with GE loss in the form of CH₄ (Pinares-Patiño et al., 2003).

We initially hypothesized that the propiogenic properties of glycerol would act as a hydrogen sink in the rumen and thus reduce CH₄ emissions. Glycerol fermentation to propionate does not constitute a hydrogen sink in itself, as glycerol has to donate electrons before entering glycolysis (Zhang and Yang, 2009; Ungerfeld and Forster, 2011). However, we did expect it to alter the rumen environment in manner that would favor the fermentation of carbohydrates to propionate rather than acetate, thereby reducing CH₄ production. The lack of effect of glycerol on CH₄ in this study, together with results from previous reports, suggest that the shift toward

Table 5. Effects of increasing concentrations of glycerol in the diet on fatty acid (FA) profiles (% of total FA) in subcutaneous tail fat of lambs (Exp. 2; n = 15)

<table>
<thead>
<tr>
<th>Component</th>
<th>Glycerol, % Dietary DM</th>
<th>SEM</th>
<th>P-values¹</th>
<th>Diet</th>
<th>Linear²</th>
<th>0 vs. Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>0.35</td>
<td>0.31</td>
<td>0.30</td>
<td>0.24</td>
<td>0.034</td>
<td>0.09</td>
</tr>
<tr>
<td>12:0</td>
<td>0.15</td>
<td>0.15</td>
<td>0.17</td>
<td>0.14</td>
<td>0.026</td>
<td>0.80</td>
</tr>
<tr>
<td>14:0</td>
<td>2.61</td>
<td>2.38</td>
<td>1.72</td>
<td>1.68</td>
<td>0.295</td>
<td>0.06</td>
</tr>
<tr>
<td>15:0</td>
<td>1.13</td>
<td>1.26</td>
<td>1.33</td>
<td>1.31</td>
<td>0.101</td>
<td>0.52</td>
</tr>
<tr>
<td>16:0</td>
<td>20.9</td>
<td>20.1</td>
<td>19.4</td>
<td>18.5</td>
<td>0.589</td>
<td>0.03</td>
</tr>
<tr>
<td>17:0</td>
<td>2.69</td>
<td>3.21</td>
<td>3.92</td>
<td>3.86</td>
<td>0.266</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18:0</td>
<td>11.4</td>
<td>11.4</td>
<td>13.1</td>
<td>13.7</td>
<td>0.684</td>
<td>0.03</td>
</tr>
<tr>
<td>Total SFA</td>
<td>39.2</td>
<td>38.8</td>
<td>39.9</td>
<td>39.4</td>
<td>0.846</td>
<td>0.85</td>
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<tr>
<td>MUFA</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9c-14:1</td>
<td>0.11</td>
<td>0.09</td>
<td>0.10</td>
<td>0.88</td>
<td>0.011</td>
<td>0.46</td>
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<td>9c-16:1</td>
<td>1.46</td>
<td>1.35</td>
<td>1.33</td>
<td>0.28</td>
<td>0.086</td>
<td>0.50</td>
</tr>
<tr>
<td>6c-8t-18:1</td>
<td>0.61</td>
<td>0.89</td>
<td>0.62</td>
<td>0.59</td>
<td>0.140</td>
<td>0.45</td>
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<tr>
<td>9c-18:1</td>
<td>0.58</td>
<td>0.50</td>
<td>0.49</td>
<td>0.49</td>
<td>0.036</td>
<td>0.11</td>
</tr>
<tr>
<td>10t-18:1</td>
<td>9.80</td>
<td>9.33</td>
<td>7.77</td>
<td>7.61</td>
<td>0.655</td>
<td>0.05</td>
</tr>
<tr>
<td>11t-8:1</td>
<td>1.48</td>
<td>1.51</td>
<td>1.30</td>
<td>1.47</td>
<td>0.153</td>
<td>0.76</td>
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<td>9c-18:1</td>
<td>35.5</td>
<td>37.0</td>
<td>38.4</td>
<td>38.8</td>
<td>0.813</td>
<td>0.02</td>
</tr>
<tr>
<td>11c-18:1</td>
<td>1.12</td>
<td>1.04</td>
<td>1.05</td>
<td>1.07</td>
<td>0.036</td>
<td>0.35</td>
</tr>
<tr>
<td>9c-20:1</td>
<td>0.24</td>
<td>0.29</td>
<td>0.30</td>
<td>0.34</td>
<td>0.016</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>50.9</td>
<td>52.0</td>
<td>51.4</td>
<td>51.5</td>
<td>0.804</td>
<td>0.77</td>
</tr>
<tr>
<td>PUFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>7.85</td>
<td>7.06</td>
<td>6.07</td>
<td>6.39</td>
<td>0.380</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>0.90</td>
<td>0.89</td>
<td>0.89</td>
<td>0.93</td>
<td>0.046</td>
<td>0.88</td>
</tr>
<tr>
<td>CLA c9, t11–18:2</td>
<td>0.85</td>
<td>0.88</td>
<td>0.80</td>
<td>0.82</td>
<td>0.056</td>
<td>0.72</td>
</tr>
<tr>
<td>CLA t10, t12–18:2</td>
<td>0.07</td>
<td>0.06</td>
<td>0.07</td>
<td>0.04</td>
<td>0.013</td>
<td>0.41</td>
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<tr>
<td>20:4 n-6</td>
<td>0.18</td>
<td>0.18</td>
<td>0.17</td>
<td>0.18</td>
<td>0.014</td>
<td>0.98</td>
</tr>
<tr>
<td>20:5 n-3 EPA</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>0.03</td>
<td>0.014</td>
<td>0.47</td>
</tr>
<tr>
<td>22:5 n-3 DPA</td>
<td>0.14</td>
<td>0.12</td>
<td>0.12</td>
<td>0.13</td>
<td>0.015</td>
<td>0.85</td>
</tr>
<tr>
<td>22:6 n-3 DHA</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.006</td>
<td>0.72</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>10.0</td>
<td>9.3</td>
<td>8.2</td>
<td>8.6</td>
<td>0.429</td>
<td>0.02</td>
</tr>
<tr>
<td>Total UFA³</td>
<td>60.9</td>
<td>61.3</td>
<td>59.6</td>
<td>60.1</td>
<td>0.955</td>
<td>0.63</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>0.26</td>
<td>0.24</td>
<td>0.21</td>
<td>0.22</td>
<td>0.014</td>
<td>0.08</td>
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<tr>
<td>Trans FA⁴</td>
<td>13.4</td>
<td>13.1</td>
<td>11.1</td>
<td>10.0</td>
<td>0.762</td>
<td>0.05</td>
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<tr>
<td>CLA+VA⁵</td>
<td>2.40</td>
<td>2.44</td>
<td>2.17</td>
<td>2.34</td>
<td>0.198</td>
<td>0.77</td>
</tr>
<tr>
<td>n-3 FA⁶</td>
<td>1.08</td>
<td>1.08</td>
<td>1.08</td>
<td>1.11</td>
<td>0.055</td>
<td>0.96</td>
</tr>
<tr>
<td>Trans- (CLA+VA)</td>
<td>11.0</td>
<td>10.7</td>
<td>8.90</td>
<td>8.70</td>
<td>0.732</td>
<td>0.05</td>
</tr>
<tr>
<td>C18:1 trans⁷</td>
<td>12.4</td>
<td>12.2</td>
<td>10.2</td>
<td>10.2</td>
<td>0.724</td>
<td>0.05</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>7.57</td>
<td>6.80</td>
<td>5.88</td>
<td>5.93</td>
<td>0.268</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

¹When fixed effects of diet are not significant (P > 0.05), values for contrasts are not reported.
²Linear effects of increasing concentrations of glycerol.
³UFA: unsaturated fatty acids = MUFA + PUFA.
⁴Trans FA = C18:1 t6–8 + C18:1 t9 + C18:1 t10 + C18:1 t11 (VA) + 18:2 c9-t11 (CLA) + 18:2 t10-t12 (CLA).
⁵VA = Vaccenic acid, C18:1 t11.
⁶n-3 FA = C18:3 + EPA + DHA + DHA.
⁷C18:1 trans = C18:1 t6–8 + C18:1 t9 + C18:1 t10 + C18:1 t11 (VA).
propionate fermentation may be more likely to occur in high forage (Wang et al., 2009; Avila et al., 2011; Lee et al., 2011) than in high grain diets (Mach et al., 2009).

**Growth Performance**

The linear decrease in DMI with increasing concentrations of glycerol in the diets is in accordance with previous studies (Musselman et al., 2008; Gunn et al., 2010b) in which replacing corn with increasing concentrations of crude glycerin (0 to 45% of DM) linearly decreased DMI when concentrations exceeded 15% of the dietary DM. Parsons et al. (2009) also reported linear decreases of intake when crude glycerin was included at more than 2% of the diet fed to finishing beef heifers. However, a previous study with steers found no effects on DMI when including crude glycerin (0 to 12% of DM) as a replacement of barley grain in a barley-corn grain based diet (Mach et al., 2009). Likewise, Gunn et al. (2010a) reported no changes in DMI when increasing concentrations of crude glycerin (0 to 20% of DM) were used to replace dry rolled corn in lamb diets.

The trend to reduced ADG with increasing concentrations of glycerol in the diets (ADG (g/d) = −2.06 × (% glycerol in diet DM) + 341.6; r² = 0.49) is likely due to reductions in DMI. The 12.4% reduction in DMI between the 7% and the 21% glycerol diets resulted in a 15.8% reduction in ADG. Decreases in ADG were reported when glycerol replaced 15% or more of rolled corn grain DM in diet for lambs (Mach et al., 2008; Gunn et al., 2010b). These authors report reductions of about 39% in ADG as a result of adding glycerin at levels up to 45% of diet DM. A quadratic effect was reported when including crude glycerin (0, 2, 4, 8, 12, and 16% of DM) in finishing heifer diets as a replacement for steam rolled corn (Parsons et al., 2009). These authors report a 23.1% reduction in ADG associated with a 12.2% reduction in DMI between diets that contained 2% or 16% glycerol. Results presented here seem to concur with those of Parsons et al. (2009) who reported increased ADG in finishing cattle fed crude glycerin at up to 8% of dietary DM, but ADG decreased when glycerin accounted for 12 or 16% diet DM. Gunn et al. (2010a) suggest that reduced BW gain at concentrations above 15% could be due to an altered ruminal environment stemming from reduced pH, and decreased cellulosic activity and thus, reduced DMI. However, previous studies in cattle using crude glycerin as a replacement of barley (Mach et al., 2009), and in vitro studies with glycerol incubated with corn and alfalfa (Lee et al., 2011) have reported no or only slight reductions in pH. No effects of crude glycerin addition on ADG were reported in the aforementioned studies of Mach et al. (2009) in cattle and Gunn et al. (2010a) in lamb diets.

The lack of effect of treatments on feed efficiency is most probably explained by decreased ADG in treatments with reduced DMI. Similarly, feeding crude glycerin up to 12% DM did not affect feed efficiency in cattle (Mach et al., 2009). Similar results were also obtained by Gunn et al. (2010a) using up to 20% crude glycerin in feedlot diets for lambs. Other studies report linear decreases in feed efficiency when crude glycerin was included in concentrations over 15% DM (Musselman et al., 2008; Gunn et al., 2010b).

The lack of effects of glycerol inclusion on HCW, dressing and grading rule are in accordance with previous reports in lambs (Gunn et al., 2010a) and beef cattle (Mach et al., 2009) that found no effects on carcass traits when replacing corn and barley grain with crude glycerin in concentrations of up to 20 and 16% of DM, respectively.

**Fatty Acid Composition**

The shift in total SFA toward a reduced palmitic acid (16:0) and increased stearic acid (18:0) is interesting because palmitic acid has been considered detrimental to serum cholesterol concentrations and stearic acid has been shown to have a net neutral impact on serum cholesterol in humans (Yu et al., 1995). This pattern of SFA has been described as more likely to be present in grass fed than grain fed cattle (Daley et al., 2010) because grass diets are richer in stearic and linoleic acid and cereal grain diets are richer in palmitic acid. Because the diets in this study were formulated to be isonitrogenous and isofibrous, with increasing concentrations of alfalfa (6.3% DM in the control treatment to 16.8% DM in the 21% glycerol treatment), it is yet to be clarified if this change is attributable to the changes in rumen fermentation pattern due to different concentrations of glycerol, the reduction in barley grain, or changes in other ingredients of the diet.

Because 10t-18:1 was the major 18:1 trans FA in our study, the linear reduction in proportions of 10t-18:1 implied linear reductions in the total trans and trans 18:1 FA. Previous studies (Dukan et al., 2007; Aldai et al., 2008) have reported that 10t-18:1 is the major 18:1 trans FA in beef from cattle fed diets high in barley (i.e., low fiber) and this FA has been associated with increased risk of coronary heart disease in humans (Hodgson et al., 1996) and negative effects on plasma lipid profiles in rabbits (Roy et al., 2007). Previous reports (Bauman and Griinari, 2003; Kramer et al., 2004; Mohammed et al., 2010) attributed this increase in 10t-18:1 to lower ruminal pH with a consequent alteration of rumen bacterial flora that shifts lipid biohydrogenation pathways mainly from Butyrivibrio fibrisolvens, which forms the t11 double bond in vaccenic acid to Megasphaera elsdenii, which forms the t10 double bond, resulting in production of 10t-18:1 instead of vaccenic acid (11t-18:1). A fraction of the 10t-
18:1 produced under these conditions will reach the tissues were it cannot be desaturated to the t10,c12–18:2 CLA (Kramer et al., 2004). Although in our study 11t-18:1 was not modified by diet, these findings support a previous report (Krehbiel, 2008) stating that an important fraction (>50%) of glycerol is evacuated from the rumen through passage or absorption through the rumen wall (Rémont et al., 1993) and is thus not fermented to propionate in the rumen environment. Studies with DDGS have reported reductions of 10t-18:1 when WDDGS (Dugan et al., 2010) or triticale DDGS (McKeown et al., 2010) were included as replacement of barley grain. Future research testing the effects of different sources and concentrations of DDGS in combination with glycerol on overall trans fatty acids in ruminants will be necessary to determine optimal inclusions of these ingredients.

Oleic acid has been reported to be the most abundant fatty acid in beef (Turk and Smith, 2009) and lamb (Diaz et al., 2005). The increase of this fatty acid has been associated with greater beneficial high-density lipoprotein (HDL) cholesterol plasma concentrations in humans (Gilmore et al., 2011). Glycerol addition to the diet linearly reduced the proportions of linoleic acid (18:2 n-6) and thus led to linear reductions in the total PUFA proportions and tended to reduce the PUFA/SFA ratio (P = 0.08). These results are in accordance with Diaz et al. (2005) who found a negative correlation between linoleic and oleic acid. This is attributable to the inhibiting effect of linoleic acid on Δ9-desaturase, the enzyme responsible for oleic acid synthesis (Jeffcoat and James, 1984). In our study, the linoleic acid content of the diets increased with greater glycerol concentrations. High concentrate diets are associated with lower ruminal pH, which decreases hydrogenase activity, producing less conversion of linoleic to stearic acid (Tove and Matrone, 1962). These changes in fatty acid profiles further suggest that animals fed glycerol added diets had better ruminal pH probably due in part to partial escape of glycerol from rumen fermentation. The n-6/n-3 ratio was linearly reduced from 7.57 to 5.93, but was still above the recommended ratio of 5:1 or less (World Health Organization, 2003).

The only previous study assessing crude glycerin effects on fatty acid composition of meat (Terre et al., 2011) found no differences in fatty acid profiles of light lambs fed 0, 5, or 10% glycerol. These authors used a 4-wk feeding period and finished the lambs at a very young age (4 wk after weaning) and low BW (24.5 ± 0.4 kg), which might not be long enough to find differences in fatty acid profiles.

The lack of effects on diet digestibility and methane emissions contrasts with our initial hypothesis. However, results from the lamb performance and fatty acid profiles experiment support the hypothesis that glycerol does not affect lamb performance or carcass traits and has the potential to beneficially affect fatty acid profiles from subcutaneous adipose tissue in lambs.


