Apparent Metabolizable Energy of Glycerin for Broiler Chickens[1,2]

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ABSTRACT Three energy balance experiments were conducted to determine AME_n of glycerin using broiler chickens of diverse ages. In experiment 1, two dietary treatments were fed from 4 to 11 d of age. Dietary treatments consisted of a control diet (no added glycerin) and a diet containing 6% glycerin (94% control diet + 6% glycerin). Four dietary treatments were provided in experiment 2 (from 17 to 24 d of age) and 3 (from 38 to 45 d of age). Diets in experiment 2 and 3 were 1) control diet (no added glycerin); 2) 3% added glycerin (97% control diet + 3% glycerin); 3) 6% added glycerin (94% control diet + 6% glycerin); and 4) 9% added glycerin (91% control diet + 9% glycerin). Diets in experiment 1 and 2 were identical, but the diet used in experiment 3 had reduced nutrient levels based on bird age. In experiments 2 and 3, broilers were fed 91, 94, 97, and 100% of ad libitum intake so that differences in AME_n consumption were only due to glycerin. A single source of glycerin was used in all experiments. Feed intake, BW, energy intake, energy excretion, nitrogen intake, nitrogen excretion, AME_n, and AME_n intake were determined in all experiments. In experiment 1, AME_n determination utilized the difference approach by subtracting AME_n of the control diet from AME_n of the test diet. In experiments 2 and 3, AME_n intake was regressed against feed intake with the slope estimating AME_n of glycerin. Regression equations were Y = 3,331x – 72.59 ($P \leq 0.0001$) and Y = 3,348.62x – 140.18 ($P \leq 0.0001$) for experiments 2 and 3, respectively. The AME_n of glycerin was determined as 3,621, 3,331, and 3,349 kcal/kg in experiments 1, 2, and 3, respectively. The average AME_n of glycerin across the 3 experiments was 3,434 kcal/kg, which is similar to its gross energy content. These results indicate that AME_n of glycerin is utilized efficiently by broiler chickens.

Key words: broiler, metabolizable energy, glycerin

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INTRODUCTION

Biofuel production is experiencing exponential growth to provide an alternative fuel source to reduce dependence upon petroleum-based fuel products. In the United States, annual production capacity of biodiesel is approximately 5.26 billion L, and with another 7.15 billion L/yr of additional production through new construction or plant expansion (National Biodiesel Board, 2007). Glycerin, a coproduct from biodiesel production, represents about 9% of the starting feedstocks on a weight basis. Glycerin is known to be a valuable ingredient for producing food, soaps, cosmetics, and pharmaceuticals (Thompson and He, 2006). Furthermore, glycerin may also be a valuable dietary energy source for poultry. It is a 3-carbon compound, and pure glycerin contains approximately 4,100 kcal/kg of gross energy (Brambilla and Hill, 1966).

During digestion, triglycerides are hydrolyzed by pancreatic lipase to form free fatty acids and glycerol (Brody, 1994). The resulting glycerol is water soluble and freely enters the portal blood (Sambrook, 1980). Intestinal absorption of glycerol in rats has been shown to range from 70 to 89% (Hober and Hober, 1937), with the high absorption rate of glycerol likely due to its small molecular weight and it being passively absorbed rather than forming a micelle like that noted for medium and large chain fatty acids with bile salts (Guyton, 1991). Once digested, absorbed, and transferred to liver and tissues, glycerol is converted to glucose via gluconeogenesis (Emmanuel et al., 1983) or oxidized for energy production via glycolysis and the citric acid cycle (Rosebrough et al., 1980).

Several researchers have reported that glycerin is an acceptable feed ingredient for poultry (Campbell and Hill, 1962; Lessard et al., 1993; Simon et al., 1996; Cerrate et al., 2006). Adding glycerin up to an inclusion level of 5%

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[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA, Iowa State University, or Mississippi State University.

[2]The term glycerol is discussed when it is produced from metabolism, whereas glycerin is used when it is produced from fats and oils as a by-product of manufacturing of soaps, fatty acids, and biofuel.

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Table 1. Characterization of crude glycerin fed to broilers in experiments 1, 2, and 3

Specification[1]	Value	Analytical method
Total glycerol, %	86.95	ASTM D 6584-00E01 (ASTM, 2006)
Methanol, %	0.028	Gas chromatography (proprietary method)
pH	5.33	Orion 230A pH meter with 9107 BN probe
Moisture, %	9.63	AOCS Ca 2e-84 (AOCS, 2000)
NaCl, %	3.13	AOCS Db 7-48 (AOCS, 2000)
Ash, %	3.19	AOCS Ca 11-55 (AOCS, 2000)
Total fatty acid, %	0.29	AOCS G 4.40, modified for glycerin (AOCS, 2000)
Analysis[2]		
Moisture, %	9.22	AOAC 984.20 (AOAC, 1996)
CP, %	0.41	AOAC 990.03 (AOAC, 1996)
Crude fat, %	0.12	AOAC 920.39 (A) (AOAC, 1996)
Ash, %	3.19	AOAC 942.05 (AOAC, 1996)
Na, %	1.26	AOAC 956.01 (AOAC, 1996)
Chloride, %	1.86	AOAC 9.15.01, 943.01 (AOAC, 1996)
K, %	<0.005	AOAC 956.01 (AOAC, 1996)
Color, fat analysis committee color standard	<1	AOCS Cc 13a-43 (AOCS, 2000)
Gross energy[3] (kcal/kg)	3,625 ± 26	Adiabatic bomb calorimeter

[1]Values reported by AGP Inc., Sergeant Bluff, IA, Lot # GB605-03.

[2]Analysis by University of Missouri-Columbia experiment Station Chemical Laboratories, Columbia, MO.

[3]Analysis by USDA, National Swine Research and Information Center, Ames, IA.

has shown no adverse effects on growth or carcass yield (Lessard et al., 1993; Simon et al., 1996; Cerrate et al., 2006). However, increasing dietary glycerin above 10% has been shown to adversely affect growth performance and meat yield of broiler chickens (Simon et al., 1996; Cerrate et al., 2006), although this may be due to feed flowability and associated feed consumption (Cerrate et al., 2006).

Previous research has used the ME of glycerin as 95 to 100% of its gross energy (**GE**) in dietary formulation (Brambilla and Hill, 1966; Lin et al., 1976; Cerrate et al., 2006). To our knowledge, AME_n of glycerin with broiler chickens has not been previously reported. The objectives of this research were to determine AME_n of glycerin using broiler chickens at various ages to 1) delineate AME_n of glycerin, and 2) determine if broilers from diverse ages utilize glycerin differently.

MATERIALS AND METHODS

Bird Husbandry

Three energy balance experiments were conducted. Ross × Ross 708 broilers were obtained from a commercial hatchery that had been vaccinated for Marek's disease, Newcastle disease, and infectious bronchitis. Three days

Table 2. Ingredient and nutrient composition of the basal diets[1]

Ingredient, % (as-is)	Experiments 1 and 2 (4 to 11 d of age and 17 to 24 d of age)	Experiment 3 (38 to 45 d of age)
Ground corn	60.33	72.95
Soybean meal (48% CP)	35.72	23.75
Dicalcium phosphate	1.81	1.30
Calcium carbonate	1.17	0.99
Sodium chloride	0.52	0.50
DL-Methionine	0.20	0.20
L-Lysine·HCl	—	0.06
Mineral and vitamin premix[2]	0.25	0.25
Total	100.00	100.00
Calculated analysis		
AME_n, kcal/kg	2,900	3,033
CP, %	22.1	16.7
TSAA, %	0.94	0.78
Lys, %	1.25	0.93
Thr, %	0.88	0.67
Ca, %	0.94	0.73
Nonphytate P, %	0.47	0.36

[1]Diets were formulated to contain 1,500, 1,500, and 1,250 ppm choline in Exp. 1, 2, and 3, respectively.

[2]Vitamin and mineral premix include per kilogram 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; B_{12}, 0.01 mg; folic acid, 0.6 μg; choline, 379 mg; D-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin, 33 mg; 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; selenium, 0.1 mg.

Table 3. Dietary treatments fed to broilers from 7 to 10 d of age in experiment 1[1]

Item	Control diet[2]	6% glycerin[3]	SEM
BW, kg	0.174	0.177	0.003
Feed intake, kg	0.085	0.087	0.001
Energy excretion, kcal	86	84	2
AME_n,[4] kcal/kg	2,518[b]	2,737[a]	21
AME_n intake, kcal	214[b]	222[a]	3
Glycerin AME_n,[5] kcal/kg	—	3,621	221

[a,b]Mean values within a column with no common letters are significantly different ($P \le 0.05$) as determined by least significant difference comparison.

[1]Values are least squares means of 12 replicate pens each pen having 12 chicks with a mean BW of 0.044 kg on 1 d of age.

[2]Control diet contained 100% basal diet.

[3]Glycerin diet contained 94% basal diet and 6% glycerin.

[4]$AME_n = [GEI - GEE] - [8.73 \times (NI - NE)] \div FI$, where GEI = gross energy intake; GEE = gross energy output in the excreta; NI = nitrogen intake from the diet; NE = nitrogen output from excreta; FI = feed intake; and 8.73 = nitrogen correction factor reported from previous research (Titus, 1956).

[5]Determined by using the difference method (Adeola, 2001).

prior to experimentation, broilers were randomly distributed into grower battery cages (Alternative Design Mfg., Siloam Springs, AR). In experiment 1, 288 chicks were used from 4 to 11 d of age (12 chicks per cage; 6 males and 6 females), whereas 576 chicks from 17 to 25 d of age (12 chicks per cage; 6 males and 6 females) were used in experiment 2, and 240 male broilers from 37 to 45 d (5 birds per cage) were used in experiment 3. Each cage (66 cm × 66 cm × 76 cm) was equipped with 1 trough feeder and 1 nipple waterer. The experimental facility was a solid-sided house with temperature control. Temperature was set at 30, 23, and 19°C in experiments 1, 2, and 3, respectively. Lighting was continuous with intensities of 10 (experiments 1 and 2) or 3 (experiment 3) lx. All procedures relating to the use of live birds were approved by the USDA-ARS Animal Care and Use Committee at the Mississippi State location.

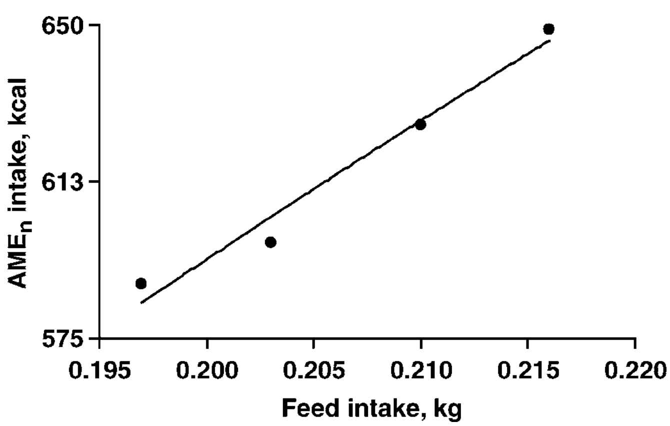

Figure 1. Regression of AME_n intake vs. feed intake from 21 to 24 d of age in experiment 2. Dietary glycerin addition of 0% = 0.197 kg of feed intake; dietary glycerin addition of 3% = 0.203 kg of feed intake; dietary glycerin addition of 6% = 0.210 kg of feed intake; dietary glycerin addition of 9% = 0.216 kg of feed intake. $Y = 3{,}331x - 72.59$ ($P \le 0.0001$; SE of the slope = 266; $r^2 = 0.80$).

Dietary Treatments

A single source of glycerin was added to the basal diets to create treatment diets in all experiments and contained 86.95% glycerol (Table 1). Basal diets were formulated to meet or exceed NRC (1994) nutrient recommendations for broilers in each experiment (Table 2). Because no dietary fat was added, all diets were formulated to be low in AME_n. In addition, all diets differed in nutrient composition due to the diverse age of broilers used in experimentation. Experimental diets were created by the addition of glycerin to the basal diet. In experiment 1, 2 dietary treatments were formulated, consisting of a control diet (100% basal diet) and a diet containing 6% glycerin (94% basal diet + 6% glycerin). In experiments 2 and 3, dietary treatments included the addition of glycerin at 0 (100% basal diet), 3% (97% basal diet + 3% glycerin), 6% (94% basal diet + 6% glycerin), or 9% (91% basal diet + 9% glycerin). Experiment 1 was a preliminary study that estimated AME_n by difference, where AME_n of the control diet was subtracted from the AME_n of the diet containing 6% glycerin. Birds were fed ad libitum. In experiments 2 and 3, broilers were fed 91, 94, 97, and 100% of ad libitum intake as determined from previous research at our laboratory. Feeding varying proportions of ad libitum intake allowed for each treatment group to consume the same amount of basal diet so differences in AME_n consumption were due to glycerin. Subsequently, AME_n intake was regressed against feed intake with the slope representing AME_n of glycerin (Adeola, 2001). One advantage of using regression analysis is that the slope estimate involves multiple inclusion levels instead of estimating from 1 level.

Measurements

The following procedures were common to all experiments. Body weight was determined when broilers were allocated to battery cages and also at the end of experimentation to ensure that dietary treatments did not limit growth. A 72-h total excreta collection period was conducted to evaluate AME_n of glycerin. After a 3-d acclimation period, feed refusal and feed allocation were weighed daily throughout the 72-h collection period. Total amount of excreta voided at the end of the collection was weighed (wet basis). Multiple subsamples were collected from the total amount of excreta and homogenized, and then 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 Thomas-Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) equipped with a 1-mm screen to ensure a homogeneous mixture. Gross energy content of feed and excreta were determined on a 2-g sample using an isoperbol oxygen bomb calorimeter (model 1281, Parr Instruments, Moline, IL), and analysis was performed in duplicate. Nitrogen content of feed and excreta was determined on a 0.2-g sample with a Combustion N analyzer (Truspec N Deter-

Table 4. Energy balance of broilers fed graded levels of glycerin from 21 to 24 d of age in experiment 2[1]

Added percent glycerin	24-d BW (kg)	Feed intake (kg)	Gross energy intake (kcal)	Energy excretion (kcal)	AME_n[2] (kcal/kg)
0[3]	0.698[b]	0.197[d]	766[d]	164	2,984[ab]
3[4]	0.708[a]	0.203[c]	785[c]	173	2,946[b]
6[5]	0.705[ab]	0.210[b]	807[b]	162	2,983[ab]
9[6]	0.707[a]	0.216[a]	839[a]	174	3,004[a]
SEM	0.003	0.001	0.2	4	18
Source of variation, *P*-value					
Linear	0.19	0.001	0.001	0.34	0.34
Quadratic	0.26	0.001	0.001	0.34	0.34

[a–d]Mean values within a column with no common letters are significantly different ($P \leq 0.05$) as determined by least significant difference comparison.

[1]Values are least squares means of 12 replicate pens each pen having 12 chicks with a mean BW of 0.457 kg at 17 d of age.

[2]$AME_n = [GEI - GEE] - [8.73 \times (NI - NE)] \div FI$, where GEI = gross energy intake; GEE = gross energy output in the excreta; NI = nitrogen intake from the diet; NE = nitrogen output from excreta; FI = feed intake; and 8.73 = nitrogen correction factor reported from previous research (Titus, 1956).

[3]Control diet contained 100% basal diet.

[4]Glycerin diet contained 97% basal diet and 3% glycerin.

[5]Glycerin diet contained 94% basal diet and 6% glycerin.

[6]Glycerin diet contained 91% basal diet and 9% glycerin.

minator, Leco Corp., St. Joseph, MI) in duplicate using a previously established method (AOAC, 1996). Feed consumption and excreta weights during the 72-h collection period were used to calculate energy and nitrogen intake and excretion. Apparent ME_n was calculated using the following equation: $AME_n = [GEI - GEE] - [8.73 \times (NI - NE)] \div FI$, where GEI = GE intake, GEE = GE output in the excreta, NI = nitrogen intake from the diet, NE = nitrogen output from excreta, FI = feed intake, and 8.73 = nitrogen correction factor reported from previous research (Titus, 1956).

Statistics

Data were statistically evaluated by the GLM and MIXED procedures (SAS, 2004) involving a randomized complete block design. Cage location was the blocking factor. Three analyses were used: 1) ANOVA with treatment means separated by the least significance comparison; 2) regression analysis to evaluate linear and quadratic effects of dietary glycerin addition; and 3) AME_n intake was regressed against feed intake to determine AME_n of glycerin. Model effects included block, diet, and block × diet (error). Statistical significance was considered at $P \leq 0.05$. Observations were removed when the response criteria exceeded 2 SD from the mean.

RESULTS

Experiment 1

Apparent ME_n of glycerin was determined as 3,621 kcal/kg with 7- to 10-d-old broiler chicks (Table 3). Broil-

Table 5. Energy balance of broilers fed graded levels of glycerin from 42 to 45 d of age in experiment 3[1]

Added percent glycerin	42-d BW (kg)	Feed intake (kg)	Gross energy intake (kcal)	Energy excretion (kcal)	AME_n[2] (kcal/kg)
0[3]	2.550[b]	0.417[d]	1,591[d]	285	3,012[b]
3[4]	2.555[ab]	0.431[c]	1,640[c]	281	3,025[ab]
6[5]	2.551[ab]	0.449[b]	1,714[b]	298	3,039[ab]
9[6]	2.583[a]	0.460[a]	1,749[a]	300	3,054[a]
SEM	0.011	0.002	8	8	16
Source of variation, *P*-value					
Linear	0.27	0.001	0.001	0.07	0.03
Quadratic	0.24	0.001	0.001	0.06	0.03

[a–d]Mean values within a column with no common letters are significantly different ($P \leq 0.05$) as determined by least significant difference comparison.

[1]Values are least squares means of 12 replicate pens each pen having 5 broilers. Average BW at 37 d was 2.155 kg.

[2]$AME_n = [GEI - GEE] - [8.73 \times (NI - NE)] \div FI$, where GEI = gross energy intake; GEE = gross energy output in the excreta; NI = nitrogen intake from the diet; NE = nitrogen output from excreta; FI = feed intake; and 8.73 = nitrogen correction factor reported from previous research (Titus, 1956).

[3]Control diet contained 100% basal diet.

[4]Glycerin diet contained 97% basal diet and 3% glycerin.

[5]Glycerin diet contained 94% basal diet and 6% glycerin.

[6]Glycerin diet contained 91% basal diet and 9% glycerin.

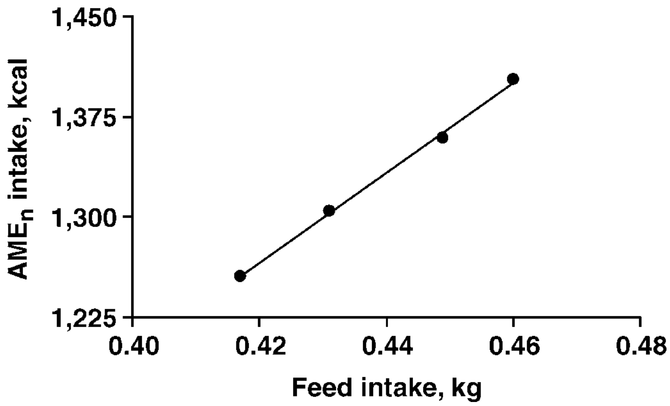

Figure 2. Regression of AME$_n$ intake vs. feed intake from 42 to 45 d of age in experiment 3. Dietary glycerin addition of 0% = 0.417 kg of feed intake; dietary glycerin addition of 3% = 0.431 kg of feed intake; dietary glycerin addition of 6% = 0.449 kg of feed intake; dietary glycerin addition of 9% = 0.460 kg of feed intake. Y = 3,349x − 140.18 ($P \leq 0.0001$; SE of the slope = 222; $r^2 = 0.84$).

ers fed the diet containing 6% glycerin had higher ($P \leq 0.05$) AME$_n$ and AME$_n$ intake than the control-fed birds. Feed intake, energy excretion, and BW were not affected by the dietary treatments.

Experiment 2

Apparent ME$_n$ of glycerin was estimated as 3,331 kcal/kg using 21- to 24-d-old broilers (Figure 1). Regressing AME$_n$ intake against feed intake resulted in an equation of Y = 3,331x − 72.59 ($P \leq 0.0001$; $r^2 = 0.80$). Gradient increments of glycerin ($P \leq 0.001$) increased feed intake and gross energy intake, but final BW, energy excretion, and AME$_n$ were not affected (Table 4). Feed intake and gross energy intake increased ($P \leq 0.05$) with each increment of glycerin.

Experiment 3

Glycerin was estimated to contain 3,349 kcal AME$_n$/kg with 42- to 45-d-old broilers (Figure 2). Apparent ME$_n$ intake was regressed against feed intake to estimate AME$_n$ of glycerin as Y = 3,349x − 140.18 ($P \leq 0.0001$; $r^2 = 0.84$). Progressive addition of glycerin increased ($P \leq 0.03$) feed intake, gross energy intake, and AME$_n$ (Table 5). Feed intake and gross energy intake increased ($P \leq 0.05$) with each incremental level of glycerin. Broilers fed 9% glycerin had higher ($P \leq 0.05$) AME$_n$ than control fed broilers, but AME$_n$ was similar to broilers fed either 3 or 6% glycerin.

DISCUSSION

Glycerin obtained from biodiesel production contained 3,596 kcal/kg of GE (Cerrate et al., 2006). In the current research, glycerin was analyzed to contain 3,625 kcal/kg of GE, 86.95% glycerol, and 9.63% moisture. The lower GE content of the glycerin source used in experimentation compared with pure glycerol (4,100 kcal/kg of GE; Brambilla and Hill, 1966) is probably related to the lower glycerol content. The mean AME$_n$ content of glycerin across the 3 experiments was estimated as 3,434 kcal/kg, which was 95% of GE content. A numerical difference of 290 kcal/kg (3,621 vs. 3,331 kcal/kg) existed between experiments 1 and 2. This should not be statistically significant because the SE was 221 and 266 for experiments 1 and 2. In addition, the AME$_n$ values between experiments 1 and 2 cannot be directly related to an age effect because experiment 1 used only 2 treatments, whereas experiment 2 evaluated 4 treatments. Conversely, only an 18 kcal of AME/kg numerical difference (3,331 vs. 3,349 kcal/kg) was observed between experiments 2 and 3.

In experiment 2, broilers fed 3% glycerin had more variability in AME$_n$ intake (2.6 vs. 1.6% coefficient of variation) than broilers consuming diets formulated to contain 6 or 9% glycerin. The higher variability associated with 3% glycerin was probably due to the low amount test product added compared with the 2 higher treatment levels. Moreover, ME$_n$ determination can be a highly variable measurement (Dozier et al., 2001, 2003; Batal and Dale, 2006). In addition, variability associated with feed intake and excreta measurements in balance experiments can mask differences due to treatments with low inclusion levels of a test ingredient. The basis of using 3, 6, and 9% glycerin was to create treatments that would be relevant to commercial practice. It would be expected that the broiler industry would use a maximum inclusion level of 5 to 6% glycerin; hence, 3 and 6% would be within the maximum range. The 9% glycerin level was chosen to create adequate spread so an AME$_n$ value could be determined with regression analysis. Furthermore, Cerrate et al. (2006) demonstrated that feeding glycerin at the 10% level had a negative effect on growth and carcass yield.

For comparative purposes, AME$_n$ of glycerin is approximately 40% of poultry oil (Cullen et al., 1962; Lessire and Leclercq, 1982) and 36% of corn oil (NRC, 1994). However, AME$_n$ of glycerin is only 10 to 12% higher than corn and grain sorghum, respectively (NRC, 1994). Hence, the energy value of glycerin is a replacement of carbohydrates and only a partial replacement of fats and oils.

In conclusion, AME$_n$ of glycerin was efficiently utilized with an AME$_n$ of 3,434 kcal/kg, which was very similar to its GE. Based on this research, AME$_n$ can be assigned 92 to 95% of its GE. Future research should determine if AME$_n$ and GE are closely related with glycerin sources derived from different feedstocks.

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