

# Metabolizable Energy Value of Crude Glycerol for Laying Hens

## A.S. Leaflet R2333

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### Summary and Implications

An experiment was conducted with laying hens to determine the nitrogen-corrected apparent metabolizable energy AMEn value of crude glycerol, a co-product of biodiesel production. Crude glycerol (87% glycerol, 9% water) was obtained from a commercial biodiesel production facility. A total of 48 40-week-old laying hens (Hy-Line W-36) was placed in metabolic cages (2 hens/cage) and given free access to the experimental diets. A corn and soybean meal-based basal diet was formulated with 15% glucose•H<sub>2</sub>O. Four dietary treatments were created by substituting 0, 5, 10, or 15% crude glycerol for glucose•H<sub>2</sub>O (3,640 kcal/kg AMEn). After 7 days of dietary adaptation, excreta were collected twice daily for 3 days, freeze-dried, and analyzed for contents of dry matter, nitrogen, acid-insoluble ash, and gross energy. Egg production was recorded daily, and eggs were collected on days 7 and 8 of the experiment for calculation of egg mass (egg production × egg weight). No significant treatment effects ( $P > 0.1$ ) were apparent for egg-production rate (93.0%), egg weight (56.1 g), egg mass (52.2 g/d), or feed consumption (104 g/d). Linear regression analysis ( $P < 0.001$ ,  $r^2 = 0.92$ ,  $n = 24$ ) revealed that the AMEn value of the crude glycerol used in this study was 3,805 kcal/kg (as-is basis) for laying hens, slightly higher than that reported for corn grain and less than half of that reported for vegetable oil.

### Introduction

In the United States, the production of diesel fuel from vegetable oil has increased exponentially from less than 2 million liters in 1999 to almost 1 billion liters in 2005. The major co-product of biodiesel production is crude glycerol (glycerin), which may become available for use as poultry feed at relatively economical costs. When consumed, glycerol can be converted to glucose or fatty acids by the hens and can therefore serve as a source of dietary energy.

Studies examining the effects of feeding either chemically pure or crude glycerol from biodiesel production to broiler chickens, turkey hens, and pigs have shown that glycerol can be used a source of dietary energy for livestock. There are no reports, however, in which the nitrogen-corrected apparent metabolizable energy (AMEn) content of crude glycerol has been directly determined. The objective of the study was to determine the AMEn of crude glycerol when fed to laying hens.

### Material and Methods

#### *Housing and Management*

A total of 48 40-wk-old Single-Comb White Leghorn laying hens (Hy-Line W-36) was obtained from a commercial source and placed in metabolic cages (30.5 × 50.8 × 40.6 cm, width × depth × height, respectively), equipped for collection of excreta, in a light-controlled, fan-ventilated room at the Iowa State University Poultry Science Research Center. Each cage contained 2 hens and was equipped with a steel self-feeder and a trough waterer. Upon arrival, hens were given free access to a laying hen diet (17% crude protein, 2,875 kcal/kg AMEn, 4.40% calcium, 0.43% non-phytate phosphorus) and water for a 2-week acclimation period. Hens were provided with 16 hours of light and 8 hours of darkness per day and the ambient temperature was maintained at 26°C throughout the study. After the 2-week acclimatization period, hens were weighed and randomly assigned to cages. Each cage was randomly assigned to 1 of the 4 experimental diets. Hens were given free access to the experimental diets for a 7-day adaptation period followed by a 3-day collection period. All procedures relating to the use of live animals were approved by the Iowa State University Institutional Animal Care and Use Committee.

#### *Dietary Treatments*

A total of 4 experimental diets was used, formulated from a basal diet (Table 1) in which 5, 10, or 15% crude glycerol (Ag Processing, Inc. Sergeant Bluff, IA; Table 2) was substituted for glucose•H<sub>2</sub>O on an equal weight basis. All diets were formulated to meet or exceed the National Research Council<sup>2</sup> nutrient recommendations for laying hens and contained 1.0% Celite to increase the content of acid-insoluble ash (an indigestible marker). The AMEn contents of the experimental diets were not equalized. The basal diet was mixed in 1 large batch in a horizontal ribbon mixer without glucose•H<sub>2</sub>O or crude glycerol additions; the treatment diets were subsequently prepared by mixing a

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<sup>2</sup>National Research Council. 1994. Nutrient requirements of poultry. 9th ed. Natl. Acad. Press, Washington, DC.

portion of the basal diet with the specified amounts of glucose•H<sub>2</sub>O and crude glycerol in a Hobart mixer. The diets were fed in mash form.

#### Data Collection and Analyses

Egg production was recorded daily and the feed consumption was determined for the 10-day-long experiment. Eggs collected over a 48-hour period on day 7 and 8 of the experiment were weighed, and egg mass was calculated as egg production × egg weight. After 1 week of adaptation to the experimental diets, excreta was collected twice daily for 3 days and stored at -20°C until analysis.

Excreta samples were pooled within cage, freeze-dried, and allowed to equilibrate with room moisture prior to analysis. The moisture contents of the experimental diets and freeze-dried excreta were determined in duplicate by drying at 135°C for 3 hours. Diet and excreta nitrogen were determined in duplicate using the micro-Kjeldahl method on a Kjeltech 1028 distilling unit (U.S. Tecator, Inc., Herndon, PA). The gross-energy contents of the experimental diets and the excreta were determined in duplicate using an adiabatic bomb calorimeter (model 1281, Parr Instrument Company, Moline, IL). In addition, the gross-energy content of the crude glycerol was determined in triplicate. The contents of acid-insoluble ash in the experimental diets and the excreta were analyzed in triplicate.

#### Calculations and Statistical Analyses

The AMEn content of the crude glycerol was estimated by a linear regression equation relating the experimental-diet AMEn values to proportion of crude glycerol in each diet. The contribution of AMEn from glucose•H<sub>2</sub>O in all diets, calculated from the glucose percentage inclusion rate and the glucose AMEn value (3,640 kcal/kg), was subtracted from glucose-containing diets. The AMEn value of the experimental diet was calculated as:

$$\text{AMEn} = \text{GE}_{\text{Diet}} - \frac{\text{GE}_{\text{Excreta}} \times \text{AIA}_{\text{Diet}}}{\text{AIA}_{\text{Excreta}}} - 8.22 \times \text{N}_{\text{Retained}}$$

where AMEn (kcal/kg) = nitrogen-corrected apparent metabolizable energy content of the diet; GE<sub>Diet</sub> and GE<sub>Excreta</sub> (kcal/kg) = gross energy of the diet and excreta, respectively; AIA<sub>Diet</sub> and AIA<sub>Excreta</sub> (%) = acid insoluble ash in the diet and excreta, respectively; 8.22 (kcal/kg) = energy value of uric acid; and N<sub>Retained</sub> (g/kg) is the nitrogen retained by the hens per kilogram of diet consumed. The retained nitrogen was calculated as:

$$\text{N}_{\text{Retained}} = \text{N}_{\text{Diet}} - \frac{\text{N}_{\text{Excreta}} \times \text{AIA}_{\text{Diet}}}{\text{AIA}_{\text{Excreta}}}$$

where N<sub>Diet</sub> and N<sub>Excreta</sub> (%) = N contents of the diet and excreta, respectively.

The experimental design was a completely randomized design with 4 dietary treatments and 6 replications per treatment. The cage containing 2 hens was the experimental

unit. The AMEn value of the crude glycerol was estimated as the slope of the linear relationship between the inclusion rate of dietary crude glycerol (independent variable) and the glucose-corrected AMEn value of the experimental diet (dependent variable) using JMP 6.0.3 (SAS Institute, Inc., Cary, NC). The effects of dietary crude glycerol on egg production, egg weight, egg mass, and feed consumption were analyzed by analysis of variance (ANOVA) using JMP. The ANOVA model included only the effects of dietary crude glycerol content, and treatment means were separated using linear, quadratic, and cubic orthogonal polynomial contrasts. The gross energy value of crude glycerol used in the study was compared to its AMEn value using a 2-tailed t-test with n = 3 for gross-energy values and n = 24 for AMEn values. Probability values less than or equal to 0.05 were considered significant. Where appropriate, means and associated SEM are reported in the text on an as-is basis.

#### Results

The mean body weight of the hens was 1.37 ± 0.01 kg (n = 24) at the start of the experiment with no significant difference among treatments (P = 0.60). The increases in dietary AMEn values attributed to substitution of crude glycerol for glucose•H<sub>2</sub>O increased linearly with increasing crude glycerol content (P < 0.001); there were no quadratic or cubic effects (P > 0.1). The AMEn value of the crude glycerol tested was 3,805 ± 238 kcal/kg (Figure 1), and was not different (P > 0.1) from its gross energy value (3,625 ± 26 kcal/kg) as revealed by a 2-tailed t-test. Feed consumption, egg production, egg weight, or egg mass were not affected (P > 0.1) by the dietary treatments in the 10-day-long experiment (Table 3).

#### Discussion

The AMEn value of crude glycerol was found to be 3,805 kcal/kg and was not different from its gross-energy value. In comparison, the National Research Council<sup>2</sup> lists a nitrogen-corrected metabolizable energy (MEn) content of corn grain of 3,350 kcal/kg and that of crude soybean oil between 8,020 to 8,650 kcal/kg. Hence, crude glycerol is estimated to contain about 14% more MEn than that of corn grain, and less than half of that contained in crude soybean oil. Moreover, crude glycerol supplies only energy and not nutrients (e.g., amino acids, phosphorus, and essential fatty acids) as do corn grain and soybean oil, which should be considered when assigning a monetary value to crude glycerol.

Although the present experiment was not designed to specifically evaluate the effects of dietary crude glycerol on egg production, egg weight, egg mass, feed consumption, or feed utilization, these parameters were not affected by the dietary crude glycerol in the relatively short experiment. Moreover, the experimentally determined AMEn content of the crude glycerol was similar to that of its gross-energy

content. These observations indicate that the energy in crude glycerol is used with high efficiency for egg production by laying hens.

The crude glycerol tested in the present experiment had a relatively high content of sodium and—because the sodium content were not equalized among the treatment diets—the excreta from hens fed the 15% crude glycerol diet was considerably wetter than that from other treatments. Moreover, crude glycerol is a viscous liquid, and flow characteristics of diets containing 10 and 15% crude glycerol were noticeably poorer than that of the control diet, suggesting that crude glycerol should not be included in laying-hen diets at more than 5 to 10% unless the diets are pelleted.

#### Acknowledgements

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**Table 1. Composition of the basal diet (as-is basis).**

Item	Amount, %
Ingredient	
Corn	39.82
Soybean meal (48% CP)	21.00
Glucose•H <sub>2</sub> O	15.00
Meat and bone meal (50% CP)	9.60
Calcium carbonate <sup>1</sup>	9.23
Vegetable oil	3.25
Celite	1.00
Vitamin premix <sup>2</sup>	0.35
Trace mineral premix <sup>3</sup>	0.30
DL-Methionine	0.27
L-Threonine	0.08
Sodium chloride (iodized)	0.10
Total	100.00
Calculated composition	
Crude protein	17.85
AMEn, kcal/kg	2,875
Ether extract	5.87
Linoleic acid	2.16
Calcium	4.51
Phosphorus (non-phytate)	0.51
Potassium	0.69
Sodium	0.21
Chloride	0.29
Methionine	0.52
Methionine + cystine	0.77
Lysine	0.96

<sup>1</sup>Supplied as a 50:50 mix of fine (0.14-mm mean diameter) and coarse (2.27-mm mean diameter) particles.

<sup>2</sup>Supplied per kilogram of diet: Vitamin A, 9,259 IU; vitamin D<sub>3</sub>, 3,086 ICU; vitamin E, 15 IU; vitamin B<sub>12</sub>, 12 µg; riboflavin, 6 mg; niacin, 31 mg; D-pantothenic acid, 11 mg; choline, 386 mg; vitamin K, 2 mg; folic acid, 0.5 mg; vitamin B<sub>6</sub>, 2 mg; thiamine, 2 mg; D-biotin, 0.05 mg.

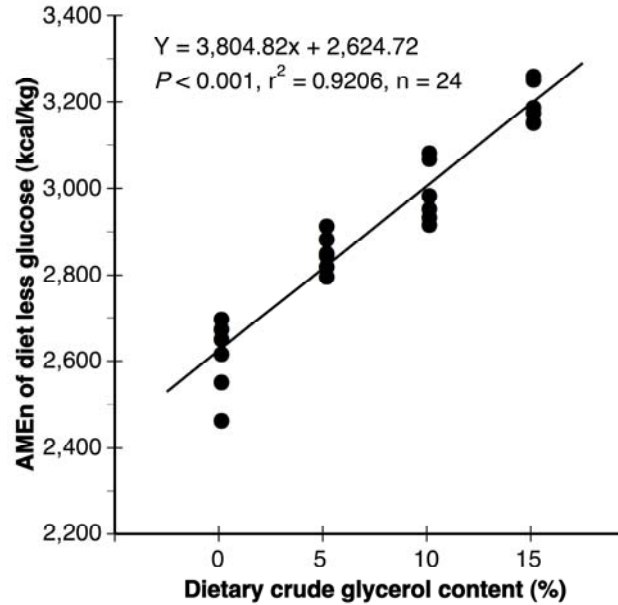
<sup>3</sup>Supplied per kilogram of diet: Manganese, 70 mg; zinc, 90 mg; iron (ferrous sulfate), 60 mg; copper, 12 mg; selenium (sodium selenite), 0.15 mg; sodium chloride, 2.5 g.

**Table 2. Characterization of the crude glycerol<sup>1</sup> fed to laying hens (as-is basis).**

Characteristic	Amount
Glycerol, %	86.95
Moisture, %	9.22
Methanol, %	0.028
CP, %	0.41
Crude fat, %	0.12
Ash, %	3.19
Na, %	1.26
Cl, %	1.86
K, %	< 0.005
Color, Fat Analysis Committee color standard	< 1
pH	5.33

<sup>1</sup>Supplied by Ag Processing, Inc., Sergeant Bluff, IA; lot number GB605 03.

**Figure 1. Relationship between the dietary inclusion rate of crude glycerol and the glucose-corrected AMEn value of the experimental diets for laying hens (each dot represents data from 1 cage containing 2 hens).**



**Table 3. Production data<sup>1</sup> of hens fed increasing levels of crude glycerol replacing 15% glucose•H<sub>2</sub>O in a basal diet.**

	Dietary crude glycerol (%)				SEM <sup>2</sup>	P
	0	5	10	15		
Feed consumption, g/day	108.0	102.3	100.1	105.6	4.3	0.59
Egg production, %	90.9	94.7	93.2	93.2	2.6	0.79
Egg weight, g/egg	55.9	55.4	57.0	56.3	0.3	0.65
Egg mass, g/day	50.8	52.5	53.2	52.5	1.9	0.83
Feed utilization, g egg mass/g feed	0.472	0.534	0.532	0.498	0.024	0.24

<sup>1</sup>Data for the entire 10-day-long experiment are shown.

<sup>2</sup>Pooled standard error of the mean, n = 6.