

Research Note

Effect of crude glycerin level in the diet of laying hens on egg performance and nutrient utilization

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ABSTRACT An experiment was conducted with 72 Bovans Brown laying hens to determine the effect of dietary crude glycerin on laying performance; egg quality; retention of N, Ca, and P; and metabolizability of energy. The dietary treatments consisted of a control corn-soybean diet containing 6% corn starch (17% CP, 2,775 kcal/kg of AME_n, 0.81% lysine, 0.36% methionine, 3.60% Ca, and 0.37% available P) and 3 experimental diets. In the experimental diets, 2, 4, or 6% crude glycerin (a coproduct of commercial biodiesel production from rapeseed) was substituted for corn starch. During the experimental period (28 to 53 wk of hen age), the dietary level of glycerin had no significant effects on performance indices [i.e., egg production (mean value of all 4 dietary treatments was 95.6%), egg weight (60.4

g), daily egg mass (57.8 g/hen), daily feed consumption (121 g/hen), and feed conversion (0.477 g of egg mass/g of feed consumed)]. No significant treatment effects were found for egg quality parameters (albumen height, Haugh units, yolk color and thickness, density and breaking strength of eggshell), excretion and retention of N, Ca and P, or metabolizability of energy. Linear regression analysis revealed that the AME_n value of crude glycerol was 3,970 kcal/kg (as-is basis). The results of this study demonstrated that crude glycerin may be incorporated to a level of 6% in the diet of laying hens without any detrimental effect on egg performance, egg quality, nutrient retention, and metabolizability of energy.

Key words: crude glycerin, laying hen, performance, egg quality, nutrient retention

2009 Poultry Science 88:615–619
doi:10.3382/ps.2008-00303

INTRODUCTION

Crude glycerin is a coproduct of biodiesel production, obtained in the process of transesterification of triacylglycerols of plant oils or animal fats, usually using methanol and a catalyst (sodium methylate). Typically in this process, approximately 90 to 110 kg of glycerin is produced from each 1,000 kg of oil. Because biofuels are a renewable, relatively clean source of energy, and the burning of biofuels releases less CO₂ than conventional fossil fuels, the global production of biodiesel from vegetable oils has increased rapidly in recent years and is expected to continue to increase in the future. Traditionally, glycerin is used in the cosmetic, pharmaceutical, and food industries, but growing interest in biodiesel production will lead to increases in the amounts of crude glycerin available to the feed industry.

Crude glycerin generally contains glycerol (80 to 90%), water (10 to 20%), ash (mainly NaCl), free fatty

acids, and traces of protein and methanol. The intestinal absorption rate of glycerol is high, and after absorption, glycerol can be converted to glucose through the process of gluconeogenesis or oxidized for energy production. The gross energy content of chemically pure glycerol (4,310 kcal/kg) is efficiently used by poultry. The metabolizability of energy in chickens is about 98%; thus, the content of AME_n in pure glycerol is 4,200 kcal/kg (Barteczko and Kaminski, 1999).

Results of previous studies have demonstrated that pure glycerol or crude glycerin can be a useful energy source in the diets for broiler chickens (Simon et al., 1996, 1997; Barteczko and Kaminski, 1999; Cerrate et al., 2006), pigs (Mourot et al., 1994; Kijora et al., 1995, 1997; Lammers et al., 2008b), and ruminants (Schröder and Südekum, 1999; DeFrain et al., 2004). In the study of Dozier et al. (2008), the AME_n of crude glycerin for broiler chickens was similar to its gross energy and was, on average (across 3 experiments), 3,434 kcal/kg. Lammers et al. (2008a) found that the energy in crude glycerol was used efficiently by laying hens and had an AME_n content of 3,805 kcal/kg. Recently, Südekum et al. (2008) reported that glycerol may help to stabilize the hygienic quality of pelleted compound feeds (mea-

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Received July 25, 2008.

Accepted November 12, 2008.

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sured as the ergosterol concentration, which was used as a marker of fungal activity) without compromising the physical quality of pellets.

The objective of the present study with laying hens was to investigate the effect of the dietary level of crude glycerin on egg performance; egg quality; retention and excretion of N, Ca, and P; and metabolizability of dietary energy. The AME_n of crude glycerin for laying hens was also determined in this study.

MATERIALS AND METHODS

Birds, Diets, and Management

The Local Krakow Ethic Committee for Experiments with Animals approved all experimental procedures relating to the use of live animals. A total of 72 eighteen-week-old Bovans Brown hens, obtained from a commercial source, were placed in individual cages on a wire-mesh floor, under controlled climate conditions in the poultry house of the Experimental Station of National Research Institute of Animal Production. Cage dimensions were 40 × 40 cm equaling 1,600 cm² of total floor space. During the preexperimental period (up to 26 wk of age), a commercial laying hen diet was offered for ad libitum consumption (17% CP, 2,770 kcal/kg of AME_n, 3.70% Ca, and 0.38% available P).

At 28 wk of age, hens were randomly assigned to 1 of 4 dietary treatments, 18 individually caged layers for each treatment. During the experiment, hens were provided with water and feed ad libitum and were exposed to a 14 L:10 D lighting schedule with a light intensity of 10 lx.

Ingredient and nutrient composition of the control diet used in the experiment is shown in Table 1. The control diet was formulated to meet or exceed nutrient recommendations (NRC, 1994) and contained 6% corn starch. In the experimental diets, 2, 4, or 6% crude glycerin was substituted for cornstarch. The crude glycerin sample (73.8% DM, 0.04% CP, 0.4% crude fat, 3.7% ash) used in this study was obtained from a commercial biodiesel production facility (GES Inc., Bidziny, Wojciechowice, Poland) using rapeseed oil as an initial substrate.

The experimental diets were fed from 28 to 53 wk of age. The nutrient content of the diets was calculated according to the chemical composition of raw feedstuffs and ME value according to equations from European Tables (Janssen, 1989). Samples of feed components were analyzed, using standard methods (AOAC, 1990), for moisture (method 930.15), CP (984.13), crude fat (920.39), and ash (942.05). Amino acids were analyzed in acid hydrolysates, after initial performic acid oxidation of sulfur amino acids and after alkaline hydrolysis of tryptophan (method 982.30; AOAC, 1990). The Ca content was analyzed by flame atomic absorption spectrophotometry (method 968.08; AOAC, 1990) and total P content by colorimetry using the molybdovanadate method (method 965.17; AOAC, 1990).

Table 1. Composition and nutrient content of control diet (%)

Item	Control diet
Ingredient	
Corn	22.00
Wheat	30.10
Soybean meal	22.00
Grass meal	2.00
Rapeseed cake	4.00
Cornstarch ¹	6.00
Rapeseed oil	2.50
Limestone	8.80
Dicalcium phosphate	1.70
NaCl	0.30
DL-Methionine (99%)	0.10
Vitamin-mineral premix ²	0.50
Total	100.00
Calculated composition (on as-is basis)	
ME, ³ kcal/kg	2,775
CP	17.00
Lysine	0.81
Methionine	0.36
Methionine + cystine	0.67
Ca	3.70
Total P	0.60
Available P	0.37

¹0, 2, 4, and 6% crude glycerin replaced cornstarch on a weight basis to create the 4 dietary treatments.

²The premix provided per 1 kg of diet: vitamin A, 10,000 IU; vitamin D₃, 3,000 IU; vitamin E, 50 IU; vitamin K₃, 2 mg; vitamin B₁, 1 mg; vitamin B₂, 4 mg; vitamin B₆, 1.5 mg; vitamin B₁₂, 0.01 mg; Ca pantothenate, 8 mg; niacin, 25 mg; folic acid, 0.5 mg; choline chloride, 250 mg; manganese, 100 mg; zinc, 50 mg; iron, 50 mg; copper, 8 mg; iodine, 0.8 mg; selenium, 0.2 mg; cobalt, 0.2 mg.

³Calculated according to European Table (Janssen, 1989) as the sum of the ME content of components.

Measurements

During the experiment, the number and weight of eggs were registered daily, feed consumption was recorded monthly, and egg production, egg mass, daily feed intake, and feed utilization (egg mass in g per 1 g of feed consumed) were calculated.

At 36 and 48 wk of age, 1 egg from each hen (18 eggs from each treatment) was collected to determine egg quality indices (i.e., albumen height, Haugh units, yolk color, eggshell thickness, and eggshell density). The eggs were analyzed using semiautomated egg quality equipment [QCM+, Technical Services and Supplies (TSS), York, UK]. The eggs were weighed, cracked, and albumen height was measured with an electronic gauge (QCH device, TSS). The albumen height was converted to Haugh units using the HU formula (Eisen et al., 1962) by Eggware software (TSS). Yolk color was measured using an electronic colorimeter (QCC device, TSS) and was expressed in Roche scale points. Shell thickness was measured near the equator of the egg using an electronic micrometer (QCT device, TSS). Eggshell density (dried shell weight per unit of shell area, mg/cm²) was calculated by Eggware software (TSS).

A further 18 eggs from each treatment were collected for measurement of eggshell breaking strength, using an Instron Testing Machine, model 5542 (Instron Ltd., High Wycombe, UK) equipped with a 500-N load cell.

The eggs were compressed at a constant crosshead speed of 10 mm/min, and breaking strength was determined at the moment of eggshell fracture.

At 38 wk of age, 5 hens from each treatment were replaced into individual balance cages. After a 1-wk adaptation period, total collection of excreta was carried out during 5 d and feed consumption for each hen was recorded. Excreta was stored in plastic bags at -20°C for 2 wk and, after thawing, was dried in an oven at 50°C to a constant weight, weighed, and finely ground. Nitrogen content in the excreta was analyzed using the Kjeldahl procedure (method 984.13; AOAC, 1990), gross energy using an adiabatic bomb calorimeter, Ca content by flame atomic absorption spectrophotometry (method 968.08; AOAC, 1990), and total P content by colorimetry using the molybdovanadate method (method 965.17; AOAC, 1990). Nitrogen (Ca, P) retention (mg) was calculated as: N intake $-$ N excretion. Nitrogen (Ca, P) retention as percentage of N (Ca, P) intake was calculated as: $(\text{N intake} - \text{N excretion})/\text{N intake} \times 100$. Dietary AME_n was calculated by the following formula: $\text{AME}_n = (\text{gross energy intake} - \text{gross energy excretion}) - [(\text{N intake} - \text{N excretion}) \times 8.73]/\text{feed intake}$, where 8.73 is the N correction factor (Titus et al., 1959). Energy utilization in the diets with different level of crude glycerin was calculated as percentage of AME_n in the gross energy of the diets. The AME_n content of crude glycerin was calculated by a linear regression equation using the procedure described by Lammers et al. (2008a). The AME_n of cornstarch taken for calculations was 4,100 kcal/kg of DM (Janssen, 1989).

Statistical Analysis

Data were subjected to statistical analysis using a completely randomized design according to the GLM procedure of Statistica 5.0 (Statsoft Inc., Tulsa, OK). The experimental unit was a cage containing 1 hen. All data were analyzed using 1-way ANOVA. Treatment means were also tested using orthogonal polynomial contrasts for evaluation of the linear and quadratic effects of increases in the dietary crude glycerin level. The AME_n of the crude glycerin was determined as the slope of the linear relationship between the dietary level of crude glycerin (independent variance) and the starch-corrected AME_n of the experimental diets (dependent variance). Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

The mean egg production, averaged across all dietary treatments, during the whole experimental period (26 to 53 wk of hen age) was 95.6%, egg weight was 60.4 g, daily egg mass was 57.8 g/hen, daily feed consumption was 121 g/hen, and feed utilization was 0.477 g of egg

mass/1 g of feed consumed (Table 2). The inclusion of 2, 4, or 6% crude glycerin had no significant effect on laying performance parameters as compared with the control group ($P > 0.05$). There were no treatment effects, either at 36 or at 48 wk of age, on egg quality parameters (i.e., albumen height, Haugh units, and egg shell thickness, density, and breaking strength; Table 2). The results of the present study are similar to those reported by Lammers et al. (2008a), who, in a 10-d experiment with Single Comb White Leghorn hens, found that egg production, egg weight, egg mass, and feed intake were not affected when 5, 10, or 15% crude glycerol was incorporated into the diet. Also, in turkey hens, glycerol used as an energy source had no negative effect on laying rate, egg weight, and feed conversion (Rosebrough et al., 1980). In a study with broiler chickens, birds fed diets containing 2.5 or 5% glycerin had a similar performance and greater breast meat yield as compared with birds fed the control diet, but the greater dietary level of glycerin (10%) negatively affected feed consumption, BW gain, feed conversion, dressing percentage, and breast meat weight (Cerrate et al., 2006). The authors indicated that this decrease in performance parameters was probably due to reduced feed consumption, caused in large part by lower pellet quality and flow rate of the diet with 10% glycerin in the tube feeders. In contrast, Barteczko and Kaminski (1999) found a beneficial effect of the inclusion of 10% glycerol in the diet on broiler performance. In a study with fattening pigs, Kijora et al. (1995) observed a positive effect of glycerol addition to the diet on feed intake, probably due to the sweet taste and better physical structure of diets that contained glycerol (i.e., feed mixture with glycerol had a better consistency and was less dusty than the control diet).

The incorporation of 2, 4, or 6% crude glycerin into the diet had no significant effect on results of N balance (Table 3). Similar results were reported by Simon et al. (1997), who observed no effect of the inclusion of 10% pure glycerol into a low-protein diet on N retention in broiler chickens; though in an earlier experiment (Simon et al., 1996), using diets rich in protein, they found that N retention was positively correlated with glycerol level in the diet (up to 20%). However, in nursery pigs fed diets with 3 or 6% crude glycerol, N retention tended to decrease as compared with control diets with 3 or 6% soybean oil (Goesbeck et al., 2008).

There were also no treatment effects of crude glycerin dietary inclusion level on Ca and P balances (Table 3). Because of the high requirement of laying hens for Ca and P, each new feed component should be tested for its potential effect on utilization of these macroelements. To our knowledge, there is thus far no experimental data indicating a negative influence of dietary glycerol on the availability of minerals; however, Barteczko and Kaminski (1999) speculated that a high glycerol level in the diet could increase the rate of digesta passage and, in this way, impair nutrient utilization. The results of

Table 2. Effect of dietary level of crude glycerin on laying performance and egg quality¹

Item	Dietary crude glycerin, %					Probabilities		
						Main (treatment) effect	Contrasts	
	0	2	4	6	SEM		Linear	Quadratic
Hen-day egg production, ² %	94.6	96.0	96.9	95.1	0.52	0.43	0.58	0.15
Egg weight, g	60.9	60.5	59.2	61.1	0.38	0.30	0.83	0.25
Daily mass of eggs, ³ g/hen	57.6	58.0	57.4	58.1	0.47	0.95	0.84	0.88
Daily feed consumption, g/hen	122	122	120	121	0.56	0.71	0.51	0.75
Feed utilization, mass of eggs in g/1 g of feed	0.474	0.477	0.479	0.480	0.04	0.94	0.57	0.90
Albumen height at 36 wk of age, mm	6.91	6.63	6.48	6.38	0.14	0.58	0.18	0.75
Albumen height at 48 wk of age, mm	6.80	6.53	6.32	6.22	0.15	0.56	0.16	0.78
Haugh units at 36 wk of age	83.0	81.0	80.3	79.0	0.97	0.52	0.15	0.84
Haugh units at 48 wk of age	80.5	78.9	78.6	77.0	0.94	0.63	0.20	0.97
Yolk color at 36 wk of age, Roche scale points	3.50	3.25	3.25	3.25	0.68	0.48	0.22	0.36
Yolk color at 48 wk of age, Roche scale points	3.33	3.25	3.42	3.17	0.66	0.59	0.58	0.54
Eggshell thickness at 36 wk of age, μm	409	403	401	405	3.62	0.84	0.59	0.46
Eggshell thickness at 48 wk of age, μm	403	391	385	395	3.88	0.42	0.38	0.16
Eggshell density at 36 wk of age, mg/cm^2	101.0	96.5	97.0	97.1	1.24	0.55	0.32	0.37
Eggshell density at 48 wk of age, mg/cm^2	96.2	93.2	94.1	91.7	1.25	0.64	0.26	0.90
Eggshell breaking strength at 36 wk of age, N	34.9	36.1	34.4	32.9	1.19	0.81	0.46	0.57
Eggshell breaking strength at 48 wk of age, N	33.2	32.2	32.3	32.8	1.02	0.98	0.93	0.71

¹Values are means of 18 replicates of 1 hen kept in individual cages.

²Hen-day egg production = $(100 \times \text{number of eggs laid})/(\text{number of hens} \times \text{days})$.

³Daily mass of eggs = $(\text{hen-day egg production} \times \text{egg weight})/100$.

our study showed that crude glycerin included at up to 6% in the diet of layers has no effect on retention or excretion of Ca and P.

There were no treatment effects on utilization of dietary energy (i.e., percentage of AME_n in gross energy); however, the dietary AME_n increased linearly ($P < 0.001$) with an increasing level of substitution of crude glycerin for corn starch (Table 3). A similar tendency was found by Lammers et al. (2008a) when crude glycerin was substituted for glucose. A linear regression equation constructed for the relationship between the dietary inclusion level of crude glycerin and the corn starch-corrected AME_n value of the experimental diets ($Y = 3970x + 2896$, $r^2 = 0.9098$, $P < 0.0001$, $n = 20$) revealed that the AME_n value of used glycerin was 3,970

± 295 kcal/kg (mean \pm SEM, as-is basis). This value was greater by 165 kcal than the value reported for laying hens by Lammers et al. (2008a) and by 536 kcal/kg than the mean value determined by Dozier et al. (2008) for broiler chickens at different ages (2, 4, and 7 wk of age), but lower by 230 kcal/kg than the AME_n of pure glycerol estimated for broilers (Barteczko and Kaminski, 1999).

In conclusion, the results of the current study demonstrated that crude glycerin, obtained from biodiesel production from rapeseed, is a relatively rich source of energy (3,970 kcal of AME_n/kg) for laying hens and could be included at the 6% level to the diet without any detrimental effect on egg performance, egg quality parameters, or retention of N, Ca, and P.

Table 3. Effect of dietary level of crude glycerin on N, Ca, and P balance and on ME of diet¹

Item	Dietary crude glycerol, %					Probabilities		
						Main (treatment) effect	Contrasts	
	0	2	4	6	SEM		Linear	Quadratic
N intake, mg/hen per day	3,611	3,485	3,547	3,623	33.6	0.86	0.87	0.44
N excretion, mg/hen per day	2,062	1,975	1,932	2,033	39.7	0.69	0.73	0.27
N retention, mg/hen per day	1,549	1,510	1,614	1,590	33.6	0.74	0.48	0.91
N retained, % of N intake		43.0	43.3	45.5	43.9	0.59	0.50	0.44
Ca intake, mg/hen per day	3,671	3,801	3,744	3,803	61.3	0.88	0.57	0.79
Ca excretion, mg/hen per day	1,559	1,733	1,620	1,793	50.2	0.36	0.20	0.99
Ca retention, mg/hen per day	2,115	2,068	2,124	2,009	52.4	0.88	0.63	0.76
Ca retained, % of Ca intake		57.2	54.5	56.7	53.0	1.07	0.50	0.31
P intake, mg/hen per day	784	765	751	763	12.9	0.86	0.51	0.64
P excretion, mg/hen per day	595	601	586	610	15.2	0.96	0.84	0.78
P retention, mg/hen per day	189	168	165	153	8.84	0.57	0.19	0.83
P retained, % of P intake		24.3	22.0	21.9	20.3	1.22	0.75	0.31
AME_n value of experimental diets, kcal/kg	2,888	2,987	3,053	3,131	21.3	0.0001	0.0001	0.43
Metabolizability of energy, % of AME_n in the gross energy of diet	68.6	69.3	69.1	69.2	0.15	0.46	0.43	0.35

¹Values are means of 5 replicates of 1 hen kept in individual balance cages.

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