Requirement of Digestible Methionine + Cystine to Molted Laying Hens

ABSTRACT

The present study aimed to determine the ideal requirement of digestible methionine + cystine to molted laying hens. The experimental period lasted 105 days, divided into five periods of 21 days. 144 Hisex White laying hens with 84 weeks-of-age were used. The experimental design was completely randomized with treatments constituted for six levels of digestible methionine + cystine (0.45, 0.50, 0.55, 0.60, 0.65, and 0.70%) in the diets, with four replicates of six birds each. Data collected were evaluated by polynomial regression at 5%. There was a significant effect \( p<0.05 \) of digestible methionine + cystine on feed intake, energy intake, protein intake, egg production, and egg mass. Internal egg quality presented positive linear effect \( p<0.05 \) on albumen height and yolk color. External egg quality was affected \( p<0.05 \) in eggshell %, eggshell thickness and eggshell resistance, where the level of 0.60% of digestible methionine + cystine in the diets provided eggshells with better quality (higher percentage, thicker and breaking resistance). Differences \( p<0.05 \) were also observed in glucose and triglycerides concentration, with 0.60% of digestible methionine + cystine in the diets presenting better equilibrium. Results of the present study suggested that higher levels of digestible methionine + cystine improved the performance and internal egg quality of molted laying hens. The level of 0.60% of digestible methionine + cystine provided better eggshell and equilibrium on blood biochemistry.

INTRODUCTION

Induced molt of laying hens is used by commercial egg producers to extend the productive period of their flock (Gongruttananun et al., 2017). Typical molt programs cause a plumage renewal, providing changes in feed intake (Macari et al., 2008; Sartório, 2011), and rejuvenation of reproductive tract, that is related to ovarian and oviducal regression (Brake & Thaxton, 1979). Feed withdrawal of various durations with photoperiod restriction is the most commonly used technique (Keshavarz & Quimby, 2002), although other alternative methods may also be used (Berry, 2003).

The main purpose of molting is to cease egg production in order for the hens to enter a nonreproductive state, which increases egg production and egg quality post molt (Webster, 2003; Donaldson et al., 2005). Schmidt et al. (2011) reported that molt programs normally improve the quality of the eggs and extend the egg production up to 25 to 30 weeks, presenting around 85% of efficiency. According to Teixeira & Cardoso (2011), the nutritional readjustment of all nutrients of the diet is essential to a successful molt program. Studies about nutritional requirements for laying hens, especially amino acids, are frequently updated in the literature (Domingues et al., 2016). However,
there is a great lack of information about requirements to molted laying hens.

The amount of protein used in diets before and after the molt has shown to affect not only feather replacement (Andrews et al., 1987) but also various post molt performance factors (Harms, 1983). Amino acids are extremely important to the hens’ metabolism due to having—a direct relationship with the egg production, feed efficiency, egg quality, and efficiency in the use of nitrogen (Lesson & Summers, 2001). The correct meeting of amino acids requirement increases the protein content of albumen, positively influencing the egg production, egg size, egg mass produced, and feed efficiency (Bateman et al., 2008; Cupertino et al., 2008; Figueredo Júnior et al., 2014).

Methionine is the first limiting amino acid in poultry diets, being the precursor of cystine production, and influencing on weight and internal content of eggs (Laurentiz et al., 2005; Brumano et al., 2010a). General recommendations for methionine in laying hens’ diets have been studied to determine an ideal requirement (Koelkebeck et al., 1999; Strathe et al., 2011).

Thus, the present study aimed to determine the ideal requirement of digestible methionine + cystine to molted laying hens.

**MATERIAL AND METHODS**

This study was conducted in the facilities of the Poultry Sector, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas State, Brazil. The experimental procedures were approved by the Ethics Committee in Use of Animals (protocol number 028/2017) of Federal University of Amazonas.

The experimental period lasted 105 days divided into five periods of 21 days. The birds were subjected to an adaptation period of seven days to feed and facilities. The aviary had galvanized wire cages, trough feeders, and nipple drinkers. Throughout the experimental period, 16 hours of light/day were provided to the birds (12 hours of natural + 4 hours of artificial), with water and feed ad libitum. Egg collection was performed two times at day (9 a.m. and 3 p.m.). The temperature and relative humidity were recorded in the same times using a digital term-hygrometer positioned above the birds’ cages, presenting average results of 30.31±0.08 °C and 77.46%, respectively.

144 Hisex White laying hens with 84 weeks-of-age were used. The birds were weighed at the beginning of the experiment in order to standardize the experimental plots, presenting a mean weight of 1.56 ± 0.03 kg. The experimental design was completely randomized with treatments constituted for six levels of methionine + cystine (0.45; 0.50; 0.55; 0.60; 0.65 and 0.70%) in the diets, with four replicates of six birds each.

For the determination of digestible sulfur amino acid requirement in the diets, they were formulated with the digestible methionine + cystine levels obeying the methionine + cystine: lysine ratios of 68, 76, 84, 91, 99 and 107, with lysine fixed at 0.653%. Levels of methionine + cystine were obtained from a basal diet deficient in methionine + cystine (0.45%) and supplemented with DL-methionine (99%). For each experimental level, the ratio of the main essential amino acids to lysine was maintained. The other nutrients in the diets met the requirements by Rostagno et al. (2017), with the experimental diets (Table 1) formulated by Supercrac (2004) computational software.

From the data collected during the 105 days of the experimental period, we calculated the feed intake (g/bird/day), egg production (%), feed efficiency (kg of feed used / kg of egg), feed efficiency (kg of feed used / dozen eggs), and egg mass (g). At the end of every 21 day period, four eggs from each plot were randomly selected to evaluate egg weight (g), specific gravity (g/cm³), yolk (%), albumen (%), eggshell (%), yolk height (mm), albumen height (mm), yolk color, eggshell thickness (μm), Haugh unit and eggshell resistance (N).

The eggs were stored up to one hour in room temperature and weighed using an electronic balance (0.01 g). The eggs were placed in wire baskets and immersed in buckets containing different levels of sodium chloride (NaCl) with density variations from 1.075 to 1.100 g/cm³ (interval of 0.005) to evaluate the specific gravity.

Then, the eggs were placed on a flat glass plate to determine albumen and yolk height, and yolk diameter using an electronic caliper. To separate albumen and yolk a manual separator was used. Each one was placed in a plastic cup and weighted in an analytical balance.

Eggshells were washed, dried in an oven (50 °C) for 48 hours, and weighed. Dry eggshells were used to determine the eggshell thickness using a digital micrometer. Average eggshell thickness was analyzed considering three regions: basal, meridional, and apical.

The yolk color was evaluated using a ROCHE© colorimetric fan with a scale of 1 to 15. Haugh unit was calculated using the egg weight and albumen height values in the formula $H_{unit} = 100 \times \log (H + 7.57)$.
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RESULTS

There was a significant reduction (p<0.05) on feed intake (y = 1.0066x² + 1.5745x + 105.47; R² = 0.75), energy intake (y = 2.8141x² - 4.306x + 284.05; R² = 0.78) and protein intake (y = 0.1788x² - 0.2575x + 15.193; R² = 0.79). The level of 0.60% of digestible methionine + cystine in the diets presented lower intake (Table 2).

There was a quadratic effect (p<0.05) on egg production (y = 1.0891x² - 1.13655x + 89.651; R² = 0.75), and egg mass (y = 0.7302x² - 0.75518x + 51.487; R² = 0.88), where the level of 0.60% of the digestible methionine + cystine in the diets presented worse results. However, there was not effect (p>0.05) on feed efficiency per mass or per dozen results, as well as on viability results.

Internal egg quality (Table 3), presented positive linear effect (p<0.05) on albumen height (y = 0.0846x + 15.474; R² = 0.97) and yolk color (y = 0.1029x + 5.66; R² = 0.93). There was an increase in albumen height and yolk color from the increase of digestive methionine + cystine levels in the diets.

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Table 1 – Experimental diets composition.

| Ingredients                  | Methionine + cystine levels (%) |
|------------------------------|--------------------------------|
| Corn (7.88%)                 | Corn (7.88%)                   |
| Soybean meal (46%)           | Soybean meal (46%)             |
| Limestone                    | Limestone                      |
| Dicalcium phosphate          | Dicalcium phosphate            |
| Salt                         | Salt                            |
| PREMIX vitaminic/mineral     | PREMIX vitaminic/mineral       |
| DL-Methionine                | DL-Methionine                  |
| L-Lysine                     | L-Lysine                       |
| L-Tryptophan                 | L-Tryptophan                   |
| L-Threonine                  | L-Threonine                    |
| Total                        | Total                           |

| Nutrients Nutritional levels | Nutritional levels |
|-------------------------------|--------------------|
| E.M, kcal.kg⁻¹               | 2.755.20           |
| Crude Protein, %              | 14.50              |
| Calcium, %                    | 4.200              |
| Available phosphorus, %       | 0.400              |
| Sodium, %                     | 0.250              |
| Total methionine, %           | 0.269              |
| Digestible methionine, %      | 0.252              |
| Digestible met. + cys., %     | 0.450              |
| Digestible lysine, %          | 0.653              |
| Digestible threonine, %       | 0.498              |
| Digestible tryptophan, %      | 0.172              |

1 Guaranteed levels per kilogram of the product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg, Selenium 40 mg, Vehicle q.s.p. 1,000 g.

-- 1.7 x W0.37), where H = albumen height (mm), and W = egg weight (g).

To determine the eggshell resistance, a resistance machine located in the Materials Laboratory of the Superior College of Technology of the State of Amazonas University was used. This machine was connected to a computer, generating the power levels (represented in Newton) used to break the eggshell.

At the end of the experimental period, four birds were selected from each treatment and submitted to the analysis of serum parameters. The blood was collected from each bird through the ulnar vein and the samples immediately sent to the Laboratory of Poultry Technology, being evaluated: glucose (mg.dl⁻¹), triglycerides (mg.dl⁻¹) and cholesterol (mg.dl⁻¹) using a portable biochemical analyzer (Accucheck Trend, ROCHE®).

All data collected in this study were analyzed using the GLM procedure of SAS (Statistical Analysis System, v. 9.2) and estimates of treatments were subjected to ANOVA and subsequent polynomial regression analysis. Results were considered significant at p≤0.05.
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External egg quality presented differences ($p<0.05$) in %eggshell ($y = -0.053x^2 + 0.06452x + 9.801; R^2 = 0.92$), eggshell thickness ($y = -0.1616x^2 + 1.991x + 45.591; R^2 = 0.83$) and eggshell resistance ($y = -0.9657x^2 + 6.9329x + 27.56; R^2 = 0.86$). The level of 0.60% of digestible methionine + cystine in the diets provided eggshells with better quality (higher percentage, thicker, and resistance).

Differences ($p<0.05$) were observed in glucose ($y = -2.6911x^2 + 2.875x + 181.63; R^2 = 0.92$), triglycerides ($y = -12.345x^2 + 13.7759x + 524.46; R^2 = 0.78$), with 0.60% of digestible methionine + cystine in the diets presenting most balanced results (Table 4).

**DISCUSSION**

The increase methionine and cystine intake caused a significant effect on birds’ performance. The level of 0.60% caused a significant decrease in energy and protein intake, consequently affecting the feed intake. High methionine levels reduced egg production and egg mass. Baião et al. (1999), and Brumano et al. (2010b) disagree with these results, where the authors reported that the performance improved by increasing methionine + cystine in the diets of laying hens.

The feed efficiency, although did not present significant differences in its results, reflected the reduction of feed intake and the drop of egg production.
(Domingues et al., 2016), where high methionine levels present a better equilibrium among the nutrients used and the egg production. Schmidt et al. (2011) reported that post molt hens normally present lower performance than birds in the first cycle, especially due to its metabolism being more susceptible to changes in diet and for presenting more difficulty to transform the diets' nutrient to eggs.

Baker et al. (1981b) also reported that potential improvements in molted hens' performance may be related with an increase in body weight loss of hens up to 31% of their original body weight, and better arrangement of metabolic pathways. Reductions of body weight above 35% may provide bad effects on the life of flock egg production. And the degree of improvement in post molting performance also may be associated with the number of days during which no eggs were produced, the named "rest period" (Berry, 2003).

According to Gongruttananun et al. (2017), the molted hens return to egg production at a slow rate after receiving re-adequate diets, and then rapidly increase during the postmolt period. Hormonal changes are typically associated with molting, where the hen responds using physical, chemical, anatomical, and physiological mechanisms at its disposal to maintain a better productive status for the longest period possible (Clarenburg, 1986; Freeman, 1987; Koelkebeck & Anderson, 2007).

However, there is not sufficient information about ideal levels of amino acids to molted hens, where the tables of requirements and manuals do not provide this information (Murakami et al., 2003). We observed that the better requirements of methionine + cysteine to molted hens was higher than recommendations reported by Rostagno et al. (2011) for laying hens in the first cycle. The ideal use of protein and amino acids for laying hens may provide a balance in the requirement, improving performance and the quality of the eggs. And an optimal supplementation of methionine and cystine to hens' diets can increase the efficiency of protein use, affecting the egg weight, yolk, and albumen percentage (Gomez & Angeles, 2009; Del Vesco et al., 2014).

Baiano et al. (1999) reported that an increase in methionine and methionine + cystine supplementation did not present significant responses on eggshell quality. However, our results presented a different response, with significant effect in the quality of eggshell. These changes in the eggshell may be associated with an increase in egg size due to methionine and cysteine supplementation, besides metabolic changes resulting in the molting process.

Naturally, eggshell quality decreases with increasing hen age (Washburn, 1982), and the incidence of cracked eggs can exceed 20% at the end of the laying period (Nys, 2001). Furthermore, the lower eggshell percentage may result in less eggshell thickness, lower internal quality, and a higher percentage of non-commercial eggs (Brumano et al., 2010b). However, the quality of the eggs may be changed from the amino acid adjustment in diets, especially the eggshell. This adjustment reduces these problems observed before and after the molt (Pavan et al., 2005; Brumano et al., 2010a).

According to Brake & Thaxton (1979), the improvements in postmolt eggshell quality occur due to changes in the reproductive tract at the cellular level. These changes include the removal of lipid (Baker et al., 1980; Baker, 1981; Baker et al., 1981a), increased uptake of 1α25-dihydroxycholecalciferol by uterine tissue (Abe et al., 1982), or the activation of some transport system such as calcium-binding protein (Berry et al., 1987).

It is important to state that the eggshell quality is an essential factor to a good egg quality (Gongruttananun, 2017). A strong eggshell is significantly important for egg producers in reducing the economic losses from broken eggs (Tumova et al., 2014) due to its function to provide a container for the eggs contents and protect them from bacterial contamination (Hamilton et al., 1979). Previous studies reported that there are 8% egg production losses due to poor eggshell quality (Nys, 2001; Abdallan et al., 2009). Thus, the objective of poultry nutritionists is to formulate diets that maximize egg size and that reduce eggshell problems late in the production cycle (Schutte & De Jong, 1994; Sohail et al., 2002).

**CONCLUSIONS**

It was concluded that higher levels of digestible methionine + cystine improved the performance and internal egg quality of molted laying hens. The level of 0.60% of digestible methionine + cystine provided better eggshell and equilibrium on blood biochemistry.

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