

Levels of energy and protein for brown-egg laying hens reared in cage free systems from 75 to 90 weeks of age

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Abstract

Two levels of apparent metabolizable energy (AME) and three levels of crude protein (CP) were evaluated in diets of brown laying hens raised in cage-free systems on performance, egg quality, internal organ index and abdominal fat. A total of 288 birds, from 75 to 91 weeks of age, were distributed in a completely randomized design in a factorial arrangement (2 x 3), with two levels of AME (2,800 and 2,550 kcal/kg) and three levels of CP (170, 160 and 150 g /kg), totaling six treatments with four repetitions of 12 birds. The results were analyzed using ANOVA, evaluating AME and CP factors and their respective interaction. Regarding performance, diets containing 2,550 kcal/kg increased ($P < 0.05$) feed intake (FI), egg production (EP) and feed conversion ratio (FCR), while for CP levels no effect was observed ($P > 0.05$), there was an interaction ($P < 0.05$) between AME and CP, where at the level of 2,550 kcal/kg, worse values were obtained in FI, EP and FCR with 150 g/kg. In egg quality, internal organ index and abdominal fat, no differences and interactions were observed ($P > 0.05$), except for the intestine index, which was higher ($P < 0.05$) in the diet with 2,550 kcal/kg of AME. It is concluded that diets for brown laying hens raised in cage-free systems from 75 to 91 weeks of age with 2,550 kcal/kg of AME increase FI, FCR, and EP compared to diets formulated with 2,800 kcal/kg. However, diets with 2,550 and 2,800 kcal/kg of AME can be formulated with 160 g/kg of CP without affecting egg quality, internal organs, or abdominal fat, except for intestinal weight, which is higher in diets with 2,550 kcal/kg.

Introduction

Cage-free rearing systems have been proposed as an appropriate alternative for animal welfare, offering better freedom of movement and allowing birds to express a wide range of behavioural patterns (Leite et al., 2021). Cage-free systems demonstrate significant adoption; for instance, Spain accounted for 27% of birds reared without cages in 2020, while France has implemented a ban on cage utilisation starting in 2022 (MAYER et al., 2023). However, birds raised in a cage-free system show an increase in feed consumption compared to those raised in cages, which is directly associated with the increase in energy expenditure due to higher physical activity.

Poultry modulate their feed intake (FI) to meet their energy requirements, an increase in the apparent metabolizable energy (AME) level of a diet result in reduced consumption (Leeson and Summers, 2009), whereas lower levels in AME leads to increased consumption (Pérez-Bonilla et al., 2012; Guzmán et al., 2016). The decrease in AME of the diet results in a higher inclusion of fiber-rich feedstuff, which provide a lower AME content and affect the transit rate of digesta, potentially causing negative impact on nutrient digestion and absorption, affecting the weight and development of the gastrointestinal tract organs (Suyama and Gewehr, 2026) and the deposition of abdominal fat (S. Jiang et al., 2013). Furthermore, the level of AME affects performance (dePersio et al., 2015), egg quality (Pérez-Bonilla et al., 2012), and bone integrity (Sha Jiang et al., 2013; S. Jiang et al., 2013) in layers.

Crude protein (CP) consists of amino acids, which support several physiological functions: tissue development and maintenance, secondary energy source, glandular secretions, hormone production,

nucleoprotein synthesis, transport, and antibody formation (Marques Bittencourt et al., 2021). Elevated dietary CP levels are economically impractical, since they also contribute to higher nitrogen excretion and exacerbate environmental pollution (Silva et al., 2006). Reducing the CP content of laying hen diets aims to maximize performance by optimizing production at a lower cost. Nevertheless, the CP level must be modified in relation to the AME level, since a decrease in the AME level increases feed intake (Pérez-Bonilla et al., 2012; Guzmán et al., 2016), leading to CP intake exceeding the requirements of laying hens. Dietary consumption is affected by several parameters, with ambient temperature being a significant one, including the productive status of the birds (Rostagno et al., 2017).

The hypothesis of the study is that the birds will require lower levels of AME and CP in the diet to meet their nutritional requirements without compromising their productive performance, as a result of the increased FI. Thus, this study aims to evaluate different levels of AME and CP in the diet of laying from 75 to 90 weeks of age, raised in a cage-free system, on productive performance, egg quality, internal organ index, and abdominal fat.

Materials and methods

The experiment was conducted in Lages, SC Brazil, Latitude – 27.8167° S, Longitude: -50.3264 27° W, and elevation 930 m, in accordance with the regulations issued by the National Council for Animal Experimentation and approved by the Ethics Committee on Animal Use of the State University of Santa Catarina, under approval number 5501010216.

Experimental design, treatments and animals

A total of 288 Hy-Line Brown laying hens, 75 weeks old, were allocated in a completely randomized design with a 2 x 3 factorial arrangement, totaling six treatments with four replicates of 12 hens per pen (experimental unit). This arrangement comprised two levels of AME (2,800 and 2,550 kcal/kg) and three levels of CP (150, 160, and 170 g/kg) in the diet. The experimental diets (Table 1) were formulated according to the nutritional requirements and feed composition recommended by (Rostagno et al., 2017) for brown laying hens, altering only the AME and CP levels. The experimental period was 112 days, consisting of four consecutive periods of 28 days each.

Table 1

Ingredients and calculated nutritional composition of experimental diets containing different levels of apparent metabolizable energy (AME, kcal/kg) and crude protein (CP, g/kg).

Ingredients, %	AME	2,800 kcal/kg			2,550 kcal/kg		
	CP	170	160	150	170	160	150
Corn		53.6	57.0	60.3	57.2	60.4	62.8
Soybean meal (45% CP)		28.8	25.9	23.1	28.2	25.3	22.3
Wheat bran		0.00	0.00	0.00	0.00	0.11	1.00
Limestone		11.5	11.5	11.5	11.5	11.5	11.5
Dicalcium phosphate		1.35	1.38	1.41	1.34	1.37	1.38
Salt		0.24	0.25	0.26	0.24	0.25	0.26
Vegetable oil		3.76	3.18	2.65	0.85	0.31	0.00
DL-Methionine		0.15	0.16	0.17	0.15	0.16	0.17
L-Lysine		0.00	0.01	0.08	0.00	0.02	0.09
Premix ¹		0.40	0.40	0.40	0.40	0.40	0.40
Adsorbent		0.20	0.20	0.20	0.20	0.20	0.20
Total		100	100	100	100	100	100
Calculated nutritional composition							
Calcium, %		4.20	4.20	4.20	4.20	4.20	4.20
Phosphorus disponible, %		0.30	0.30	0.30	0.30	0.30	0.30
Digestible lysine, %		0.77	0.77	0.77	0.77	0.77	0.77
Digestible methionine+cistine, %		0.70	0.70	0.70	0.70	0.70	0.70
Digestible threonine, %		0.59	0.59	0.59	0.59	0.59	0.59
Sodium, %		0.23	0.23	0.23	0.23	0.23	0.23
Potassium, %		0.59	0.59	0.59	0.59	0.59	0.59
¹ Premix – Mineral e vitamin: Vitamin A: 2,333 UI; Vitamin D3: 666 UI; Vitamin E: 1,666; UI. Vitamin K3: 533 UI; Vitamin B2: 1,000 g; Vitamin B12: 2,666 mg; Vitamin B3: 6,666 mg; Choline: 78,120 mg; Pantotenic acid: 1,166 mg; Copper: 2,667 mg; Iron: 16,670 g; Manganese: 23,330 g; Zinc: 16,670 g; Iodine: 400 mg; Selenium: 66,670 mg; Zinc bacitracin: 6,667 mg.							

The birds were housed in an experimental aviary with open-air ventilation (30 x 5 m), with a ceiling height of 2.65 meters, bird netting, and external plastic curtains on the sides. The shed contained 24 pens of 2.1 m² (1.5 x 1.4 m), each pen considered an experimental unit, equipped with a tubular feeder (20 kg), two "nipple" drinkers, a perch (1.4 m), three nests with dimensions of 27 x 30 x 30 cm (front x height x depth) lined with wood shavings with weekly replacement, masonry floor covered with 10 cm of wood shavings bedding, which was periodically turned over.

Feed in mash form and water were provided *ad libitum*, and the birds underwent a 14-day adaptation period to the diets. The temperature inside the shed during the experimental period was 16°C (+/- 8°C), and the lighting program used was 16 h of light and 8 h of darkness.

Data collection

The performance of the birds was evaluated through average daily feed intake (FI, g/hen/day), egg production (EP, % eggs/hen/day), feed conversion ratio (FCR, kg/kg), egg mass (EM, g/hen/day), final hen body weight (BW, kg) and weight gain (WG, g).

Egg quality was evaluated through average egg weight (EW, g), Haugh unit (HU), and yolk index (YI), albumen index (AI) and eggshell index (SI) were expressed as a percentage in relation to the EW. The analysis of six whole eggs from each experimental unit was conducted in the last three days of each period. After weighing, the eggs were sectioned along the equatorial segment, and the internal content was exposed to determine the height (mm) of the albumen. The determination of the albumen height was taken in the region closest to the yolk. The HU was calculated using the formula described by Silversides and Villeneuve (1994) in which: $HU = 100 \times \log (h + 7.57 - 1.7 \times EW^{0.37})$, where h represents the height of the albumen (mm) and EW the weight of the egg (g). The yolk was separated from the albumen for weighing on a precision scale, and the weight of the albumen was obtained by subtracting the weight of the egg from the weights of the yolk and the shell. The percentages of yolk, albumen and eggshell, in relation to the weight of the egg, were obtained according to the equation: Component (%) = (weight of component / weight of egg) x 100.

At the end of the experiment, two birds from each experimental unit were stunned and sacrificed by cervical dislocation after a 6-hour fast. Weighings were performed on a precision balance with 0.01 g to obtain the organ index (liver, intestine, oviduct, and ovary) and abdominal fat index (AFI), obtained by dividing the organ weight by the hens live weight, expressed as a percentage (%).

Statistical analysis

The results were analyzed using two-factor ANOVA considering the independent effects of AME and PB levels and their interaction. For AME levels, the F-test was used, and for PB levels, Dunnett's test was used, which compared the 150 and 160 levels with 170 g/kg (control). The residuals were tested for

normality using the Shapiro-Wilk test. The statistical procedures were carried out using SAS statistical package (Version 9.4, SAS Institute Inc., Cary, NC), considering a significance level of $P < 0.05$.

Results

In terms of performance (Table 02), the diet with 2,550 kcal/kg of AME resulted in higher FI ($P < 0.0001$), EP ($P < 0.019$), and FCR ($P < 0.0001$) compared to those fed 2,800 kcal/kg, with no difference ($P > 0.05$) in EW, EM, BW and WG. No effect ($P > 0.05$) of CP level was observed on FI, EP, EW, FCR, EM, BW, and WG. There was an interaction ($P < 0.05$) between the AME and CP factors on FI, EP, and FCR (Table 2). The diets containing 2,800 kcal/kg and the lower level of CP of 150 g/kg, resulting in a reduction in FI and EP compared to the control CP group (170 g/kg) (Table 3). However, birds that consumed diets with 2,550 kcal/kg, and the lower protein level (150 g/kg), increased FI and FCR compared to the CP level of 170 g/kg.

Egg quality parameters (Table 4) were not affected ($P > 0.05$) by AME and CP levels, and no interaction ($P > 0.05$) was observed between the factors.

No effect ($P > 0.05$) of AME and CP levels was observed in the internal organ and abdominal fat index, except for the intestine index, which was higher ($P < 0.05$) in hens that received a diet with 2,550 kcal/kg of AME (Table 5). No interactions ($P > 0.05$) were observed between the factors.

Discussion

The hypothesis that reducing the AME level in diets increases the FI, where laying hens adjust their feed intake to meet their energy requirements, was confirmed. Birds fed diets with 2,550 kcal/kg of AME increased their FI by 11% compared to birds fed 2,800 kcal/kg. This higher FI likely induced the higher EP obtained with 2,550 kcal/kg (Table 2), also facilitated by the higher intake of other nutrients present in the diet, since they were isonutritive, except for the AME. In fact, the low temperature observed during the experimental period (16°C, $\pm 8^\circ\text{C}$) may have been a factor influencing the result obtained, as it is known that birds increase FI to maintain body homeothermy (Han et al., 2023).

The higher FCR of the hens receiving 2,550 kcal/kg of AME with 150 g/kg of CP in the diet justifies the maintenance of daily EP compared to diets with a higher level of PB, due to the increase in FI. Furthermore, it was noted that hens on the diet with the lowest energy and protein levels exhibited an estimated 5% increase in FCR compared to those receiving 2,550 kcal/kg of AME and 170 g/kg of CP. This increase in consumption indicates that the hens modulate their intake based on the calorie density and protein content of the feed. The average daily protein intakes of the hens were 20.7, 19.6, and 18.2 g/day for protein levels of 170, 160, and 150 g/kg, respectively, corresponding to a decrease in daily intake of 5.3% and 12.1%. Despite the decrease in CP intake, there was no effect in EP, EW, EM, and FCR, attributable to the addition of synthetic amino acids, which maintained constant levels of digestible lysine and methionine across experimental diets. Multiple hormones are involved in egg formation, and one of the main functions of amino acids is the formation of hormones that are involved in the egg

formation process (Wu et al., 2005). The level of CP used in the diet and the availability of amino acids have a direct effect on the plasma concentration of hormones (Namroud et al., 2008). These findings validate those reported by (Silva et al., 2006), who investigated decreases in dietary protein levels from 165 to 152.5 and 140 g/kg, without affecting the FCR and conversions per mass and per dozen eggs.

The parameters FI, EP, and FCR were influenced by the interaction between CP and AME, as hens consuming 150 g/kg of CP in diets with 2,800 kcal/kg of AME exhibited decreased EP relative to those consuming diets with 160 and 170 g/kg of CP, which was attributed to the reduced FI of the birds with 150 g/kg of CP. For birds that consumed 2,550 kcal/kg of AME, the levels of CP in the diet do not affect the FCR, justified by the FI that also suffered the effect of the interaction between CP and AME in the diet. With the reduction of the dietary level of CP, there was an increase in FI, which highlights that a reduction in the energy level of the diets increases consumption (Leeson and Summers, 2009), causing the layers to adjust their consumption to meet their energy requirements (Gunawardana et al., 2008; Sha Jiang et al., 2013). It can also be inferred that the reduction in the energy level combined with the dietary reduction of CP to 150 g/kg in the diet further increases FI.

Research demonstrates that EM is closely correlated with the CP level of the diet (Summers et al., 1991). Meluzzi et al. (2001) observed that commercial layers fed 150 g/kg of CP showed higher EM than those provided with 130 g/kg. In the current study, the birds that were fed 160 and 170 g/kg of CP had higher EM compared to the 150 g/kg level, suggesting that the decrease in CP mainly impacted EW partly due to the reduced availability of other essential amino acids, since only lysine and methionine were provided in the feed.

Egg quality characteristics were not affected by the AME content of the diet, which corroborates results obtained by Saldaña et al. (2016) and Guzmán et al. (2016). According to Safaa et al. (2008), EW depends mainly on the methionine, fat, and linoleic acid content of the diet, considering that as the energy concentration of the diet increases, the level of fat and linoleic acid also increases, which can result in heavier eggs (Grobass et al., 1999; Pérez-Bonilla et al., 2012). However, in this study, no difference was observed in egg quality characteristics in relation to the intake of CP in diets, since CP is related to protein synthesis in the oviduct, influencing egg quality (Wu et al., 2005). Guzmán et al. (2016) emphasizes that when amino acid intake and the composition of diet ingredients are similar, there are no differences in egg quality characteristics.

Abdominal fat correlates with total body fat (Becker et al., 1979), because when birds are fed high-energy diets, fat deposition increases due to the loss in energy use efficiency and limitations in metabolizing excess energy, consequently affecting production (Pérez-Bonilla et al., 2012). However, the AFI of the laying hens was not affected by the levels of AME in the diet, which can be explained by the WG that did not differ between the treatments. Concerning the internal organs, the concentrations of AME and CP did not affect these parameters, except for the intestine index, which were higher in the birds that received 2,550 kcal/kg of AME. This outcome is associated with the increased volume of feed in the gastrointestinal tract, resulting from the higher FI.

The findings of this research indicate that reducing the AME to 2,550 kcal/kg increases FI and EP in cage-free hens, while without adversely affecting egg quality, body weight, or abdominal fat deposition. Although the lower combination of CP and energy resulted in an increase in the FCR, indicating a less efficient use of the feed. The dietary treatments did not significantly affect the indices of internal organs, except for an elevated intestine index associated with reduced AME, due to the increased FI. In conclusion, the results suggest that cage-free laying hens from 75 to 90 weeks of age can be fed diets with lower levels of AME and CP without compromising egg production or quality. This strategy can be advantageous for both economic and environmental sustainability by reducing the levels of protein and energy in the diet and the associated nitrogen excretion, without compromising the productive performance of the laying hens.

Declarations

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Author's Contributions

Conceptualization: Aline Felix Schneider Bedin, Cleverson de Souza; Methodology: Aline Felix Schneider Bedin, Cleverson de Souza; Formal analysis and investigation: Aline Felix Schneider Bedin, Cleverson de Souza; Writing - original draft preparation: Aline Felix Schneider Bedin; Marcelo Suzuki Suyama; Writing - review and editing: Aline Felix Schneider Bedin, Marcelo Suzuki Suyama, Clóvis Eliseu Gewehr; Supervision: Clóvis Eliseu Gewehr.

Data Availability

All available data is included in the manuscript. The original contributions presented in the study are included in the article, further inquiries can be directed at the first author.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the State University of Santa Catarina, under approval number 5501010216.

Consent to participate

All authors provided consent to participate.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Consent for publication

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Table 2 To 5

Table 2 To 5 are available in the Supplementary Files section.

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