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Determination of Apparent Metabolizable Energy of Crude Glycerin in Broilers Chickens

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ABSTRACT

The objective of the experiment was to determine the Apparent Metabolizable Energy corrected for nitrogen (AMEn) of a glycerin product derived from biodiesel production for broilers. A number of 35 male Ross 308 broilers, with 35 days of age was fed *ad libitum* diets containing 0, 5, 10, 15, or 20% glycerin. AMEn was determined using total excreta collection, with four days of adaptation and three days of collection. Increasing glycerin intakes resulted in increased water excretion. Glycerin AMEn varied as a function of dietary inclusion levels, and a cubic equation was identified by regression analysis. Maximum AMEn level- 4890 kcal/kg DM- was obtained with 15% glycerin inclusion. However, this level caused excessive water excretion, making its use unpractical.

INTRODUCTION

Crude glycerin, a byproduct of biodiesel production, which consists of glycerol, water, fat, and minerals, has been studied as a potential energy source for poultry and pigs. The gross energy value of this byproduct, depending on its percentage of residual oil, is approximately 3600 kcal/kg (Lammers *et al*., 2008; Cerrate *et al*., 2006).

In order to use glycerol as an energy source for non-ruminant animals, it is essential to understand how this substance is metabolized. Glycerol utilization depends on the enzymes glycerol kinase and dehydroxiacetone phosphate dehydrogenase (Lin *et al*., 1976). When the capacity of these two enzymes is exceeded, glycerol is no longer used, and consequently, it is excreted from the body. It was observed in fasting animals that fatty acid and glycerol blood concentrations are similar, and therefore, it is assumed that both have similar distribution (Bergman, 1968). The dietary addition of glycerol favors the synthesis of glucose (Emmanuel & Robblee, 1983), pyruvate, and other products of the Krebs' cycle, such as malate and oxalacetate (Rosebrough *et al*., 1980). In broilers, dietary glycerol may induce anatomical, physiological, and biochemical changes, particularly in the liver and kidneys (Cryer & Bartley, 1973).

A digestibility of 95% has been attributed to glycerol in many studies with broilers. However, Simon *et al*. (1996) determined 75% digestibility for that compound. Cerrate *et al*. (2006) carried out two experiments where glycerol was used as energy source for broilers. Glycerol levels of 0, 5, and 10% were used in the first trial, and 0, 2.5, and 5% in the second. An AME content of 2527 kcal/kg was assumed for glycerol. In the first experiment, broilers fed the diet containing 5% glycerol presented similar weight gain and feed conversion ratio as those fed the control diet (no glycerol addition), whereas those fed 10% glycerol reduced their feed intake and had significantly lower weight gain. In the second experiment, the dietary inclusion of glycerol did not influence feed intake, weight gain, or feed conversion ratio, but breast muscle yield and carcass weight were significantly higher in the broilers fed diets containing 2.5 and 5% glycerol as compared to the control birds. It was also observed that water retention capacity during carcass chilling was not affected by glycerol, which had already been demonstrated in pigs (Mourot *et al*. 1994). The authors also verified the litter of broilers fed 10% glycerol was wetter than that of the control birds, indicating the possible presence of diarrhea.

This study aimed at determining the energy value of a crude glycerin product and the effects of its dietary inclusion on broilers metabolism.

MATERIAL AND METHODS

In this experiment, 35 male Ross® 308 broilers, with 35 days of age and average initial weight of 1938±116 g were used. Birds were housed in individual metabolic cages, and received artificial lighting for 24 h.

Broilers were individually weight and designated to the experimental treatments according to weight to have even weight distribution among treatments. One experimental unit consisted of an individual bird. A completely randomized experimental design with five treatments was applied. Treatments were defined as combinations of the basal diet with different crude glycerin inclusion levels (Table 1). The glycerin product used was a byproduct of biodiesel processing from soybean oil, containing 4198 kcal of GE/kg DM, 92% DM, <0,05% of methanol, 2,05% of sodium and 0,10% of potassium.

Table 1 - Diet composition of the different treatments according to the glycerin percentage of inclusion.

Treatment	Basal diet (%)	Glicerin (%)
1	100	0
2	95	5
3	90	10
4	85	15
5	80	20

The basal diet was based on corn and soybean meal, and contained 2980 kcal AME/kg, 21.2% crude protein, 1.03% digestible lysine, 0.86% digestible methionine+cystine, 0,99% calcium and 0,43% phosphorus.

AMEn was evaluated by the balance procedure in which food intake over a period, is related to the excreta output over the same period, also called total collection excreta, with the basal diet replaced by glycerin (Sibbald & Slinger, 1963, Matterson *et al*., 1965). The experimental period included four days of adaptation and three days of total excreta collection. Birds were submitted to 6-h fasting before the first collection and on the last day of excreta collection. Excreta were collected twice daily and stored at -16 °C. Birds were weighed at housing, in the beginning of the collection period, and at the end of the experiment. On the last day of the experimental period, the excreta produced during the interval between the last meal and the end of the fasting period were equaly considered to analyses.

Experimental feeds and collected excreta were analyzed for gross energy, crude protein, and humidity (AOAC, 1995). Feed intake, weight gain, feed conversion ratio, and water excretion were determined for each individual bird. Daily water excretion was calculated according to the formula: excreted water =(excreta quantity * excreta DM %) -100).

Data were submitted to ANOVA using the GLM module of SAS software package (SAS, 1999). Means were compared by the LSmeans test. Analysis of regression was performed using the REG procedure of SAS.

RESULTS AND DISCUSSION

No differences in performance were detected, although the experiment was not designed to determine this type of response due to the low number of birds and the short experimental period. However, water excretion significantly increased as glycerin intake increased (p≤0.001); the regression equation produced an ascending quadratic curve (Figure 1).

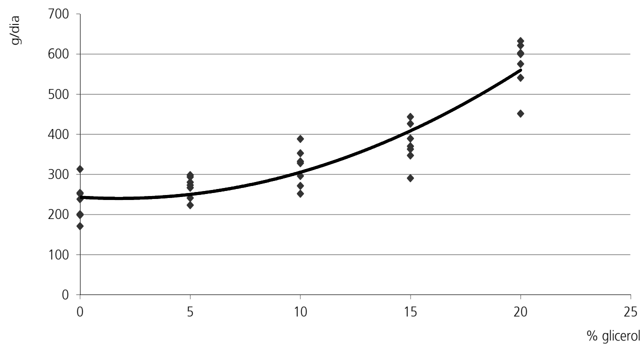

Figure 1 - Daily excretion of water in the excreta as a function of dietary glycerol percentage**.
Means followed by different letter are significantly different by the t-test ($p \leq 0.001$). $Y = 204.31 + 11.81X + 0.18 X^2$ $r^2 = 0.76$ ($p \leq 0.001$). Y = water intake in mL; X = glycerin inclusion level

Due to the increasing water excretion, three birds per treatment were sacrificed at the end of the experimental period to investigate the origin of the diarrhea. The post-mortem examination showed that the ileal content of the animals fed 20% glycerin presented normal texture. This fact indicates that diarrhea was due to excessive urine production, and not to water retention in the intestine (Figure 2).

In 1951, Doerschuk (1951) determined in rats that glycerol can be metabolized by the animal body. Glycerol metabolization depends on several enzymes, out of which glycerol kinase limits its use (Vernon & Walker, 1970). When increasing glycerol levels are fed, there seems to be a metabolization limit, above which glycerol blood concentration increases, requiring its excretion in the urine. As glycerol is water soluble, it increases water excretion.

The high level of sodium observed in the used glycerin (2,05%) also could help the water intake and urine production increase. When 20% of glycerin was supplemented a 0,6% of sodium also was incorporated to the diet. Murakami *et al*. (2001) and Borges *et al*. (1998) observed a linearity between sodium increment and litter humidity. Nevertheless, independently of sodium effect, Gianfelici (2008) supplementing pure glycerol (p.a) in another experiment, also observed the same phenomenon, i.e, the increase in urine production above 10% of glycerol in the diet .

Glycerin AMEn varied as a function of its inclusion level in the feed ($p \leq 0{,}001$) (Figure 3). A cubic response was observed, with the highest AMEn value (4890 kcal/kg DM) obtained with 15% glycerin, after which AMEn decreased. Glycerol average AMEn was calculated as 3561 kcal/kg on dry matter basis or 3276 kcal/kg "as is". However, it is important to consider the variation of this response as a function of the different levels of glycerin intake, making the average value of little importance. A minimum AMEn of 1527 kcal/kg was obtained with the inclusion of 5% glycerol. This low value may be attributed to variability due to the small quantity of glycerol intake as compared to total feed intake. Therefore, the low energy value obtained with that inclusion level may be more associated to methodological errors than to metabolic phenomena.

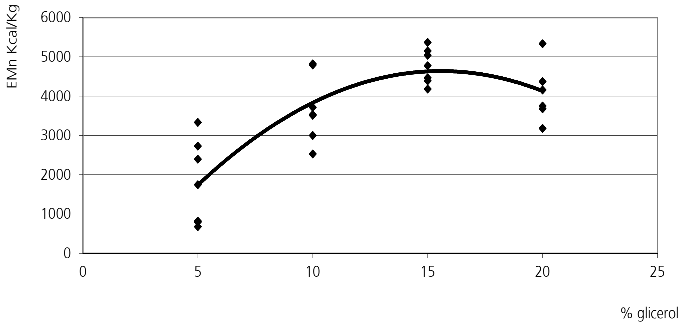

Figure 2 - Glycerin Apparent Metabolizable Energy corrected for nitrogen (kcal/kg DM) for the different dietary glycerin inclusion levels.
$Y = 567.37 – 12.47X + 51.26X^2 – 2.08X3$; $r^2 = 0.77$ ($p \leq 0.001$). Y=glycerin AMEn; X= glycerin inclusion level.

The average AMEn values obtained in the present study are consistent with those of Dozier *et al*. (2008), who found 3331 kcal AMEn/ kg "as is" in 38- to 45-day-old broilers when 0, 3, 6, or 9% glycerol was added to the feed, and 3621 kcal AMEn/kg "as is" in 0- to 14-day-old broilers fed 0, 3, and 6% glycerol. In layers, Lammers *et al*. (2008) determined an average AMEn of 3800 kcal/kg when 0, 5, 10, and 15% glycerol was fed. In agreement with the results of the present experiment, Bartlet & Schenieder (2002) showed that pure glycerol ME values for broilers, layers, and pigs varied according to its dietary inclusion levels. Those authors suggest that AME is reduced when glycerol is no longer reabsorbed by the kidneys, and thus the

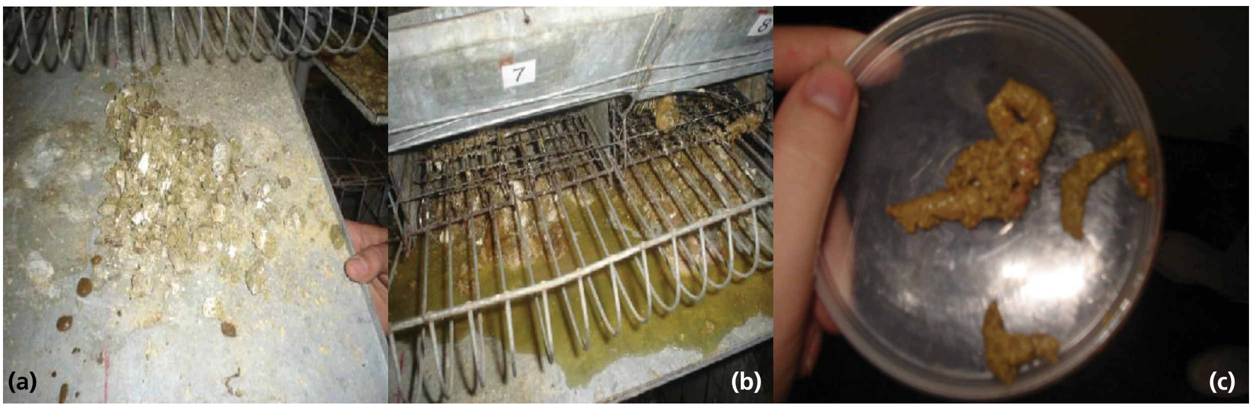

Figure 3 - Excreta of birds fed 0 (a) and 20% (b) glycerol, and ileal content of a bird fed 20% glycerol (c).

excess is excrete in the urine, as observed in the present experiment.

CONCLUSIONS

Glycerin AMEn depends on the characteristics of the raw material and on glycerin inclusion level in the diet. It is difficult to determine an average value. It is possible to use glycerin as an energy source in broiler feeding. However, its inclusion levels cannot be excessive as they cause wet excreta problems.

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