Crude Glycerin and Energy Density of Diets for Growing, Pre-Lay and Pre-Peak Backcob Brown Egg-Laying Hens

ABSTRACT

A total of 320 13-weeks-old pullets were weighed and randomly allocated to treatments comprised by four levels of crude glycerin (0, 3, 6 or 9%) and two levels of ME (metabolizable energy, difference of 100 kcal/kg) in the diets growing (14 to 17 weeks of age), pre-lay and pre-peak (low: 2750, 2800 and 2750 kcal/kg and high: 2850, 2900 and 2850 kcal/kg, respectively). During the study, body weight was registered until 30 weeks of age, feed intake, egg weight and egg-production for each repetition and for individual hens were measured every week. The information was analyzed through a completely randomized design with a 4x2 factorial arrangement. During the growing phase, hens that received low energy diets consumed more feed ($p<0.05$), gained less body weight ($p<0.05$) and recorded lower feed conversion ($p<0.05$). Besides, a positive linear effect ($p<0.05$) was observed when including glycerin on feed conversion and weight gain. During the initial egg-laying phase, hens fed low-energy diets consumed more feed ($p<0.05$) and laid lighter eggs ($p<0.05$). Furthermore, a positive linear effect ($p<0.05$) of including crude glycerin on egg yield and feed intake was observed. After the egg-laying peak, the hens of high energy groups consumed 1.6 g/d less feed ($p<0.05$) and lay 0.9% less eggs, also, a quadratic effect of inclusion of glycerin on laying-eggs was observed. In conclusion, the use of high energy diets decrease feed intake, increase egg weight until peak, but decrease the percentage of eggs post-peak; glycerin used in diets increases feed intake and improves egg-laying rate in different ways during the laying phase.

INTRODUCTION

Egg production in recent years has experienced a progressively rapid growth (Alexandratos & Bruinsma, 2012) and a significant increase in consumption from 8.1 kg in the year 2000 to 8.9 kg in the year 2009 (FAOSTAT), up to an estimate of 8.9 and 13.8 kg in developing and industrialized countries by the year 2030, respectively (Bruisma, 2003). The previous situation means that bird feeding systems must be continuously updated since the profitability of the poultry sector is correlated with the price of the diet (Altahat et al., 2012). Thus, the design of feeding strategies that reduce production costs without altering the productive performance of current lines is a priority for the poultry sector.

In egg-laying hens, the period around the beginning of the egg-laying phase constitutes a critical moment for the subsequent productive performance of the hen (Sujatha & Rajini, 2015). Two to three weeks before laying the first egg, there is a significant increase in the weight gain of the egg-laying hens that seek to accumulate body reserves to support the production and size of the eggs during the egg-laying cycle (Summers, 1993). Besides, towards the end of the growth period, there
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Is a rapid development of the ovary and the oviduct and an increase in the size of the liver (Sujatha et al., 2014). From the nutritional point of view, adequate contribution of energy and protein in the diet should be considered at this stage, as this will allow the correct accumulation of body reserves (Cankaya et al., 2008) and appetite stimulation in the animal, to increase its feed intake capacity (Leeson & Summers, 2000; Lazaro & Mateos, 2008).

The energy concentration of diets affects the productive performance of both growing chicks and egg-laying hens (Pérez-Bonilla et al., 2012; Saldaña et al., 2015). In general, it is widely recognized that energy is one of the main nutritional factors that control feed intake and contributes significantly to the cost of the diet (Classen, 2017). Studies suggest that birds consume to meet energy requirements (Fisher & Wilson, 1974). The previous approach has been questioned, since certain strains of egg-laying hens fail to adjust their consumption, resulting in responses that are not uniform or predictable (Jalal et al., 2007; Classen, 2017), resulting in the limited ability to increase consumption when low energy diets are used (van Krimpen et al., 2007), a situation that could be revealed and affect economic return of a poultry company. This would occur if – due to the cost of raw materials – the production of low energy diets is implemented; thus, birds must sacrifice egg-laying or the size of the eggs due to the limited energy intake (de Persio et al., 2015). On the other hand, it is also necessary to economically assess the compensation in feed efficiency achieved by using high energy diets (Leeson & Summers, 2009).

Glycerin production from the biodiesel industry reached more than 2 million tons in 2011 (Ciriminna et al., 2014), which has caused the price of this co-product to decrease in the market (Yang et al., 2012) giving the possibility for its use as an alternative resource in animal feeding systems (Makkar, 2012; Silva et al., 2014; Beserra et al., 2016; Vongsamphanh et al., 2017). Glycerol, the main component of glycerin, is a molecule that has a rapid intestinal absorption (Kato et al., 2004), and due to the low caloric increase it has a high net or effective energy value (AVINESP, 2014). In egg-laying hens this co-product has been used at levels of up to 15% for short periods (Lammers et al., 2008; Nemeth et al., 2013) or at 7.5% for several weeks in the egg-laying cycle (Boso et al., 2013; Cufadar et al., 2016).

Glycerin consumption during the transition stage (period around the beginning of the egg-laying phase, in this study from 14 weeks to 50% of laying-eggs) of laying-hens that receive low energy diets might stimulate feed consumption and improve growth during the last phase of the growth period and thus, improve production during egg-laying. This study aims to evaluate the effect of the inclusion of crude glycerin in diets with different metabolizable energy values during the growing (weeks 14 to 16), pre-lay (week 17 until reaching 5% egg-laying) and pre-peak phases (from 5% to 50% egg-laying) on the productive performance of Backcob Brown egg-laying hens until 40 weeks of age.

MATERIAL AND METHODS

This study was carried out in the Poultry Unit of the CI Tibaitatá of the Colombian Agricultural Research Corporation, Agrosavia, located at 2516 masl (4.685222 -74.204722), with average temperature of 14 °C and a relative humidity of 76%.

Experimental unit

For this study, 320 chicks of the Backcob Brown line of 13 weeks of age were selected from a commercial lot and distributed according to their weight to comprise homogeneous experimental units. The birds were placed individually in two-level battery cages, each equipped with an automatic nipple drinker and a lateral channel feeder. Five chicks that shared the same feeder and were separated from the others were considered an experimental unit (eight per treatment).

Experimental diets

Animal management protocols were endorsed by the Bioethics Committee of the Faculty of Veterinary Medicine and Zootechnics, National University of Colombia, Bogotá (act 05). The glycerin used in this study was obtained from an oil-palm biodiesel production plant. The composition of the crude glycerin was as follows: 3547 kcal/kg of GE (Adiabatic Parr calorimetric pump), 8.21% humidity (Karl Fischer volumetric titrator), 84.2% glycerol (gas chromatograph PERKIN ELMER), 4.52% ash, 1.73% sodium (PERKIN ELMER 2380 atomic absorption spectrophotometer) and 19.7 ppm of methanol (gas chromatograph PERKIN ELMER). For the formulation of diets a value of 3340 kcal of ME/kg was utilized for crude glycerin, which was obtained from its metabolism (86%) determinate in a previous balance bioassay carried out under similar conditions (Ariza et al., 2012). During each feeding phase, four levels of crude glycerin (0, 3, 6 and 9%)
and two levels of ME (low: with 2750 kcal of ME/kg in growing and pre-peak, and 2800 kcal in pre-laying; and high: with 2850 kcal of ME/kg in growing and pre-peak, and 2900 kcal in pre-laying) were used in the diets. A phased feeding system was employed, where the growing phase was established from week 14 to week 16; the pre-laying phase from week 17 up to 5% of egg-laying; and the pre-peak phase from 5% to 50% egg-laying, per repetition.

Ingredients of diets was analyzed in analytical chemistry laboratory Agrosavia for gross energy (Adiabatic Parr calorimetric pump), ethereal extract (Soxhlet methodology), nitrogen (Kjeldahl method), ash (calcination total), crude fiber (acid and alkaline hydrolysis). ME of each ingredient was calculated according to Rostagno et al., (2011). Details on the ingredients and the calculated nutritional profile of the different diets used in the study are presented in Tables 1, 2, and 3. Once each experimental repetition reached 50% of egg-laying, they began to receive feed for their production peak (17.5% of CP and 3.8% of Ca) without glycerin, until 40 weeks of age.

**Table 1** – Ingredients and nutritional composition of the experimental diets used from 14 to 17 week (g/kg on a fresh base).

| Ingredients                  | 2750 kcal of ME/kg | 2750 kcal of ME/kg | 2850 kcal of ME/kg | 2850 kcal of ME/kg |
|------------------------------|--------------------|--------------------|--------------------|--------------------|
| Corn                         | 483                | 442                | 408                | 370                | 370                |
| Crude glycerin               | 0                  | 30                 | 60                 | 90                 | 90                 |
| Wheat middlings              | 170                | 170                | 170                | 170                | 170                |
| Rice polishing               | 120                | 120                | 120                | 120                | 120                |
| Soybeans whole extruded      | 0                  | 0                  | 0                  | 60                 | 60                 |
| Soybean meal 49              | 186                | 192                | 198                | 204                | 204                |
| Fish meal                    | 0                  | 0                  | 0                  | 1                  | 1                  |
| Soybean oil                  | 3                  | 6                  | 6                  | 8                  | 15                 |
| Common salt                  | 3                  | 3                  | 3                  | 3                  | 3                  |
| Sodium bicarbonate           | 3                  | 5                  | 3                  | 4                  | 3                  |
| Calcium carbonate            | 10                 | 9                  | 9                  | 9                  | 9                  |
| Tricalcium phosphate         | 10                 | 11                 | 11                 | 12                 | 10                 |
| DL-Methionine                | 2                  | 2                  | 2                  | 2                  | 2                  |
| Choline chloride 60%         | 7                  | 7                  | 7                  | 7                  | 7                  |
| Premix vit-min               | 3                  | 3                  | 3                  | 3                  | 3                  |

**Calculated analysis**

| ME (kcal/kg) | 2750 | 2750 | 2750 | 2750 | 2850 | 2850 | 2850 | 2850 |
|--------------|------|------|------|------|------|------|------|------|
| Crude protein (%) | 16.5 | 16.5 | 16.5 | 16.5 | 16.6 | 16.6 | 16.7 | 16.5 |
| EE (%) | 3.4 | 3.4 | 3.4 | 3.5 | 5.4 | 5.4 | 5.3 | 5.3 |
| CF (%) | 4.8 | 4.8 | 4.8 | 4.7 | 4.9 | 4.9 | 4.8 | 4.8 |
| Calcium (%) | 0.90 | 0.90 | 0.90 | 0.90 | 0.88 | 0.89 | 0.90 | 0.90 |
| Available P (%) | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| Digestible P (%) | 0.43 | 0.43 | 0.44 | 0.44 | 0.42 | 0.42 | 0.43 | 0.43 |
| DEB (mEq/kg) | 270 | 270 | 250 | 250 | 250 | 250 | 250 | 250 |
| Digestible lysine (%) | 0.72 | 0.73 | 0.74 | 0.74 | 0.73 | 0.76 | 0.74 | 0.75 |
| Digestible Met+Cys (%) | 0.59 | 0.59 | 0.60 | 0.60 | 0.59 | 0.63 | 0.60 | 0.63 |
| Diet price (USD/kg) | 0.30 | 0.30 | 0.29 | 0.29 | 0.31 | 0.31 | 0.30 | 0.30 |

1 Premix of vitamins and minerals (contribution per kilogram of diet): vitamin A, 7500 IU; vitamin D3, 1,900 IU; vitamin E, 28 IU; vitamin K, 1.5 mg; thiamine, 2.0 mg; riboflavin, 5.0 mg; niacin, 30 mg; pantothenic acid, 10 mg; pyridoxine, 2.8 mg; biotin, 0.01 mg; folic acid, 0.7 mg; vitamin B12, 0.01 mg; choline, 10 mg; Zn, 65 mg; Mn, 70 mg; Fe, 50 mg; Cu, 10 mg; I, 1.0 mg; and Se, 0.3 mg. 2 Ethereal extract. 3 Crude fiber. 4 Dietary electrolyte balance. 5 Digestible Methionine+Cysteine (%).

**Experimental protocol**

Hens were weighed weekly until they reached an age of 24 weeks and then biweekly until they were 30 weeks of age, to establish weight gain. Besides, feed residuals were recorded weekly to calculate the average feed intake and feed conversion. During the egg-laying phase, the eggs of each repetition were collected daily and timely (11:00 hours), and at the end of the week, they were totaled, weighed and classified to calculate the feed conversion per dozen eggs and egg-laying percentage. Furthermore, the individual record of each hen was kept until reaching 40 weeks of age, in which at the beginning of the egg-laying phase, the number of consecutive eggs laid, as well as the resting period between each brood, were counted. Finally, at 25 and 35 weeks of age, two AA-sized eggs were selected for each repetition to assess shell thickness (Ames brand micrometer, model 25-5),...
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yolk color (Roche scale) and the height of the albumin (Ames brand micrometer, model S-8400) in animal nutrition laboratory. Moreover, Haugh units (100*log (albumin height – 1.7 * (egg weight 0.37 ) + 7.6)) were calculated as an indicator of egg quality.

With the weekly results of feed conversion per dozen eggs for each repetition, and the price and the time the diets were used during the growing, pre-laying and pre-peak phases, the feeding cost from week 20 to week 28 was calculated. Subsequently, with the price of the peak diet, the feeding cost from week 29 to week 40 was calculated. On the other hand, with the weekly distribution of eggs in the different categories, the calculation of the income per dozen eggs was made using the average sales prices in Colombia. With the above information, gross profitability was calculated as the difference between the income per dozen eggs and the cost of feed per dozen eggs.

Statistical analysis

Dates were grouped in the four following ranges: from 14 to 16 weeks, from 17 weeks to the beginning of laying and pre-peak and post-peak phase. Data were evaluated through a completely randomized design with a 4x2 factorial arrangement corresponding to four levels of glycerin (0, 3, 6 and 9%) and two energy densities (low and high), using as a covariate in the design, the initial weight of each repetition. A test of adjusted means was used to assess the effect of the interaction, setting the glycerin level factor or energy density of the diet for the corresponding comparison. When the main effects were significant, Tukey’s means test was performed comparing glycerin levels or energy density of the diets. Additionally, the type of effect (linear, quadratic, or cubic) of the glycerin inclusion in the diet was evaluated through contrasts. The information was processed using the SAS® statistical package version 9.2 (2008), with a mixed procedure.

Table 2 – Ingredients and nutritional composition of the experimental diets for the pre-laying phase (g/kg on a fresh base).

| Ingredients                  | 2800 kcal of ME/kg | 2900 kcal of ME/kg |
|------------------------------|--------------------|--------------------|
|                              | 0%     | 3%     | 6%     | 9%     | 0%     | 3%     | 6%     | 9%     |
| Corn                         | 464    | 440    | 403    | 365    | 440    | 438    | 405    |        |
| Crude Glycerin               | 0      | 30     | 60     | 90     | 0      | 30     | 60     | 90     |
| Wheat middling              | 190    | 190    | 190    | 190    | 169    | 139    | 135    |        |
| Rice polishing              | 25     | 0      | 0      | 0      | 5      | 0      | 0      |        |
| Soybeans whole extruded     | 100    | 100    | 100    | 100    | 130    | 125    | 125    |        |
| Soybean meal 49             | 123    | 140    | 152    | 176    | 118    | 135    | 140    |        |
| Fish meal                   | 10     | 10     | 10     | 10     | 10     | 10     | 10     |        |
| Soybean oil                 | 12     | 15     | 16     | 18     | 22     | 22     | 17     | 18     |
| Common salt                 | 3      | 3      | 3      | 3      | 3      | 3      | 3      |        |
| Sodium bicarbonate          | 4      | 3      | 3      | 3      | 4      | 6      | 4      | 4      |
| Calcium carbonate           | 58     | 51     | 50     | 51     | 50     | 51     | 51     |        |
| Tricalcium phosphate        | 10     | 11     | 12     | 12     | 10     | 11     | 11     |        |
| DL-Methionine               | 3      | 3      | 3      | 3      | 3      | 3      | 3      |        |
| L-Lysine HCl                | 1      | 0      | 0      | 0      | 0      | 0      | 0      |        |
| L-Threonine                 | 1      | 0      | 1      | 1      | 0      | 1      | 1      |        |
| Choline chloride 60%        | 1      | 1      | 1      | 1      | 1      | 1      | 1      |        |
| Premix vit-min              | 3      | 3      | 3      | 3      | 3      | 3      | 3      |        |

Calculated analysis

| ME (kcal/kg) | 2800 | 2800 | 2800 | 2800 | 2900 | 2900 | 2900 | 2900 |
|--------------|------|------|------|------|------|------|------|------|
| Crude protein (%) | 16.9 | 17.1 | 17.1 | 17.1 | 17.1 | 17.1 | 17.1 | 17.0 |
| EE (%)<sup>2</sup> | 4.8  | 4.6  | 4.7  | 4.8  | 6.0  | 6.1  | 5.4  | 5.4  |
| CF (%)<sup>3</sup> | 4.4  | 4.2  | 4.2  | 4.2  | 4.2  | 4.2  | 4.1  | 3.8  |
| Calcium (%)    | 2.5  | 2.5  | 2.5  | 2.5  | 2.5  | 2.5  | 2.5  | 2.5  |
| Available P (%) | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Digestible P (%) | 0.39 | 0.40 | 0.41 | 0.41 | 0.39 | 0.39 | 0.38 | 0.38 |
| DEB (mEq/kg)<sup>4</sup> | 252  | 250  | 250  | 250  | 260  | 260  | 251  | 250  |
| Digestible lysine (%)<sup>5</sup> | 0.80 | 0.79 | 0.79 | 0.79 | 0.77 | 0.77 | 0.80 | 0.82 |
| Digestible Met+Cys (%)<sup>5</sup> | 0.73 | 0.71 | 0.71 | 0.72 | 0.70 | 0.72 | 0.73 | 0.75 |
| Diet price (USD/kg) | 0.31 | 0.30 | 0.30 | 0.30 | 0.31 | 0.31 | 0.31 | 0.31 |

<sup>1</sup>Premix of vitamins and minerals (contribution per kilogram of diet): vitamin A, 7500 IU; vitamin D3, 1900 IU; vitamin E, 28 IU; vitamin K3, 1.5 mg; thiamine, 2.0 mg; riboflavin, 5.0 mg; niacin, 30 mg; pantothenic acid, 10 mg; pyridoxine, 2.8 mg; biotin, 0.01 mg; folic acid, 0.7 mg; vitamin B12, 0.01 mg; choline, 10 mg; Zn, 65 mg; Mn, 70 mg; Fe, 50 mg; Cu, 10 mg; I, 1.0 mg; Se, 0.3 mg.<sup>2</sup>Ethernel extract.<sup>3</sup>Crude fiber.<sup>4</sup>Dietary electrolyte balance.<sup>5</sup>Digestible Methionine+Cysteine (%).
To establish the effect of the experimental treatments on the parameters of the egg-laying curve, the data were adjusted to the McMillan model (1981), an equation that registered the best fit, given the evaluation time (40 weeks), and whose parameters explain the biological process (López, 2008). The equation used was the following:

\[ y_t = a \left( e^{-kt1} - e^{-kt2} \right) \]

Where \( y_t \) is egg-laying at week \( t \); \( a \) is the asymptotic egg-laying value; \( k1 \) is the rate of decrease in egg production after the egg-laying peak; \( k2 \) is the instant rate of increase in egg production.

The data was processed using the NLIN procedure of the SAS statistical package. The test described by Motulsky & Christopoulos (2003) was used to evaluate the existence of different egg-laying curves based on experimental treatments, using the error information of the reduced model (regardless of clustering) and the complete model (with different parameters for each group).

### RESULTS AND DISCUSSION

In all the variables evaluated, no interaction was observed; therefore, the results are presented and discussed with the main effects. During the growing phase, i.e., from week 14 to 16, a significant effect of the energy density of the diet on the response of the hens was observed (table 4). Birds that received low energy diets consumed more \((p<0.05)\) feed (+1.6 g/d) but recorded a lower \((p<0.05)\) weight gain (-0.4 g/d) and higher \((p<0.05)\) feed conversion (+0.70 g/g). On the other hand, a linear effect \((p<0.05)\) of including crude glycerin in the diets was observed, where the higher the inclusion of this co-product, the higher the weight gain and the lower the feed conversion.

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The productivity increase (weight gain and feed efficiency), observed during the growing phase (14 to 16 weeks of age) in pullets that received high energy diets can be associated with the higher inclusion of soybean oil (1.5 vs. 0.6% for high and low, respectively) and higher concentration of ethereal extract in the diets (5.4 vs. 3.4% for high and low, respectively). In this sense, birds fed high-fat diets have a higher feeding efficiency since they have a low caloric increase (Baiao & Lara, 2005) and make the bolus retention rate higher, allowing a higher enzyme action time (Mateos & Sell, 1981). On the other hand, Saldaña et al. (2015) report that the use of diets with different concentrations of metabolizable energy during the growth period did not affect the weight at 17 weeks of age, similar to what was observed in this study, since the birds managed to compensate the energy use through the increase in feed consumption. The previous results support the theory that birds, within a certain limit of energy concentration and/or diet presentation form, consume feed until they meet the energy requirement of their genetic potential (Leeson et al., 1996; Veldkamp et al., 2005).

### Table 4 – Performance of Backcob Brown hens from 14 weeks to laying beginning fed low or high energy diets and crude glycerin.

| Treatment | 14 to 16 weeks | 17 weeks until beginning laying | 19 weeks (g) |
|-----------|----------------|--------------------------------|--------------|
|            | Weight gain (g/d) | Feed intake (g/d) | Feed conversion (g/g) | Weight gain (g/d) | Feed intake (g/d) | Feed conversion (g/g) | Weight at |
| ME        |                |                  |                        |                |                  |                        | week 19 (g) |
| High      | 7.79 a         | 63.2 b           | 8.23 a                  | 13.4           | 81.9 b           | 6.11                    | 1.558        |
| Low       | 7.38 b         | 64.8 a           | 8.95 b                  | 13.8           | 84.5 a           | 6.14                    | 1.550        |
| MSE1      | 0.131          | 0.344            | 0.163                   | 0.231          | 0.072            | 0.062                   | 5.10         |
| Glycerin  |                |                  |                        |                |                  |                        |             |
| 0         | 7.34           | 63.6             | 8.82                    | 13.2           | 82.3             | 6.26                    | 1.536        |
| 3         | 7.46           | 63.7             | 8.92                    | 13.8           | 82.7             | 6.01                    | 1.556        |
| 6         | 7.66           | 64.2             | 8.46                    | 13.6           | 83.7             | 6.18                    | 1.557        |
| 9         | 7.89           | 64.6             | 8.21                    | 13.9           | 83.9             | 6.06                    | 1.567        |
| MSE2      | 0.180          | 0.486            | 0.231                   | 0.327          | 1.233            | 0.088                   | 7.22         |

Effects3

| ME        | *              | **             | *              | NS            | NS            | NS            | NS            |
| Glycerin  | NS             | NS             | NS             | NS            | NS            | NS            | +             |
| Glycerin contrast4 | L | L | L | NS | NS | NS | L |
| Initial weight | **3 | *4 | NS | **5 | NS | **6 |

1 mean standard error for energy, n= 32.
2 mean standard error for glycerin, n= 16.
3 +:p<0.1, *: p<0.05, **: p<0.001, NS: not significant.
4 L: linear.
5 16.7 (1.46) – 0.0083 (0.0013)* Initial weight, R²: 0.388.
6 56.2 (3.88) + 0.0070 (0.0035)* Initial weight, R²: 0.06.
7 -5.15 (1.90) – 0.0124 (0.0017)* Initial weight, R²: 0.458.
8 57.1 (9.21) + 0.023 (0.0083)* Initial weight, R²: 0.115.
9 613.2 (55.5) + 0.848 (0.049)* Initial weight, R²: 0.823.

The use of glycerin in growing and pre-laying phases of hens has not been reported in the literature; however, the increase in feed intake based on the inclusion of glycerin in the diets was described by Guerra et al. (2011) in chickens during growing phase, a response that has been associated with the increase in diet palatability (Min et al., 2010) or the improvement in feed texture (Loreska et al., 2017). On the other hand, Sehu et al. (2012) report a positive effect of the inclusion of glycerin on weight gain during the broiler phase of chicks. The increase in weight gain related to the consumption of glycerin can be associated with the increase in the net energy supply of glycerin compared to that of corn (the effective energy/metabolizable energy ratio for glycerin is 1, and 0.87 for corn) (AVINESP, 2014).

Birds fed with low metabolizable energy diets registered, from 20 to 28 weeks of age, 2.4 g/d more (p<0.05) of feed intake, laid eggs weighing 0.8 g less (p<0.05) and recorded 0.03 kg per dozen eggs (p=0.072) of feed conversion, compared to those who received high energy diets (table 5). Also, a linear trend of the inclusion of crude glycerin in the diets on the weight gain of birds during that same period was observed. In that sense, with higher inclusion of glycerin, there was less weight
gain; however, for the egg-laying percentage and feed consumption, the linear trend was directly proportional to the inclusion of glycerin in the diet. Besides, birds fed diets containing 3% glycerin laid more eggs, and these were heavier \( (p<0.05) \) compared to those of egg-laying hens that received diets with 6% glycerin. Additionally, the weight at the beginning of the study, i.e., in birds of 13 weeks of age, affected \( (p<0.05) \) the performance variables up to the egg-laying peak. 

Van Krimpen et al. (2007) observed that birds that consumed diets diluted by 10% from week 18 to 26, compensated by consuming 10% more feed, which resulted in similar nutrient intake, and therefore, the performance was not affected; these results are similar to the ones observed in this study. Further, when dePersio et al. (2014) evaluated the dilution of the diet of up to 15% in hens of the Hy-Line W-36 variety, they found that the birds compensated for feed consumption during the first evaluation period (until reaching 26 weeks of age). However, later, with a continuous supply of the diluted diets, the birds did not increase the consumption of feed, limiting the intake of nutrients and negatively affecting the percentage of egg-laying and egg weight. 

In this study, a linear trend of glycerin consumption was observed on the weight of the birds before the start of the egg-laying phase, a trend that was maintained in terms of egg production up to the egg-laying peak. Saldaña et al. (2016) reported that hens that consumed crushed diets were heavier at week 17 of age, which meant that they started production earlier and lay, until the 22\textsuperscript{nd} week of age, more eggs that were larger compared to those birds that were lighter and were fed diets in flour form (milled). Besides, these researchers report that the weight gain was higher in lighter birds at the beginning of the egg-laying phase, which coincides with the trend observed in glycerin consumption. It is generally recognized that hens that are lighter at the beginning of egg-laying produce fewer eggs that are smaller compared to those hens that are heavier (Leeson et al., 1997; Perez-Bonilla et al., 2012).

Min et al. (2010) indicate that glycerin can stimulate feed consumption as it has a sweet taste. The increase in feed consumption in egg-laying hens by using glycerin in diets was reported by Fontinele et al. (2017) in red egg-layers of 90 weeks of age, with an estimated maximum of 6%, and also by Suchy et al. (2012) with 4% glycerol, when they replaced soybean oil with glycerin. Some authors mention that the

**Table 5 – Performance until the egg-laying peak of Backcob Brown egg-laying hens fed during the transition phase with low or high energy diets and crude glycerin.**

| Treatment | Feed intake (g/bird/d) | Weight gain (g/d) | Egg-laying (%) | Egg weight (g) | Egg mass (g) | Feed conversion (kg/dozen eggs) |
|-----------|------------------------|------------------|----------------|---------------|-------------|-------------------------------|
| ME        |                        |                  |                |               |             |                               |
| High      | 103.4 b                | 2.6              | 78.4           | 54.2 a        | 42.5        | 1.58 a                        |
| Low       | 105.8 a                | 2.7              | 78.9           | 53.4 b        | 42.1        | 1.61 b                        |
| MSE\(^1\) | 0.379                  | 0.198            | 0.456          | 0.240         | 0.343       | 0.009                         |
| Glycerin  |                        |                  |                |               |             |                               |
| 0         | 103.2 b                | 3.0              | 77.6           | 53.8 ab       | 41.7        | 1.60                          |
| 3         | 104.9 b                | 2.6              | 79.1           | 53.3 b        | 42.3        | 1.59                          |
| 6         | 104.9 ab               | 2.7              | 78.1           | 54.6 a        | 42.7        | 1.60                          |
| 9         | 105.4 a                | 2.2              | 79.8           | 53.4 ab       | 42.7        | 1.58                          |
| MSE\(^2\) | 0.537                  | 0.280            | 0.646          | 0.340         | 0.485       | 0.013                         |
| Effects\(^3\) |                |                  |                |               |             |                               |
| ME diet   | **                      | NS               | NS             | *             | NS          | *                             |
| Glycerin  | *                      | NS               | +              | *             | NS          | NS                            |
| Glycerin contrast\(^4\) | L                 | L                | L              | C             | NS          | NS                            |
| Initial weight | **
\(^5\) | NS | **
\(^5\) | **
\(^7\) | **
\(^8\) | **
\(^9\) | **
\(^1\) mean standard error for energy, \( n= 32 \).
\(^2\) mean standard error for glycerin, \( n= 16 \).
\(^3\) +: \( p<0.1 \), *: \( p<0.05 \), **: \( p<0.001 \), NS: not significant.
\(^4\) C: Cubic; L: linear.
\(^5\) 85.3 (4.78) + 0.017 (0.0043)*Initial weight, \( R^2: 0.209 \).
\(^6\) 52.2 (4.96) + 0.024 (0.0044)*Initial weight, \( R^2: 0.315 \).
\(^7\) 42.8 (2.76) + 0.0098 (0.0024)*Initial weight, \( R^2: 0.203 \).
\(^8\) 19.5 (3.52) + 0.020 (0.0031)*Initial weight, \( R^2: 0.405 \).
\(^9\) 1.84 (0.10) - 0.00021 (0.00009)*Initial weight, \( R^2: 0.083 \).
sodium present in glycerin may limit feed consumption (Guerra et al., 2011), however, in this study, the use of crude glycerin with 1.1% sodium did not cause that effect. The increase in egg-laying by using glycerin in diets was reported by Fontinele et al. (2017) in brown chickens in the second egg-laying cycle, where birds fed diets containing 10% crude glycerin laid 6% more eggs compared to birds in the control group.

Boso et al. (2013) observed that egg production of laying hens increased linearly with the consumption of glycerin in a range that varied between 1.5 and 7.5% during the 16 weeks that egg-laying lasted, and this response is directly related to the increase in feed consumption. This last group of researchers also reported a quadratic response in egg weight, with an estimated minimum inclusion of 4.9% glycerin (Boso et al., 2013). However, Fontinele et al. (2017) reported a positive linear effect of the inclusion of glycerin in diets on egg mass in brown chickens associated with increased feed consumption. Increase in feed intake positively affects egg mass, as reported by Zheng et al. (2020), when the supply of diets in mash or pellet was evaluated.

Consumption of low or high energy diets from week 13 and up to 50% egg-laying affected feed intake and egg-laying in Backcob Brown egg-laying hens after the egg-laying peak (Table 6). The birds that received low-energy diets during the transition phase consumed 1.6 g more feed (p<0.05) and laid 0.9% more eggs than their counterparts. Similar to what was observed before the egg-laying peak, the weight at 13 weeks of age positively affected (p<0.05) the productive performance between the egg-laying peak and the moment when they reached 40 weeks of age.

Studies that evaluate the effect of the use of diets with different energy concentrations during the end of the growing phase and the beginning of the egg-laying phase on the subsequent performance of the egg-laying hens were not found in the literature. However, in the work developed by Saldaña et al. (2016), the use of diets with different levels of metabolizable energy during the growth phase (1 to 17 weeks of age) of egg-laying hens, contrary to what was observed in this study, did not affect subsequent performance during the egg-laying phase in terms of feed consumption, production, egg weight, feed efficiency or weight gain.

There are no reports in the literature of the dragging effect of glycerin consumption during the transition period on the subsequent performance of egg-laying hens. However, research evaluating glycerin consumption on the productive response of egg-laying hens concludes, in accordance with what was found after the egg-laying peak, that the use of this co-product in diets does not affect feed consumption, not even when it is included in the formulation of diets (Świątkiewicz & Koreleski, 2009; Boso et al., 2013; Mandalawi et al., 2015) or when it is used to replace oil in the formulation (Cufadar et al., 2016; Kanbur et al., 2017). The previous result has also been found in egg-laying quails (Erol et al., 2009; Ghayas et al., 2017).

Similarly, the use of this resource does not affect the percentage of egg-laying or the weight of an egg of egg-laying hens, when inclusions are lower than 7.5% (Świątkiewicz & Koreleski, 2009; Yalcin et al., 2010; Duarte et al., 2014, Mandalawi et al., 2015) or when up to 75% of the energy contribution of oils is replaced by glycerin (Cufadar et al., 2016; Kanbur et al., 2017). In egg-laying quails, the use of diets with inclusions of up to 10% glycerin did not affect the percentage of egg-laying or egg weight (Erol et al., 2009; Ghayas et al., 2017). Some authors conclude that feed use efficiency in egg-laying hens is not affected by the inclusion of glycerin in the diets, since in some cases, the increase in egg-laying and mass was correlated with feed consumption (Fontinele et al., 2017) or a similar productive response is evidenced in the variables that build this index (Świątkiewicz & Koreleski, 2009).

The distribution of eggs in different categories was not affected by the experimental treatments (results by treatments no presented). The average of all treatments during the study was: Jumbo: 2.7%, AAA: 23.8%, AA: 30.1%, A: 18.2%, B: 16.4%, C: 8.5% and broken eggs: 0.8%. Similarly, the physical characteristics of the eggs were not affected by the experimental treatments (results not presented), which on average, recorded the following values: 0.41 mm in shell thickness, 11.9 in color score, 5.95 mm in albumin height and 89.3 Haugh units. Similar to what was found in this study, the literature concludes that shell thickness, yolk color or the height of the albumin of the eggs are not affected by the levels of glycerin inclusion in the diets (Świątkiewicz & Koreleski, 2009 Boso et al., 2013; Duarte et al., 2014; Mandalawi et al., 2015; Cufadar et al., 2016; Fontinele et al., 2017).

The use of diets with different levels of metabolizable energy affected the shape of the egg-laying curve of egg layers (Table 7). Diets that contained less metabolizable energy had a lower rate of decline after the egg-laying peak and a higher (p<0.05) rate of increase to the peak, a parameter that was also affected by the inclusion of crude glycerin in the diets. The estimation of the performance of an egg-laying

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**Crude Glycerin and Energy Density of Diets for Growing, Pre-Lay and Pre-Peak Backcob Brown Egg-Laying Hens**

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**Crude Glycerin and Energy Density of Diets for Growing, Pre-Lay and Pre-Peak Backcob Brown Egg-Laying Hens**
cycle (until week 72 of age) through the parameters of the McMillan model indicate that birds fed low-energy diets would produce 20 eggs more compared to those fed high-energy diets (330 and 310 for low and high energy, respectively). In the same way, birds fed diets during the transition period with 9% glycerin would produce about 10 more eggs compared to those in the control group (326, 333, 335 and 336 eggs per bird for diets with 0, 3, 6 and 9% glycerin, respectively).

Table 7 – Parameters of the egg-laying dynamics of Backcob Brown egg-laying hens fed during the transition phase with low and high energy and crude glycerin diets.

| Treatment | Egg-laying curve parameters |
|-----------|-----------------------------|
|           | a1 | k1 | k2 |
| ME        |     |    |    |
| High      | 99.2 | 0.0015 a | 0.746 a |
| Low       | 102.1 | 0.0043 b | 0.663 b |
| Glycerin  |     |    |    |
| 0         | 101.2 | 0.0038 | 0.667 b |
| 3         | 101.2 | 0.0030 | 0.662 b |
| 6         | 100.0 | 0.0024 | 0.720 ab |
| 9         | 99.8  | 0.0023 | 0.774 a |

1 Asymptotic egg-laying value, 2 Rate of decrease in egg production after the egg-laying peak, 3 Rate of increase in egg production until the egg-laying peak.

Otwinowska-Mindur et al. (2016) found that the egg-laying curve of hens with low productivity was characterized by a higher value of the rates of ascent to the peak and fall after the peak. In this study, laying-hens with lower production recorded a lower rate of ascent to the peak, but a higher rate of descent. On the other hand, when the egg-laying curves of selected and unselected leghorn hens were compared, the authors found that the genetic improvement changed the pattern of the curve, mainly increasing the maximum potential parameter (a) and decreasing the rate of ascent to the peak (c) in the McMillan model (Savegnago et al., 2012).

Reproductive performance (characteristics of clutches and resting periods) up to the egg-laying peak was not affected (p>0.05) by the energy density of the diet or the level of inclusion of crude glycerin (data not shown), given the high variation between individuals in the period evaluated. Furthermore, only the start of the egg-laying phase and the number of eggs accumulated up to the peak were affected (p<0.05), due to the weight of the hens at 13 weeks of age. Until 40 weeks of age, egg-laying hens fed low energy diets recorded a higher (p<0.05) number of eggs accumulated, a larger (p<0.05) maximum brood size and also a higher (p=0.061) average brood, compared to those who received diets with more energy (table 8).
On the other hand, a linear trend of the inclusion of glycerin in diets with time was found at the beginning of the egg-laying phase, being lower ($p=0.068$) in birds that consumed diets with more glycerin in the transition phase, a difference that translates in 2.1 days less when laying the first egg. In addition, birds fed diets without glycerin laid 2.1 eggs ($p=0.076$) compared to those in the treatment with 9% glycerin.

An increase in body weight at 13 weeks of age resulted in less time at the beginning of the egg-laying phase, a higher number of cumulated eggs, larger broods, and fewer resting periods.

In egg-laying hens, the expression of the genetic potential related to productive efficiency and the degree of adaptation is associated with large brood sizes (Zerjal et al., 2013; Samiullah et al., 2016). The amount of eggs accumulated is a function of the broods and resting periods recorded by the egg-layers; in that sense, Erensayin & Camci (2003) found that the correlation of this variable with the number of broods is negative, a response that was also observed in this study ($-0.408$, $p<0.001$). In addition, a negative correlation was found between the amount of eggs accumulated and the length of the resting period ($-0.459$, $p<0.01$), which evidences the impact of the duration of this last on the performance of the birds, being similar to the results reported in the literature (Mutayoba et al., 2012; Alkan et al., 2013). On the other hand, early onset of egg-laying positively relates the number of total eggs per bird (Alkan et al., 2013), a ratio that was also found in this study ($-0.394$ $p<0.05$).

Furthermore, egg-laying hens that were fed during the transition phase with high energy diets recorded a lower ($p<0.05$) feeding cost to the egg-laying peak, compared to those that received low energy diets, which affected ($p=0.0976$) positively the gross profitability of that group of birds. On the other hand, chickens that received diets with 6 or 9% crude glycerin received $89 more ($p=0.0981$) per dozen eggs than birds fed 0 or 3% crude glycerin (table 9).

After the production peak, the economic evaluation was not affected by the experimental treatments. On the other hand, the weight of the chicks at 13 weeks of age significantly affected the economic indicators evaluated. In that sense, the higher the weight at that age, the higher the gross profitability associated mainly with an increase in income. Granghelli et al., (2019) and dePersio et al. (2015) observed that cost increased

### Table 8 – Characteristics of broods and resting periods until 40 weeks of age in Backcob Brown egg-laying hens fed during the transition phase with low or high energy diets and crude glycerin.

| Treatment | Start of egg-laying phase (s) | Eggs | Maximum broods (eggs) | Broods (eggs) | No. of broods | Resting period (d) | No. of resting periods |
|-----------|-----------------------------|------|---------------------|--------------|---------------|--------------------|------------------------|
| ME        |                             |      |                     |              |               |                    |                        |
| High      | 20.2                        | 130.1| 71.3 b              | 28.7         | 4.8           | 1.54               | 4.3                    |
| Low       | 20.4                        | 131.1| 81.5 a              | 34.7         | 4.1           | 1.58               | 3.9                    |
| MSE 1     | 0.090                       | 0.413| 3.39                | 2.42         | 0.314         | 0.116              | 0.373                  |
| Glycerin  |                             |      |                     |              |               |                    |                        |
| 0         | 20.4                        | 129.3| 75.6                | 30.0         | 4.7           | 1.66               | 4.0                    |
| 3         | 20.5                        | 130.6| 77.8                | 30.6         | 4.6           | 1.62               | 4.0                    |
| 6         | 20.2                        | 131.1| 81.5 a              | 32.3         | 4.4           | 1.48               | 4.1                    |
| 9         | 20.2                        | 131.4| 70.6                | 32.3         | 4.4           | 1.46               | 4.2                    |
| MSE 2     | 0.128                       | 0.584| 4.80                | 3.42         | 0.445         | 0.164              | 0.528                  |

**Effects**

1. ME diet NS + + NS NS NS NS
2. Glycerin + + NS NS NS NS NS NS
3. Contrast glycerin a L L NS NS NS NS NS
4. Initial weight +5 ** 5 * NS NS NS NS

*1 mean standard error for energy, n= 32.
2 mean standard error for glycerin, n= 16.
3 +: $p<0.1$, *: $p<0.05$, **: $p<0.001$, NS: not significant.
4 L: linear.
5 22.5 (0.98) - 0.002 (0.0009)* Initial weight, $R^2$: 0.076.
6 103.9 (4.65) + 0.024 (0.0041)* Initial weight, $R^2$: 0.346.
7 -46.4 (37.9) + 0.110 (0.034)* Initial weight, $R^2$: 0.144.
8 -51.9 (25.3) + 0.075 (0.023)* Initial weight, $R^2$: 0.150.
9 16.6 (3.42) - 0.010 (0.0030)* Initial weight, $R^2$: 0.159.
10 12.8 (3.80) - 0.0076 (0.0034)* Initial weight, $R^2$: 0.075.
.linearly with energy density of the diet, while gross profitability decreased. This is a response that contrasts with the results obtained in this study until week 28 since the use of diets with higher energy resulted in lower feed consumption, which reduced costs and increased profitability. In this study, glycerin inclusion in diets of transition phase of laying hens did not affect the costs, however, Pasquetti et al. (2014), observed a linear reduction in quail production costs with the use of crude glycerin, while Batista et al. (2013) and Avellaneda et al. (2018), found that the inclusion of refined glycerin negatively affects production costs.

In conclusion, laying-hens that received high energy diets during transition phase reduced feed intake during the overall assay, that reduces partial cost; besides, they lay heavier eggs in period from beginning laying to egg-laying peak and in less percentage after the egg-laying peak. A positive linear effect of glycerin consumption on weight gain and feed intake was observed from 14 to 17 weeks of age and during the pre-peak phase; also, the laying-egg rate was affected of linear form before laying-peak (higher percentage of eggs to higher inclusion of glycerin) and quadratic form after laying-peak (higher posture estimate around of 4.5% of glycerin inclusion) by glycerin intake.

### Table 9 – Economic evaluation of the feed of Backcob Brown egg-laying hens fed during the transition phase with low or high energy diets and crude glycerin (USD/dozen eggs).

| Treatments | Income Until egg-laying peak | Income-cost | From egg-laying peak until week 40 | Income-cost |
|------------|-----------------------------|-------------|-----------------------------------|-------------|
| ME         |                             |             |                                   |             |
| high       | 0.78                        | 0.51 a      | 0.28                              | 0.92        |
| low        | 0.78                        | 0.52 b      | 0.26                              | 0.92        |
| MSE¹       | 0.006                       | 0.003       | 0.006                             | 0.004       |
| Glycerin   |                             |             |                                   |             |
| 0          | 0.77                        | 0.51        | 0.25                              | 0.92        |
| 3          | 0.79                        | 0.52        | 0.26                              | 0.92        |
| 6          | 0.79                        | 0.51        | 0.28                              | 0.92        |
| 9          | 0.79                        | 0.52        | 0.28                              | 0.92        |
| MSE²       | 0.009                       | 0.005       | 0.009                             | 0.006       |
| Effects ³  |                             |             |                                   |             |
| ME diet    | NS                          | *           | +                                 | NS          |
| Glycerin   | +                           | NS          | NS                                | NS          |
| Contrast glycerin⁴ | C          | NS          | L                                 | NS          |
| Initial weight | ** *         | **          | **                                | **          |

1: mean standard error for energy, n= 32.
2: mean standard error for glycerin, n= 16.
3: +; p<0.1, *; p<0.05, **; p<0.001, NS: not significant.
4: C: Cubic; L: linear.

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