NITROGEN-CORRECTED APPARENT METABOLIZABLE ENERGY VALUE OF CRUDE GLYCEROL FOR LAYING HENS

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Abstract

An experiment was conducted with 64 Tetra SL laying hens (placed into four groups) to determine the AMEn value of feed grade crude glycerol (DM 92%; glycerol 86.80%; methanol 0.04%; fat 0.49%; ash 4.67%; GE 15.59 MJ/kg). All groups were given a 100 g basal diet per day (AMEn value 11.46 MJ/kg; protein 16.97%; Ca 3.49%; P (non-phytate) 0.56%; lysine 0.82%; methionine 0.40%; cysteine 0.29%; threonine 0.60%; tryptophan 0.19%), which was supplemented with 5%, 7.5% and 10% glycerol for the three experimental groups. Hens were placed in metabolism cages (4 hens/treatment in four replicates). After a 10-day adaptation period, excreta was collected and weighed individually for 10 days. The AMEn value of crude glycerol (86%) was calculated as 15.30 MJ/kg for laying hens. During the experiment, egg production (90.0–90.8%), egg weight (56.8–58.0 g) and egg mass (51.2–52.7 g) were not affected by dietary treatments. Feed conversion was significantly reduced by a 10% glycerol supplementation. We concluded that crude glycerol's energy is efficiently used by laying hens, and more efficiently than by broilers. The established 15.30 MJ/kg AMEn value of crude glycerol when fed in the daily ration to laying hens means that they utilized 97.6% of the glycerol's GE content.

Key words: biofuel by-product, glycerol, nutrition, metabolizable energy, hen

The decline in world stocks of fossil energy, and uncertainties in the oil and gas supply have increased the significance and use of renewable energy sources, including the production and use of biodiesel. Between 1998 and 2010 the European Union's biodiesel production increased from 100 thousand tons to 9.57 million tons (EBB Statistics, 2012). The EU's goal that at least 5.75% biodiesel should be mixed into the diesel used by vehicles, has contributed to this increase. The EU, in line with the Europe 2020 strategy, plans to further increase this percentage. To meet this goal, a biodiesel production capacity of 22.117 million tons was available in the EU in 2010 (EBB Statistics, 2012).

Crude glycerol is a by-product of biodiesel production. Depending on the refinement techniques, the production of one litre of biodiesel results in the formation of 79 g (Thompson and He, 2006) or 100 g of crude glycerol (Friedrich, 2004). Calculating with these figures, there were 0.76–0.96 million tons of glycerol generated in the EU in 2010, resulting from biodiesel production. This amount is expected to keep on growing, which allows that a certain amount of crude glycerol (exceeding the demand of conventional users in the chemical, pharmaceutical, cosmetic and food industries) shall be used for animal feed.

Depending on the energy supply glycerol may be connected in various ways to the energy metabolism of the animals. Glycerol, as a water-soluble small molecule, is easily absorbed passively (Sambrook, 1980; Guyton, 1991). Bartelt and Schneider (2002) in experiments on laying hens found that 97.1–99.4% of the glycerol was absorbed until the end of the ileum. Simon *et al.* (1997) also reported good absorption of glycerol in broilers. Under energy deficient conditions, glycerol is oxidized and used as energy source. One mole of glycerol yields 22 moles of ATP. Under energy surplus conditions, glycerol promotes fat synthesis (Doppenberg and van der Aar, 2007).

Although glycerol has been commonly used in recent years to improve the energy supply of dairy cows (Chung *et al.*, 2007; Schröder and Südekum, 2007; Donkin *et al.*, 2009), only a few research studies have been published about glycerol as a feed material in monogastric nutrition. The few available experiments in broilers studied mainly the impact of glycerol feeding on weight gain, feed conversion and meat quality (Campbell and Hill, 1962; Lin *et al.*, 1976; Lessard *et al.*, 1993; Simon *et al.*, 1996, 1997; Cerrate *et al.*, 2006). Only a few attempts were made to determine the metabolizable energy content of glycerol (Barteczko and Kamiński, 1999; Bartelt and Schneider, 2002; Dozier *et al.*, 2008; Schmidt and Zsédely, 2012). Even fewer experimental results are available on using glycerol in the feed of laying hens (Bartelt and Schneider, 2002; Lammers *et al.*, 2008; Świątkiewicz and Koreleski, 2009). Therefore, in our experiment we wanted to obtain further data on the AMEn value of the “feed grade” glycerol, containing 85–87% glycerol and only minimal amounts of methanol and crude fat, for laying hens.

Material and methods

A total of 64 Tetra SL laying hens were used in 4 consecutive metabolism experiments (4 hens/treatment in four replicates). There were four dietary treatments: one control and three experimental (5%, 7.5% or 10% glycerol supplementation). Hens were placed into individual metabolism cages (50 cm × 44 cm × 48 cm) equipped with feeders and drinking troughs. Trays beneath the cages allowed the accurate collection of excreta.

A 10-day adaptation period was followed by a 10-day experimental period, during which the feed consumption and the amount of excreta were determined individually. Collected excreta was stored in a deep freezer until analysis. At the end

of the 10-day collection period, the frozen excreta samples were thawed and then samples from hens of the same treatments were combined and homogenized. The N content of excreta was analysed from wet excreta samples to preclude the NH_3 loss. The method was the same for all four experiments. In successive experiments, hens were aged 28, 31, 34 and 37 weeks.

Table 1. Composition and nutrient content of the basal diet

Item	%
Components:	
corn	50.00
wheat	17.00
soybean meal (45% CP)	17.00
sunflower meal (38.8% CP)	6.00
limestone	9.00
sodium chloride	0.30
vitamin and mineral premix*	0.50
L-lysine	0.07
DL-Methionine	0.13
Total	100.00
Nutrient content (calculated):	
AMEn (MJ/kg)	11.46
crude protein (%)	17.15
ether extract (%)	2.40
crude fibre (%)	3.50
Ca (%)	3.52
P (non-phytate) (%)	0.36
Na (%)	0.29
lysine (%)	0.82
methionine (%)	0.41
cystine (%)	0.30
threonine (%)	0.60
tryptophan (%)	0.18

*Composition of vitamin and mineral premix: vitamin A 2 000 000 IU/kg; vitamin D₃ 700 000 IU/kg; vitamin E 6800 mg/kg; vitamin K 525 mg/kg; Mn 19543 mg/kg; Zn 19313 mg/kg; Fe 7990 mg/kg; Cu 1929 mg/kg; Se 68.31 mg/kg.

Composition and nutrient content of the diets are shown in Table 1. All diets were formulated to meet the nutrient recommendations of the Hungarian Feed Codex (2004) for laying hens. Layers were allowed to consume 100 g of the basal diet per day, which was supplemented with 5%, 7.5% and 10% of glycerol in the experimental group. Thus the daily rations of the experimental groups were 105 g, 107.5 g and 110 g. Birds in the control group were fed a basal diet (100 g/day) without glycerol.

Partially refined glycerol (86.8% glycerol content) from biodiesel production was used. The feedstock of trans-esterification was rapeseed oil. The chemical composition of the feed grade glycerol is shown in Table 2. Data show that the glycerol contained only minimal amounts of methanol and crude fat. By contrast, the crude ash content was high and 96.6% was sodium chloride.

Table 2. Composition of the crude glycerol fed to laying hens

Item	
Dry matter	92.00%
Glycerol	86.80%
Ether extract	0.49%
Methanol	0.04%
Crude ash	4.67%
Gross energy (GE)	15.59 MJ/kg

The AMEn value of the diet and glycerol was calculated using the following equation (Dozier *et al.*, 2008):

$$AMEn = [GEI - GEE] - [36.5 \times (NI - NE)] / FI$$

where:

GEI – GE intake,

GEE – GE output in the excreta,

NI – nitrogen intake from the diet,

NE – nitrogen output from excreta,

FI – feed intake,

36.5 – the nitrogen correction factor.

All procedures relating to the use of live animals were approved by the West Hungarian University Experimental Animal Committee.

The glycerol content was determined according to the ISO EN 14106 (2003) standard that employs liquid-liquid extraction to separate the free glycerol and the extract was analysed by the use of a 1,4-butanediol internal standard for free glycerol content. Methanol was determined by headspace gas chromatography according to a standard test method ISO EN 14110 (2003).

The gross energy (GE) of crude glycerol, feed and excreta was determined in an IKA C2000 Basic bomb calorimeter (Dynamic 25°). Benzoic acid was used as reference standard. All samples were analysed in duplicate. The chemical composition (dry matter, crude protein, ether extract, crude fibre, crude ash) of feed samples was characterized according to AOAC (1995). The chloride content of crude glycerol was determined according to the Hungarian Feed Codex (2004).

Statistical analysis was carried out by using GenStat 11® software (GenStat Procedure Library Release PL19.1, VSN International Ltd.). One-way ANOVA was performed to examine the effect of glycerol on egg production and egg weight. Differences were declared significant at $P < 0.05$. The AMEn values of glycerol fed to laying hens were estimated as a slope of the linear relationship between the AMEn intake from the diet (dependent variable) and glycerol content (0%, 5%, 7.5% and 10%) of the diet (independent variable).

Results

The AMEn values of the diets containing varying amounts of glycerol are shown in Table 3. We concluded that the calculated (11.46 MJ/kg) and experimentally de-

terminated AMEn values (11.82 MJ/kg) of the basal diet show good agreement. The relationship between the inclusion rate of crude glycerol and the AMEn value of the experimental diets for laying hens is shown in Figure 1. This relationship was used to determine the AMEn value of glycerol fed, which in our experiment was 15.30 MJ/kg for the product containing 86.8% glycerol. Converting this value to 100% pure glycerol, the AME_n value was obtained as 17.63 MJ/kg for laying hens. The gross energy (GE) content of pure glycerol which was reported by Doppenberg and van der Aar (2007) is 18.06 MJ/kg, so the AME_n value is 97.6% of the GE content in our experiment.

Table 3. Effect of glycerol supplement on the AMEn value of the diet

Item	Dietary glycerol content (%)			
	0	5	7.5	10
Energy intake (kJ/hen/day)	15073	15853	16243	16633
Energy excretion (kJ/hen/day)	2730	2742	2707	2896
AMEn value of the feed (MJ/kg)	11.82	12.65	13.01	13.34

Figure 1. AMEn of crude glycerol (86.8%) fed to laying hens

Data shows the combined regression AMEn content of diets over glycerol content of diets. Laying hens fed 0, 5, 7.5 or 10% crude glycerol. The slope of the regression line indicates that AMEn of crude glycerol is 15.303 MJ/kg

Table 4. Effect of dietary glycerol on the egg production, egg weight, egg mass and feed conversion

Item	Dietary glycerol content (%)			
	0	5	7.5	10
Egg production (%)	90.00±7.39	90.83±9.96 n.s.	90.76±5.17 n.s.	90.83±7.80 n.s.
Egg weight (g)	56.85±4.52	57.04±3.88 n.s.	58.04±3.49 n.s.	56.82±3.20 n.s.
Egg mass (g)	51.16±6.10	51.81±6.28 n.s.	52.68±4.13 n.s.	51.61±4.94 n.s.
Feed conversion (kg/kg egg mass)	1.95±0.09 a	2.03±0.15 a	2.04±0.10 a	2.13±0.09 b

n.s. – not significant compared to control group (0% dietary glycerol content).

a, b – means in the same row with unlike superscripts differ ($P < 0.05$).

Egg production ($90.6 \pm 7.6\%$), egg weight (57.2 ± 3.8 g) and egg mass (51.8 ± 5.4 g) were not significantly affected by the dietary treatments in the 10-day metabolism experiment (Table 4). Feed conversion was significantly reduced by a 10% glycerol supplementation.

Discussion

Few experimental results are available on the AME_n value of glycerol. Barteczko and Kamiński (1999), Dozier *et al.* (2008), and Schmidt and Zsédely (2012) produced data concerning the AME_n value of glycerol for broilers. Similarly, few data are available with regard to laying hens. Lammers *et al.* (2008) and Świątkiewicz and Koreleski (2009) conducted experiments to determine the AME_n value of glycerol for laying hens. Lammers *et al.* (2008) in metabolism experiments used 48 Hy-Line W-36 laying hens, fed 0%, 5%, 10% and 15% crude glycerol in their ration and found the AME_n value of 15.92 MJ/kg (3805 kcal/kg). According to the authors, this value does not differ significantly from the GE content of the 87% crude glycerol in the feed (15.17 MJ/kg, 3625 kcal/kg).

Świątkiewicz and Koreleski (2009) placed 20 Bovan Brown laying hens (5 per treatment) into individual cages and fed 0%, 2%, 4% and 6% crude glycerol in the daily ration to determine the AME_n value of crude glycerol (73.8% DM and 69.7% glycerol content). The established 16.6 MJ/kg AME_n value exceeds the one measured by Lammers *et al.* (2008), and by ourselves. However, our results and Lammers' results are close to each other. When converting the measured AME_n values to 100% glycerol content, the values differ only by 3.7%.

Bartelt and Schneider (2002) studied glycerol's ME content for laying hens. They found the ME values of pure glycerol of 17.59, 16.81 and 14.54 MJ/kg depending on the amount of glycerol fed in the ration (5%, 10% or 15%). The results show that the ME value decreased with increasing glycerol content of the diet. Bartelt and Schneider (2002) also observed this trend in experiments with broilers and pigs. When explaining this fact Doppenberg and van der Aar (2007) assume that the available glycerol kinase enzyme is not sufficient for the formation of glyceraldehyde-3-phosphate from the absorbed glycerol, an intermediate in the lipogenesis and gluconeogenesis pathways. Data also indicate that ME values are higher than AME_n values. This may be related to the fact that we also consider the energy of the protein when determining AME_n values.

The results of our experiment show that laying hens utilize feed glycerol as energy source more favourably than broilers do. In our previous experiment with broilers (Schmidt and Zsédely, 2012), the AME_n value of glycerol was calculated as 93.2% of the GE content, whereas in the present experiment with laying hens, the AME_n value was 97.6% of the GE content. Furthermore, results of Świątkiewicz and Koreleski (2009) demonstrated that laying hens utilize the energy content of glycerol well. Dozier *et al.* (2008) measured the AME_n value of glycerol for broilers to be 16.52 MJ/kg (converted to 100% glycerol), which is also below the value

(18.3 MJ/kg) measured by Lammers et al. (2008). The above mentioned AMEn content (15.30 MJ/kg) is 11%, 17% and 15% higher than that of corn grain, wheat and triticale respectively as reported by Jeroch et al. (1993), which indicates the potential to use glycerol as feed.

Dietary glycerol did not significantly affect egg production of hens nor egg weight (Table 4). This statement agrees with the experiment of Lammers et al. (2008) because they did not observe any significant differences in egg production and egg weight using 5%, 10% or 15% dietary glycerol. In addition, a similar result was reported by Świątkiewicz and Koreleski (2009) in the case of feeding 2%, 4% or 6% glycerol in the diet.

We concluded that crude glycerol's energy is efficiently used by laying hens, and more efficiently than by broilers. The established 15.30 MJ/kg AMEn value of crude glycerol when fed in the daily ration of laying hens means that laying hens utilized 97.6% of the glycerol's GE content.

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Współczynnik metaboliczności energii surowej gliceryny dla kur nieśnych

STRESZCZENIE

Eksperyment przeprowadzono na 64 kurach nioskach Tetra SL, przydzielonych do czterech grup, w celu określenia współczynnika metaboliczności energii (AME_n) surowej gliceryny paszowej (SM 92%; gliceryna 86,8%; metanol 0,04%; tłuszcz 0,49%; popiół 4,67%; energia brutto 15,59 MJ/kg). Wszystkie grupy otrzymywały 100 g dawki podstawowej dziennie ($AMEn$ 11,46 MJ/kg; białko 16,97%; Ca 3,49%; P-niefitynowy 0,56%; lizyna 0,82%; metionina 0,40%; cysteina 0,29%; treonina 0,60%; tryptofan 0,19%), do której w grupach eksperymentalnych dodawano glicerynę w ilości 5, 7,5 i 10%. Nioski umieszczono w klatkach metabolicznych (4 ptaki/grupę w czterech powtórzeniach). Po dziesięciodniowym okresie adaptacji przez dziesięć dni zbierano i indywidualnie ważono odchody niosek. Stwierdzono, że współczynnik $AMEn$ surowej gliceryny (86%) dla kur nieśnych wynosi 15,30 MJ/kg.

W czasie doświadczenia czynniki żywieniowe nie miały wpływu na nieśność (90,0–90,8%), wagę jaj (56,8–58,0) ani masę jaj (51,2–52,7 g). Wykorzystanie paszy uległo znacznemu pogorszeniu przy dodatku 10% gliceryny.

Ustalono, że nioski dobrze wykorzystują energię gliceryny i czynią to lepiej od brojlerów. Stwierdzona wartość pokarmowa gliceryny podawanej nioskom w dziennej dawce (15,3 MJ/kg) oznacza, że nioski wykorzystywały 97,6% energii gliceryny brutto.