Apparent metabolizable energy of crude glycerin originating from different sources in broiler chickens

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ABSTRACT An energy balance experiment was conducted to determine the AMEn of various crude glycerin samples, and to generate an equation to predict AMEn of crude glycerin based on its chemical composition. Dietary treatments consisted of a corn-soybean meal basal diet with no added glycerin and a basal diet supplemented with 6% glycerin. Crude glycerin samples were obtained from biodiesel production facilities throughout the United States, which use a variety of lipid products as their initial feedstock. Two identical energy balance trials were conducted. In each trial, 864 male broilers (Ross × Ross 708) were fed a common starter diet until 17 d of age when they were switched to 1 of 12 experimental diets (6 replicates per treatment) from 17 to 22 d of age, with a 48-h collection period on d 21 and 22. Nitrogen-corrected apparent metabolizable energy values of crude glycerin samples were estimated by difference, whereby AMEn of the basal diet was subtracted from the complete diet containing the test ingredient. The AMEn of the basal diet and US Pharmacopeia-grade glycerin were determined to be 3,085 and 3,662 kcal/kg, respectively, whereas the AMEn of the 10 crude glycerin samples ranged from 3,254 to 4,134 kcal/kg. Two crude glycerin samples had high levels of fatty acids compared with the other samples (24 and 35% vs. <0.30%), and even though their AMEn was higher than that of the other glycerin samples (3,806 vs. 3,611 kcal/kg, P < 0.01, respectively), their AMEn as a percentage of gross energy (GE) was lower than that of the other samples (65.5% vs. 97.4%, respectively; P < 0.01). Including all of the glycerin samples, the stepwise regression equation to predict AMEn was determined to be: [AMEn (kcal/kg) = 1,605 – (19.13 × % methanol) + (39.06 × % fatty acid) + (23.47 × % glycerin)]; (R2 = 0.25; SE = 379; P ≤ 0.01). These data indicate that glycerin is a good source of energy for broilers, and the AMEn of glycerin is dependent on fatty acid, methanol, and water contents.

Key words: apparent metabolizable energy, gross energy, glycerin

INTRODUCTION

In the United States, biodiesel production has accelerated in growth, providing an alternate fuel source, which reduces dependence on petroleum-based fuel products. Crude glycerin is a coproduct of biodiesel production, representing approximately 9% of the starting feedstock weight (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006). Although soybean oil is a major lipid used in initial feedstock, production facilities sometimes use alternate lipid sources, such as other vegetable oils, animal fat, and yellow grease, in order to reduce their biodiesel production costs.

Glycerol is readily absorbed in the intestine (Hober and Hober, 1937) and metabolized into glucose via gluconeogenesis (Emmanuel et al., 1983), or oxidized for energy production via glycolysis and the citric cycle (Rosebrough et al., 1980). Glycerin has been reported as an acceptable feed ingredient for poultry (Campbell and Hill, 1962; Lin et al., 1976; Simon et al., 1996; Barteczko and Kaminski, 1999; Cerrate et al., 2006; Światkiewicz and Koreleski, 2009). Recently, Dozier et al. (2008) determined AMEn for a single source of glycerin to be 3,434 kcal/kg for broilers, which is approximately 95% of its gross energy (GE). In a companion study, Lammers et al. (2008a) determined the AMEn for that same source of glycerin to be 3,805 kcal/kg in laying hens, and the AMEn was similar in quantity to
the GE content. Kerr et al. (2009) found that although the glycerol, fatty acid, methanol, ash, and moisture composition is known to vary among crude glycerin samples obtained from production facilities across the United States, the ME content in nursery pigs is related to crude glycerin, methanol, and fatty acid composition of a given sample.

To our knowledge, the effects of crude glycerin composition and source on AMEn in poultry have not been evaluated. The objectives of this experiment were to determine AMEn of various crude glycerin samples and to generate an equation to predict AMEn based on the composition of the samples.

MATERIALS AND METHODS

Experimental Treatments

Crude glycerin samples were obtained from various biodiesel plants that used soybean oil (hexane and expeller-derived), tallow, yellow grease, or poultry fat. Glycerol content was determined by difference (100 – % of methanol – % of moisture – % of ash). Moisture (AOAC International, 1995; method 984.20), methanol (gas chromatography proprietary method), pH (Orion 230A pH meter with 9107 probe, Orion, Thermo Electro Corp., Beverly, MN), Na (AOAC International, 1995; method 956.01), Cl (AOAC International, 1995; method 915.01 and 943.01), ash (AOAC International, 1995; method 942.05), and fatty acids (AOCS, 2000; method G 4.40 modified for glycerin) were characterized using standard techniques. The composition of each crude glycerin sample is presented in Table 1.

Dietary treatments consisted of either a common basal diet, which met or exceeded the NRC (1994) requirements (Table 2) or 1 of 12 diets containing 6% crude glycerin: USP-grade glycerin (USP), glycerin from Ag Processing Inc., Sergeant Bluff, IA (AGP-IA), Renewable Energy Group, Ralston, IA (REG-R), Renewable Energy Group, Wall Lake, IA (REG-WL), Renewable Energy Group, Albert Lea, MN (REG-MN), Archer Daniels Midland, Mexico, MO (ADM-MO), Westway Feed Products, Cincinnati, OH (WW-OH), Westway Feed Products, Houston, TX (WW-TX), Imperial Western Products acidulated glycerin, Coachella, CA (IW-AC), Imperial Western Products nonacidulated glycerin, Coachella, CA (IW-NA), and US Biofuels, Rome, GA (USB-GA). Crude glycerin samples were from hexane-derived soybean oil (AGP-IA, REG-WL, REG-MN, ADM-MO, and WW-TX), expeller-derived soybean oil (REG-R), tallow (WW-OH), yellow grease (IW-AC and IW-NA), or poultry fat (USB-GA). One crude glycerin sample was nonacidulated (IW-NA), so it had an elevated level of total fatty acid content. In addition, the sample that used poultry fat (USB-GA) also had an elevated level of total fatty acid content.

Common Procedures

All procedures relating to the use of live birds were approved by a USDA-ARS Animal Care and Use Committee (Mississippi State, MS). Two trials were conducted and the following procedures were identical for each trial. One thousand Ross × Ross 708 male broiler chicks (Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery and placed in an open-sided house. Chicks were vaccinated for Marek’s disease, Newcastle disease, and infectious bronchitis at the hatchery. Birds were managed under similar practices and fed corn-soybean meal diets from 0 to 16 d of age (AMEn was 3,040 kcal/kg of corn-soybean meal, 0.92% digestible TSAA, 1.22% digestible Lys, 0.90% Ca, and 0.45% nonphytate P). The ambient temperature set point was 33°C at placement and was decreased as the birds aged, resulting in a final set point of 28°C at 14 d of age. The photoperiod was on a continuous

<table>
<thead>
<tr>
<th>Sample</th>
<th>Source</th>
<th>Glycerin</th>
<th>Moisture</th>
<th>Methanol</th>
<th>pH</th>
<th>NaCl</th>
<th>Ash</th>
<th>Fatty acids</th>
</tr>
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<tbody>
<tr>
<td>USP</td>
<td>—</td>
<td>99.62</td>
<td>0.35</td>
<td>ND^5</td>
<td>5.99</td>
<td>0.013</td>
<td>0.01</td>
<td>0.02</td>
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<tr>
<td>ADM-MO</td>
<td>SB</td>
<td>83.88</td>
<td>10.16</td>
<td>0.01</td>
<td>6.30</td>
<td>5.997</td>
<td>5.83</td>
<td>0.12</td>
</tr>
<tr>
<td>AGP-IA</td>
<td>SB</td>
<td>83.49</td>
<td>13.40</td>
<td>0.11</td>
<td>5.53</td>
<td>2.838</td>
<td>2.93</td>
<td>0.07</td>
</tr>
<tr>
<td>REG-MN</td>
<td>SB</td>
<td>85.76</td>
<td>8.35</td>
<td>0.03</td>
<td>6.34</td>
<td>6.065</td>
<td>5.87</td>
<td>ND</td>
</tr>
<tr>
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<td>83.96</td>
<td>9.36</td>
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<td>6.346</td>
<td>6.45</td>
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<td>WW-TX</td>
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<td>81.34</td>
<td>11.41</td>
<td>0.12</td>
<td>6.59</td>
<td>6.577</td>
<td>7.12</td>
<td>0.01</td>
</tr>
<tr>
<td>WW-OH</td>
<td>TA</td>
<td>73.65</td>
<td>24.37</td>
<td>0.03</td>
<td>3.99</td>
<td>0.073</td>
<td>1.91</td>
<td>0.04</td>
</tr>
<tr>
<td>IW-AC</td>
<td>YG</td>
<td>93.81</td>
<td>4.07</td>
<td>0.04</td>
<td>6.10</td>
<td>0.162</td>
<td>1.93</td>
<td>0.15</td>
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<tr>
<td>IW-NA</td>
<td>YG</td>
<td>52.79</td>
<td>4.16</td>
<td>3.49</td>
<td>8.56</td>
<td>1.977</td>
<td>4.72</td>
<td>34.84</td>
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<tr>
<td>USB-GA</td>
<td>PF</td>
<td>51.54</td>
<td>4.99</td>
<td>14.99</td>
<td>9.28</td>
<td>0.011</td>
<td>4.20</td>
<td>24.28</td>
</tr>
</tbody>
</table>

^1Samples analyzed in duplicate by Ag Processing Inc., Omaha, NE, 68154.

^2Glycerin content determined by difference as: 100 – % methanol – % total fatty acid – % moisture – % ash.

^3USP = USP-grade glycerin; ADM-MO = Archer Daniels Midland, Mexico, MO; AGP-IA = Ag Processing Inc., Sergeant Bluff, IA; REG-MN = Renewable Energy Group, Albert Lea, MN; REG-R = Renewable Energy Group, Ralston, IA; REG-WL = Renewable Energy Group, Wall Lake, IA; WW-TX = Westway Feed Products, Houston, TX; WW-OH = Westway Feed Products, Cincinnati, OH; IW-AC = Imperial Western Products acidulated glycerin, Coachella, CA; IW-NA = Imperial Western Products nonacidulated glycerin, Coachella, CA; USB-GA = US Biofuels, Rome, GA.

^4Sources from which crude glycerin samples originated: SB = soybean oil from hexane soybean crush plant (except for REG-R where the soybean oil was obtained from extruded soybeans), TA = tallow, YG = yellow grease, PF = poultry fat.

^5ND = not detected
schedule with lighting intensities of 30 lx from 0 to 7 d of age and 10 lx from 8 to 16 d of age. Light intensity settings for each intensity adjustment were verified at bird level (30 cm) using a photometric sensor with NIST-traceable calibration (403125, Extech Instruments, Waltham, MA).

### Energy-Balance Trials

At 17 d of age, 864 Ross × Ross 708 male broilers (72 pens; 12 birds per pen) were randomly distributed into grower battery cages (Alternative Design Mfg., Siloam Springs, AR). The chicks in each battery cage had a mean BW of 517 g, ranging from 460 to 548 g. Each battery cage (66 × 66 × 76 cm) was equipped with one trough feeder and one nipple waterer. The experimental facility was a solid-sided house with temperature control. A 48-h total excreta collection period (21 to 22 d of age) was conducted to evaluate AMEn of the experimental diets. After a 3-d acclimation period, feed disappearance and total amount of excreta at the end of collection were weighed (wet basis). Multiple sub-samples were collected from the total amount of excreta and homogenized. A 250-g representative sample was placed in a plastic bag for analysis. Representative samples of feed and excreta were frozen and subsequently dried at 55°C for 48 h. Dried samples were then ground through a 1-mm screen to ensure a homogeneous mixture.

The GE content of feed and excreta was determined in duplicate for 1-g samples using an isoperibol oxygen bomb calorimeter (model 1281, Parr Instruments, Moline, IL). The nitrogen content of the feed and excreta was determined in duplicate for a 0.2-g sample with a Combustion N analyzer (Truspec N Determinator, Leco Corp., St. Joseph, MI) using a previously established method (AOAC International, 2000; method 968.06).

During the 48-h collection period, feed consumption and excreta weights were used to calculate energy and nitrogen intake and excretion. Nitrogen-corrected apparent metabolizable energy was calculated using the following equation:

$$\text{AME}_n = \left[ \text{GE intake} - \text{GE output in excreta} \right] - \left[ 8.73 \times (N \text{ intake from diet} - N \text{ output from excreta}) \right] / \text{feed intake}.$$  

From previous research, a nitrogen correction factor of 8.73 was used (Titus, 1956). Crude glycerin was substituted for 6% of the basal diet. Nitrogen-corrected apparent metabolizable energy was determined by subtracting 94% of the contribution of AMEn from the basal diet using the following equations:

$$\text{Total AMEn intake} = \left[ \text{GE intake} - \text{GE excretion} \right] - \left[ 8.73 \times (N \text{ intake} - N \text{ excretion}) \right],$$

$$\text{Basal AMEn intake} = \text{AME}_n \text{ of control diet (94% basal)},$$

$$\text{Glycerin AMEn} = \left( \text{total AMEn intake} - \text{basal AMEn intake} \right) / \text{glycerin intake}.$$

### Statistical Analyses

Data were analyzed as a one-way treatment structure in a randomized complete block design (SAS Institute, 2004). Pen location was the blocking factor. Each treatment was represented by 12 replications (6 replicates per trial) over time. Stepwise regression was used to determine the effect of the nutrient composition on AMEn. The R^2, SE of the regression estimate, and the Mallows statistic [C(p); Mallows, 1973] were used to define the best-fit equation. In addition, full and partial correlations between AMEn and nutrient composition were conducted to assist in interpreting the stepwise regression results. Six preplanned orthogonal contrasts were used to separate AMEn responses. Statistical significance was considered at $P \leq 0.05$.

### RESULTS AND DISCUSSION

Crude glycerin is an energy source for poultry (Dozier et al., 2008; Lammers et al, 2008a), but its chemical composition may vary among sources obtained from biodiesel production facilities (Thompson and He, 2006; Min et al., 2010). In the present study, crude glycerin samples ranged extensively in their chemical compositions (Table 1). Samples originating from soy oil (AGP-IA, REG-WL, REG-MN, ADM-MO, WW-TX, and REG-R) had an average glycerin content of 83.9% and exhibited small variability among the 6 sources. Conversely, samples from tallow, yellow grease, and

### Table 2. Ingredient and nutrient composition of the basal boiler diet (as-is basis)

<table>
<thead>
<tr>
<th>Item</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (%)</td>
<td>Value</td>
</tr>
<tr>
<td>Corn</td>
<td>62.43</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td>33.74</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.71</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.13</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.52</td>
</tr>
<tr>
<td>Dl-Methionine</td>
<td>0.22</td>
</tr>
<tr>
<td>Vitamin and mineral premix1</td>
<td>0.25</td>
</tr>
<tr>
<td>Calculated composition</td>
<td>2.921</td>
</tr>
<tr>
<td>CP (%)</td>
<td>20.5</td>
</tr>
<tr>
<td>TSAA (%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Lys (%)</td>
<td>1.16</td>
</tr>
<tr>
<td>Thr (%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Nonphytate P (%)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

1Vitamin and mineral premix/kg of diet: vitamin A (vitamin A acetate), 7,716 IU; cholecalciferol, 2,205 IU; vitamin E (source unspecified), 9.9 mg; menadione, 0.9 mg; vitamin B12, 0.01 mg; folic acid, 0.6 µg; thiamin, 1.0 mg; d-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; and selenium, 0.1 mg.

### Statistical Analyses

Data were analyzed as a one-way treatment structure in a randomized complete block design (SAS Institute, 2004). Pen location was the blocking factor. Each treatment was represented by 12 replications (6 replicates per trial) over time. Stepwise regression was used to determine the effect of the nutrient composition on AMEn. The R^2, SE of the regression estimate, and the Mallows statistic [C(p); Mallows, 1973] were used to define the best-fit equation. In addition, full and partial correlations between AMEn and nutrient composition were conducted to assist in interpreting the stepwise regression results. Six preplanned orthogonal contrasts were used to separate AMEn responses. Statistical significance was considered at $P \leq 0.05$. 

### RESULTS AND DISCUSSION

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poultry oil (WW-OH, IW-AC, IW-NA, and USA-GA), ranged from 51.54 to 93.94% in glycerin content. The IW-NA and USB-GA samples had the lowest glycerin content, but had the highest free-fatty acid composition (34.8 and 24.3%, respectively) compared with the other samples (<0.3%). In the production of biodiesel, the acidulation process increases the production of methyl esters, resulting in a crude glycerin source that contains a higher glycerin content, at the expense of free-fatty acid content (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006). Therefore, crude glycerin samples with a low fatty acid content imply a very efficient production process for the recovery of esterified fatty acids. The high fatty acid content in IW-NA was expected because it was a nonacclimated product. However, the basis for the relatively high fatty acid content in the USB-GA source is not readily explained. Moreover, IW-NA and USB-GA both had higher methanol concentrations than the other samples. In parallel with fatty acid content, recovery of methanol is also indicative of production efficiency, given that it is reused during the production process (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006). The high methanol content in IW-NA was expected because the final coproduct during the production process was acidulated (IW-AC) and had a low methanol content. High levels of methanol in feed ingredients may cause animal welfare concerns (Lammers et al., 2008b,c). Moderate levels of ash and NaCl were noted with several of the products. Samples WW-OH, IW-AC, and USB-GA had high ash and low NaCl content and were from biodiesel facilities using a K-based catalyst instead of a Na-based catalyst.

Methanol has warranted special consideration in the use of crude glycerin because it is not completely removed at the biodiesel production facility. Methanol is a potentially toxic compound that can elicit a variety of acute and chronic symptoms (Roe, 1982; Medinsky and Dorman, 1995; Skrzydlewska, 2003). Even though gastrointestinal disturbance is one of the chronic exposure symptoms, Kerr et al. (2009) reported no apparent effect of dietary methanol on pig performance or ME as a percentage of GE between pigs fed IW-NA (3.49% methanol) or USB-GA (14.99% methanol) and pigs fed crude glycerin sources with lower levels of methanol. Likewise, Lammers et al. (2008b) did not report any increased incidence of methanol-associated lesions due to toxicity in eye, kidney, or liver tissue in growing finishing pigs fed 5 or 10% crude glycerin (0.32% methanol). As a general-purpose feed ingredient, glycerin is regulated in the United States under 21CFR Subpart B 182.1320, which requires that levels of methanol in methyl esters of higher fatty acids should not exceed 0.015%. Recently, however, crude glycerin was defined by the Association of American Feed Control Officials (AAFCO, 2010), such that nonruminants can be fed up to 10% crude glycerin in their complete feed as long as it contains ≥80% glycerin, ≤15% water, ≤0.15% methanol, ≤8% salt, ≤0.1% sulfur, and ≤5 ppm of heavy metals.

For our 11 samples of crude glycerin, the average AMEn was 3,646 kcal/kg (Table 3). The crude glycerin samples that originated from soybean oil had a higher AMEn (P ≤ 0.01) than the sample from tallow; however, the opposite was calculated for samples from yellow grease. Crude glycerin from tallow (WW-OH) was lower than from yellow grease (IW-AC; P ≤ 0.01). The AMEn of IW-NA and USB-GA samples was higher than that of the other glycerin samples (P ≤ 0.01). Because the GE of crude glycerin differed widely between samples, we compared AMEn levels as a percentage of GE (Table 3). Crude glycerin samples that originated from soybean oil had similar AMEn as a percentage of GE compared with samples that originated from tallow (P = 0.09) and yellow grease (P = 0.91), with no difference between tallow- or yellow grease-based glycerin samples being found (P > 0.23). In contrast, crude glycerin samples that had a high fatty acid content (IW-NA and USB-GA) had a lower calculated AMEn as a percentage of GE than the other samples (P ≤ 0.01). Dozier et al. (2008) reported an average AMEn of 3,434 kcal/kg (94.7% of GE) across 3 experiments that examined broiler chickens being fed a single source of crude glycerin (87% glycerin) that originated from soy oil. In the current experiment, the AMEn as a percentage of GE was averaged to be 97.4% for all glycerin samples (excluding IW-NA and USB-GA), indicating that crude glycerin was used efficiently as a source of dietary energy in broilers. This is supported by Lammers et al. (2008a) and Swiatkiewicz and Koreleski (2009), who reported that crude glycerin was a viable energy source in laying hens.

In the current study, broilers did not utilize glycerin samples with a relatively fatty acid content or samples with a low fatty acid content, as evidenced by the lower AMEn relative to the GE. Wiseman and Salvador (1991) reported a linear reduction in AMEn in broilers fed diets of increasing concentrations of free fatty acids. This is supported by Artman (1964) and Sklan (1979), who found that free fatty acids reduce the rate of absorption compared with oil and fat sources containing triglycerides and free fatty acids, suggesting the decreased use of free fatty acids could be because of the absence of the monoglyceride backbone. The lower absorption of free fatty acids may relate to the lack of monoglycerides in the duodenum, thereby depressing the amount of fatty acids entering micellar solution, resulting in reduced absorption. The 2-monoglyceride has been shown to promote solubility, resulting in a mixed bile-salt monoglyceride fatty-acid micelle (Hofmann and Borgstrom, 1962; Johnston, 1963; Senior, 1964).

Pearson correlation coefficients between the chemical composition of crude glycerin and AMEn are presented in Table 4. Gross energy (r = 0.32) and water (r = −0.36) had the highest correlation coefficients (P


Because more than one chemical component in feed ingredients can influence AMEn, a stepwise regression was used to predict AMEn among glycerin samples (Table 5). Methanol, fatty acids, and glycerin from the crude glycerin samples resulted in the best-fit equation (Equation 5 in Table 5):

\[
\text{AMEn (kcal/kg)} = [1,605 - (19.13 \times \% \text{ methanol}) + (39.06 \times \% \text{ fatty acids}) + (23.47 \times \% \text{ glycerin})],
\]

\(R^2 = 0.25, \ P < 0.01\).

Likewise, a stepwise equation (Table 6) was used to predict AMEn relative to GE that included fatty acids, methanol, and water (Equation 3 in Table 6):

\[
\text{AMEn (kcal/kg)} = [91.63 - (0.61 \times \% \text{ fatty acids}) - (1.17 \times \% \text{ methanol}) + (0.60 \times \% \text{ water})],
\]

\(R^2 = 0.65, \ P < 0.01\).

Using nursery pigs, Kerr et al. (2009) reported an equation to predict ME between glycerin samples of the same source:

\[
\text{ME (kcal/kg)} = 3,580 - (40.52 \times \% \text{ water}) + (48.55 \times \% \text{ fatty acids}), \ (R^2 = 0.42, \ P < 0.01).
\]

The relatively low \(R^2\) for the prediction of AMEn in the current study and Kerr et al. (2009) may be from the relatively small variation in chemical composition among the crude glycerin samples, particularly the samples that originated from soybean oil. The low \(R^2\) could also be because of high inherent biological variation and the use of only 6% supplemental glycerin to evaluate energy use. Fundamentally, AMEn is 97.4% of GE if the total fatty acid content is less than 0.5%, and AMEn is 65.6% of GE if the total fatty acid content ranges from 25 to 35% (Table 3). This is supported by Bartlet and Schneider (2002) who determined prececal digestibility of glycerin to be approximately 97% (Bartlet and Schneider, 2002).

In conclusion, the results of our study indicate that crude glycerin is a good energy source for broilers, but methanol, fatty acid, and glycerin contents within the

### Table 3. Energy values of various crude glycerin products in growing broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>No. of observations</th>
<th>Source</th>
<th>GE (kcal/kg)</th>
<th>AMEn (kcal/kg)</th>
<th>% of GE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>—</td>
<td>4.038</td>
<td>3.085</td>
<td>76.40</td>
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<tr>
<td>USP</td>
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<td>—</td>
<td>4.325</td>
<td>3.662</td>
<td>84.68</td>
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<td>ADM-MO</td>
<td>12</td>
<td>SB</td>
<td>3.627</td>
<td>3.364</td>
<td>92.76</td>
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<tr>
<td>AGP-IA</td>
<td>12</td>
<td>SB</td>
<td>3.601</td>
<td>3.849</td>
<td>106.90</td>
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<td>REG-MN</td>
<td>11</td>
<td>SB</td>
<td>3.676</td>
<td>3.479</td>
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<td>REG-R</td>
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<td>SB</td>
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<td>3.889</td>
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<td>3.254</td>
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<tr>
<td>WW-OH</td>
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<td>TA</td>
<td>3.173</td>
<td>3.256</td>
<td>102.61</td>
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<td>IW-AC</td>
<td>12</td>
<td>YG</td>
<td>4.153</td>
<td>4.100</td>
<td>98.71</td>
</tr>
<tr>
<td>IW-NA</td>
<td>11</td>
<td>YG</td>
<td>6.021</td>
<td>4.135</td>
<td>68.68</td>
</tr>
<tr>
<td>USB-GA</td>
<td>11</td>
<td>PF</td>
<td>5.581</td>
<td>3.476</td>
<td>62.29</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>—</td>
<td>—</td>
<td>91.2</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>Contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vs. all others</td>
<td>—</td>
<td>—</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>USP vs. all others</td>
<td></td>
<td>—</td>
<td>0.56</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>SB vs. TA</td>
<td></td>
<td>—</td>
<td>0.01</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>SB vs. YG</td>
<td></td>
<td>—</td>
<td>0.01</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>TA vs. YG</td>
<td></td>
<td>—</td>
<td>0.01</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Low fatty acid vs. high fatty acid glycerin</td>
<td>—</td>
<td>—</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

1At 17 d of age, broilers were adapted to diets and feeding regimen for 5 d before a 48-h collection period.

2Number of observations (12 broilers/pen) per dietary treatment. Pens with AMEn values not within 2 SD were removed from the data set.

3Sources from which crude glycerin samples originated: SB = soybean oil from hexane soybean crush plant (except for REG-R, where the soybean oil was obtained from extruded soybeans), TA = tallow, YG = yellow grease, and PF = poultry fat.

4Control diet or source of glycerin: USP = USP-grade glycerin; ADM-MO = Archer Daniels Midland, Mexico, MO; AGP-IA = Ag Processing Inc., Sergeant Bluff, IA; REG-MN = Renewable Energy Group, Albert Lea, MN; REG-R = Renewable Energy Group, Ralston, IA; REG-WL = Renewable Energy Group, Wall Lake, IA; WW-TX = Westway Feed Products, Houston, TX; WW-OH = Westway Feed Products, Cincinnati, OH; IW-AC = Imperial Western Products acidulated glycerin, Coachella, CA; IW-NA = Imperial Western Products nonacidulated glycerin, Coachella, CA; and USB-GA = US Biofuels, Rome, GA.

5SE was calculated by averaging the SE calculated by PROC GLM (SAS Institute, 2004) for the variable of interest.

6Preplanned contrasts: soybean oil-based glycerin (ADM-MO, AGP-IA, REG-MN, REG-R, REG-WL, WW-TX), tallow-based glycerin (WW-OH), and yellow grease-based glycerin (IW-AC, excluding IW-NA because of its high fatty acid content).

7Excluding IW-NA and USB-GA because of their high fatty acid content.

8High fatty acid glycerin samples included IW-NA and USB-GA, and low fatty acid glycerin samples included all others.
The stepwise regression equation was determined to be:

\[
\text{AME}_n (\text{kcal/kg}) = 1,605 - (19.13 \times \text{methanol}) + (39.06 \times \text{fatty acid}) + (23.47 \times \text{glycerin}).
\]

Crude glycerin samples that originated from soy oil had an average AMEn of 3,579 kcal/kg, and an AMEn as a percentage of GE of 98.44%. Conversely, crude glycerin samples that had a relatively high fatty acid content appeared to be poorly utilized by broilers.

**Acknowledgments**

We are thankful for help from J. Cook at the USDA-ARS-NLAE (Ames, IA), D. Chamblee at the USDA-ARS Poultry Research Unit (Mississippi State), and L. N. Rose at Auburn University (Auburn, AL) for laboratory assistance.

### Table 4. Pearson correlation coefficients between chemical characteristics and energy use of crude glycerin samples in growing broilers\(^1\)

<table>
<thead>
<tr>
<th>Chemical characteristic</th>
<th>Glycerin</th>
<th>Water</th>
<th>Methanol</th>
<th>FA</th>
<th>Ash</th>
<th>GE</th>
<th>AMEn</th>
<th>PAMEn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Water</td>
<td>-0.07</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Methanol</td>
<td>-0.75</td>
<td>-0.27</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FA</td>
<td>-0.88</td>
<td>-0.35</td>
<td>0.70</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ash</td>
<td>-0.24</td>
<td>0.09</td>
<td>0.01</td>
<td>0.05</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GE</td>
<td>-0.69</td>
<td>-0.63</td>
<td>0.71</td>
<td>0.94</td>
<td>-0.10</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AMEn</td>
<td>0.01</td>
<td>-0.36</td>
<td>-0.04</td>
<td>0.23</td>
<td>-0.15</td>
<td>0.32</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>PAMEn</td>
<td>0.60</td>
<td>0.47</td>
<td>0.09</td>
<td>-0.74</td>
<td>0.02</td>
<td>-0.78</td>
<td>0.33</td>
<td>1.00</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.82</td>
<td>0.01</td>
<td>0.01</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\)FA = fatty acids; GE = gross energy; PAMEn = percentage of AMEn relative to GE.

### Table 5. Stepwise regression equations for AMEn of various crude glycerin products in growing broilers\(^1\)

<table>
<thead>
<tr>
<th>AMEn equation</th>
<th>Regression coefficient parameter</th>
<th>Statistical parameter(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>Water</td>
</tr>
<tr>
<td>Equation 1</td>
<td>3,883</td>
<td>-25.61</td>
</tr>
<tr>
<td>SE</td>
<td>65</td>
<td>5.91</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Equation 2</td>
<td>3,935</td>
<td>-28.47</td>
</tr>
<tr>
<td>SE</td>
<td>71</td>
<td>6.08</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Equation 3</td>
<td>3,855</td>
<td>-23.58</td>
</tr>
<tr>
<td>SE</td>
<td>72</td>
<td>5.99</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Equation 4</td>
<td>1,090</td>
<td>6.19</td>
</tr>
<tr>
<td>SE</td>
<td>1,517</td>
<td>17.36</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.47</td>
<td>0.72</td>
</tr>
<tr>
<td>Equation 5</td>
<td>1,605</td>
<td>—</td>
</tr>
<tr>
<td>SE</td>
<td>461</td>
<td>—</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\)FA = fatty acids; C(p) = Mallows statistic.

\(^2\)An adjusted R\(^2\) was calculated using the Noint option only when the intercept was excluded from the final model, \(P > 0.1\).
Table 6. Stepwise regression equations for AMEn relative to gross energy for various crude glycerin products in growing broilers

<table>
<thead>
<tr>
<th>PAMEn equation</th>
<th>Regression coefficient parameter</th>
<th>Statistical parameter²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>FA</td>
</tr>
<tr>
<td>Equation 1</td>
<td>97.37</td>
<td>−1.03</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Equation 2</td>
<td>97.71</td>
<td>−0.71</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.98</td>
<td>0.11</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Equation 3</td>
<td>91.63</td>
<td>−0.61</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.76</td>
<td>0.11</td>
</tr>
<tr>
<td>Estimate P-value</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1FA = fatty acids; PAMEn = percentage AMEn relative to GE; C(p) = Mallows statistic.
2An adjusted R² was calculated using the Noint option only when the intercept was excluded from the final model, P > 0.15.

REFERENCES