R. Bras. Zootec., 49:e20200063, 2020
https://doi.org/10.37496/rbz4920200063

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia
Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Performance of brown layers fed reduced dietary protein levels in two rearing systems

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Received: March 30, 2020
Accepted: May 20, 2020

How to cite: Viana, E. F.; Souza, W. J.; Costa, M. A.; Arnhold, E.; Carvalho, F. B.; Mello, H. H. C.; Café, M. B. and Stringhini, J. H. 2020. Performance of brown layers fed reduced dietary protein levels in two rearing systems. Revista Brasileira de Zootecnia 49:e20200063. https://doi.org/10.37496/rbz4920200063

ABSTRACT - An experiment was conducted aiming to evaluate the effect of different levels of crude protein, based on the ideal protein concept and two rearing systems, on productive performance of brown laying hens. A total of 400 Hisex Brown laying hens between 30 and 45 weeks of age were distributed in a completely randomized design and a 2×4 factorial arrangement, with main effects including two rearing systems (cage and floor) and levels of crude protein (140, 150, 160, and 180 g kg^{-1}), totalizing eight treatments. Five replicates with 10 birds each were used per experimental unit. The following parameters were evaluated: egg production, feed intake, body weight gain, feed conversion ratio, and quality traits such as dirty, cracked, or broken eggs. No interaction effect was observed between dietary protein levels and rearing systems for body weight gain, feed intake, egg production, egg weight, and feed conversion ratio. Feed intake and egg production were higher in the floor rearing system. Feed conversion ratio (kg/dz) was improved in birds reared in the cage system. The rate of cracked and broken eggs was higher in the cage system. The layers reared in the floor system produced a higher percentage of dirty eggs. The dietary protein level did not affect the evaluated parameters. Thus, we conclude that a floor rearing system is an option for layers, and the dietary protein level can be reduced up to 140 g kg^{-1} for Hisex Brown hens from 30 to 45 weeks of age.

Keywords: egg, ideal protein level, litter, productivity, rearing system, welfare

1. Introduction

Increasing demands for animal welfare and sustainability of egg production have motivated researchers to implement changes in rearing systems, especially in allowing conditions for birds to express their natural behaviors (Blokhuis et al., 2000). The main advantages of a cage rearing system are easy removal of excreta, better parasite control, and productivity maximization with higher stocking densities. However, birds raised in open housing systems, such as floor and free-range, with or without access to free areas, are allowed to express their natural behaviors such as scratching, flapping wings, dust bathing, and nesting. Such alternative housing systems have been widely used since they are designed to allow hens to express more natural behavior and have freedom of movement (Yilmaz

Dikmen et al., 2016). Thus, studies of these alternative rearing systems to compare laying performance in conventional cages and on the floor are of great importance. Given the constant genetic improvement of genetic lines, research on the nutritional demands of laying hens in different systems is needed.

In furnished cages and non-cage systems, hen nutrition may be influenced by the provision of litter (Lay Jr. et al., 2011). Hetland and Svihus (2007) verified that birds with access to paper as litter had higher feed intake and poorer nutrient use than laying hens reared in cages. According to Yilmaz Dikmen et al. (2016), laying hens kept in free-range system present higher egg production, feed intake, egg mass, and better feather score. Netto et al. (2018) concluded that birds raised on the floor had better results for egg production and higher weights of egg, yolk, albumen, and shell compared with Hisex Brown hens raised in conventional cages.

The benefits of diets based on digestible amino acids, given an ideal protein concept, have been widely reported in studies with broilers. Torki et al. (2015) observed that dietary protein reduction from 165 to 120 g kg^{-1} and amino acid supplementation are enough to maintain the performance of Lohmann Selected (LSL-Lite) laying hens. According to Soares et al. (2019), ideal amino acid profiles depend on experimental conditions, genetic lines, environmental factors, and age of pullets or hens. However, the above studies were carried out under cage conditions. Therefore, testing protein reduction in laying hen diet is crucial to evaluate nitrogen use efficiency, environmental effects, and feed costs.

Given the above and considering that protein requirements of laying hens in a floor system need further study to understand bird performance and respective effects on egg quality, we hypothesized that protein demands in poultry nutrition may change when moving hens from a conventional cage to a floor system. Therefore, this study aimed to evaluate the performance of Hisex Brown layers, from 30 to 45 weeks of age, in two rearing systems and receiving isonutritive diets with four levels of crude protein (140, 150, 160, and 180 g kg^{-1}).

2. Material and Methods

This research project was approved by the Ethics Committee on Animal Use (case no. 312/11). The experiment was carried out in Urutaí, Goiás, Brazil (17°27'49" S latitude, 48°12'06" W longitude, 807 m altitude).

Four hundred Hisex Brown layers, from 30 to 45 weeks of age, were allotted in a completely randomized design and a 2×4 factorial arrangement, wherein the main effects included two rearing systems (cage and floor) and four levels of dietary crude protein (140, 150, 160, and 180 g kg^{-1}), with five replicates of ten birds each.

Floor rearing system consisted of 20 boxes with a 10-cm rice-hull litter. Each box was equipped with a pendular drinker, a linear tube feeder, and a nest. Box dimensions were 2.2 × 1.5 × 3 m (length × width × height). The nests were made of wood, had three holes (33 × 40 × 45 cm), and were suspended 10 cm above the litter layer. Stocking density in the floor system was one bird per 3.3 m^2. Conventional cages measured 100 × 37 × 40 cm and had four 25-cm boxes each. Stocking density was one bird per 500 cm^2, and lighting program was 16 h of light, as indicated in the HISEX Brown Management Guide (Globoaves, 2006). Poultry house temperatures were measured using a maximum-minimum thermometer (Incoterm®). In the floor rearing system, maximum and minimum temperatures were respectively 29.9 and 22.5 °C, while in the conventional cages they were 30.1 and 22.3 °C.

Experimental diets were isonutritive and formulated on the ideal protein concept, according to Rostagno et al. (2011) (Table 1).

Laying performance and egg quality were analyzed for laying hens from 30 to 45 weeks of age. Eggs were sampled four times a day (8.00, 10.00, 14.00, and 16.00 h). Eggs and nests were handled according to the recommendations of Albino et al. (2005).

The variables studied were: egg production (%, hen-house basis), egg weight, egg mass, feed intake, body weight gain, feed conversion ratio (kg/dozen eggs and kg/egg mass), and percentages of dirty, cracked, and broken eggs. An egg was considered dirty when excreta or dirt was detected covering at least 30% of its shell surface. Cracked eggs were those with cracks of any size on their eggshell but without breaking its internal membrane. Lastly, eggs showing rupture of the shell were considered broken.

Average egg mass was determined by weighing eggs individually on the last four days of each laying period, using a Gehaka® semi-analytical scale (model BK-4000, accuracy of 0.01 g). Dirty, cracked, and broken eggs were counted daily.

Feed intake (g/bird/day) was calculated by the difference between the amount of feed supplied and leftovers on the last four days of each production period (28 days). Weight gain (kg) was estimated

Table 1 - Composition of experimental diets

Item	140 CP	150 CP	160 CP	180 CP
Ingredient (g kg^{-1})				
Corn grain	687.8	655.8	679.7	612.9
Soybean meal (450 g kg^{-1} CP)	179.1	206.2	134.8	188.7
Corn gluten meal (600 g kg^{-1} CP)	-	2.7	68.5	74.4
Soybean oil	16.2	21.2	2.3	13.2
Dicalcium phosphate	10.5	10.3	10.4	9.9
Limestone	91.8	91.8	92.0	92.0
Common salt	4.7	4.7	4.8	4.8
DL-methionine	2.9	2.7	1.8	1.2
Lysine HCL	2.1	1.2	2.9	1.1
L-valine	1.4	0.9	0.3	-
L-threonine	1.3	0.7	0.5	-
L-tryptophan	0.3	0.2	0.4	0.1
L-isoleucine	0.5	0.0	0.0	0.0
L-arginine	0.0	0.0	0.0	0.0
Vitamin supplement	1.0	1.0	1.0	1.0
Mineral supplement	0.5	0.5	0.5	0.5
BHT (butylhydroxytoluen)	0.1	0.1	0.1	0.1
Total	1000.0	1000.0	1000.0	1000.0
Nutritional values (g kg^{-1})				
Metabolizable energy (Mcal kg^{-1})	2,900	2,900	2,900	2,900
Crude protein	140.0	150.0	160.0	180.0
Digestible lysine	7.5	7.5	7.5	7.5
Digestible methionine + cystine	6.9	6.9	6.9	6.9
Digestible methionine	4.8	4.7	4.5	4.2
Digestible valine	7.1	7.2	7.2	7.7
Digestible threonine	5.7	5.7	5.7	6.0
Digestible tryptophan	1.7	1.7	1.7	1.7
Digestible isoleucine	5.7	5.7	6.0	7.0
Digestible arginine	8.1	8.9	8.0	9.5
Digestible phenylalanine	6.3	6.9	8.0	9.1
Glycine + serine	13.2	14.3	14.5	16.7
Digestible histidine	3.6	3.8	3.9	4.4
Digestible leucine	12.4	13.2	17.9	19.6
Linoleic acid	23.5	25.8	16.5	21.7
Calcium	38.5	38.5	38.5	38.5
Available phosphorus	2.8	2.8	2.8	2.8
Sodium	2.1	2.1	2.1	2.1

CP - crude protein.
Supplementation levels (amount per kg of product): 10,000 IU of vitamin A; 2,000 IU of vitamin D3; 1,833 mg of vitamin E; 2 mg of vitamin B1; 1,000 mg of vit. B2; 3 mg of vitamin B6; 0.015 mg vitamin B12; 12 mg of pantothenic acid; 3 mg of vitamin K3; 1 mg of folic acid; 0.25 mg of selenium; 33,333 mg of manganese; 6,567 mg of iron; 2,667 mg of copper; 250 mg of iodine; 26,667 mg of zinc; 6,000 mg of niacin; 70,000 mg of choline; 680 mg of ethoxyquin; 8,333 mg of halquinol.

by weighing each bird on the first day and the last day of the experiment. Feed conversion ratio was determined as a direct relationship between feed intake and dozens of eggs produced (kg/dz) and between feed intake and egg mass produced (kg/kg).

Data were subjected to analysis of variance, and means were compared by the Scott Knott test at $\alpha = 0.05$ significance level. Statistical analyses were performed by the SAEG v. 9.1 software. The proposed mathematical model was as follows:

$$y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk},$$

in which y_{ijk} = value observed in the rearing system i (i = 1, 2), level j (j = 1, 2, 3, 4), and repetition k (k = 1, 2, 3, ..., 10); μ = overall mean of the experiment; a_i = fixed effect of the system i (i = 1,2); b_j = fixed effect of the level j (j = 1, 2, 3, 4); $(ab)_{ij}$ = fixed effect of the interaction between system i (i = 1, 2) and level j (j = 1, 2, 3, 4); and e_{ijk} = random error in the system i (i = 1, 2), level j (j = 1, 2, 3, 4), and repetition k (k = 1, 2, 3, ..., 10).

3. Results

Dietary protein levels and rearing system showed no interaction for body weight gain, feed intake, egg production, egg weight, and feed conversion ratio (P>0.05) (Tables 2 and 3). Dietary protein levels did not interfere with feed intake and body weight gain (P>0.05); however, birds raised on the floor had higher feed intakes (P<0.05). Egg production was influenced by the rearing system, while dietary protein levels showed no effect on this parameter (P<0.05). Layers raised on the floor showed better egg production. Egg weight was affected neither by the increasing dietary protein levels nor by rearing system (P>0.05). Dietary crude protein levels did not affect feed conversion ratio (kg/dz and kg/kg). An effect (P<0.05) of rearing system was observed for feed conversion ratio per dozen eggs, in which hens raised in cages had better results (kg/dz), but this effect was not observed for feed conversion ratio per egg mass (kg/kg).

Statistical interaction between dietary crude protein levels and rearing systems was found for dirty eggs (P<0.05) (Table 4). Layers raised on the floor had higher percentages of dirty eggs (15.10%) compared with those raised in cages (5.67%). The percentage of dirty eggs was the same in the cage system, regardless of the dietary crude protein level (Table 5). Conversely, laying hens reared in the floor system and fed 140 g kg^{-1} CP produced less dirty eggs (Table 5). No interaction effect (P>0.05) was observed between dietary protein levels and rearing systems for percentages of cracked and broken eggs. Such rates were higher in the cage system but were not affected by dietary protein levels.

Table 2 - Body weight gain, feed intake, egg production, and egg weight of laying hens

Factor	Body weight gain (g)	Feed intake (g/bird/day)	Egg production (%)	Egg weight (g)
Rearing system (S)				
Cage	288.50	120.60b	76.86b	55.43
Floor	294.05	121.55a	80.47a	55.41
Crude protein (CP; g kg^{-1})				
140	290.70	119.80	77.28	55.20
150	289.30	122.00	78.62	55.32
160	293.40	121.50	80.62	55.19
180	291.70	121.00	78.13	55.98
ANOVA (P-value)				
CP × S	0.39	0.13	0.10	0.06
CP	0.83	0.11	0.09	0.06
S	0.09	0.02	0.002	0.08
CV (%)	3.46	1.12	1.52	2.98

CV - coefficient of variation.
Means followed by different lowercase letters in a column differ significantly from each other by the F test (P<0.05).

Table 3 - Feed conversion ratio of laying hens

Factor	Feed conversion ratio (kg/dz)	Feed conversion ratio
Rearing system (S)		
Cage	1.901b	2.456
Floor	2.093a	2.445
Crude protein (CP; g kg^{-1})		
140	1.950	2.481
150	2.124	2.454
160	2.028	2.494
180	1.888	2.374
ANOVA (P-value)		
CP × S	0.09	0.66
CP	0.08	0.36
S	0.02	0.10
CV (%)	13.12	6.89

CV - coefficient of variation.
Means followed by different lowercase letters in a column differ significantly from each other by the F test (P<0.05).

Table 4 - Percentage of dirty, cracked, and broken eggs of laying

Factor	Dirty egg (%)	Cracked egg (%)	Broken egg (%)
Rearing system (S)			
Cage	5.67b	2.51a	1.90a
Floor	15.10a	0.71b	1.24b
Crude protein (CP; g kg^{-1})			
140	10.00	1.68	1.59
150	10.55	1.66	1.54
160	10.38	1.53	1.58
180	10.78	1.59	1.56
ANOVA (P-value)			
CP × S	0.03	0.58	0.50
CP	0.13	0.18	0.55
S	<0.01	0.01	0.01
CV (%)	7.07	10.97	7.12

CV - coefficient of variation.
Means followed by different lowercase letters in a column differ significantly from each other by the F test (P<0.05).

Table 5 - Effects of the interaction between rearing system and dietary crude protein level on percentage of dirty egg

Crude protein (g kg^{-1})	Rearing system	
	Cage	Floor
140	5.91A	14.08B
150	5.83A	15.25A
160	5.47A	15.29A
180	5.69A	15.87A

Means followed by the different uppercase letters in a column differ significantly from each other by the Scott Knott test (P<0.05).

4. Discussion

According to Leeson and Summers (2005), laying hens can express their genetic potential at temperatures within the thermal comfort zone (19 to 27 °C). Silva (2000) considered that the ideal temperature for egg production would be between 21 and 26 °C, which is close to the conditions observed most of the time in our experiment. It is important to understand the effects of these systems under tropical conditions, as in Brazil, once these experimental results express the genetic potential of laying hens under such conditions.

The higher feed intake of layers raised in a floor system can justify the higher bird body weight gain. According to Netto et al. (2018), laying hens raised in a floor system are free to perform their natural behaviors, increasing movement and, hence, higher energy expenditure, which improves feed conversion per dozen eggs.

The model fitted to the data of dietary protein level was not significant for body weight gain. This result is similar to that of Sá et al. (2007), who did not verify any effect of crude protein levels on body weight gain of brown layers from 34 to 50 weeks of age.

Layers raised in a floor system had higher egg production (80.47%) than those in cages (76.86%). This parameter is one of the most important and is affected by some factors. Poultry raised on the floor can express its natural behavior and be under animal welfare conditions, which can increase egg production. According to Yilmaz Dikmen et al. (2016), in a free-range system, hens have better feather and bone traits and additional space for comfort and welfare. However, these authors also found that egg production was higher in the free-range system but statistically similar to that of the cage and enriched-cage systems.

Tactacan et al. (2009) did not observe effects on egg production for white laying hens between 21 and 60 weeks of age when housed in conventional or enriched cages. On one hand, Küçükyılmaz et al. (2012) observed that white laying hens produced 2.87% fewer eggs in an organic system (shed with access to free areas) compared with those in conventional cages. On the other hand, brown layers produced 4.23% more eggs in an organic system than hens in cages. In our study, brown hens had better performance in a floor system, which may have been due to the rusticity and easy adaptation of this strain compared with the white chickens.

Valkonen et al. (2006) compared egg production of white and brown layers raised in conventional and enriched cages (access to nest, perch, dust box, and larger available area of 750 cm^2/bird) and fed diets with reduced (14.7%) or high (190 g kg^{-1}) protein contents. The authors observed that from the 32nd week of age, birds housed in enriched cages produced fewer eggs than those housed in conventional cages but no effect was observed due to protein levels. Pérez-Bonilla et al. (2012) evaluated different levels of crude protein (165, 175, and 185 g kg^{-1}) and fat (1.8 and 3.6%) in diets on egg production and quality of brown layers between the 22nd and 50th week of age with different live weights (1,592 and 1,860 g), and observed no effect of crude protein levels on egg production. Likewise, Rama Rao et al. (2011) assessed the performance of hens between 21 and 72 weeks of age fed diets with three crude protein levels (150, 165, and 180 g kg^{-1}) and also observed no effects of crude protein levels on egg production, feed intake, and egg weight.

The similar egg weight produced by hens probably occurred because the birds used in our study were the same age. According to Şekeroğlu and Altuntaş (2009), egg weight is influenced by several factors such as lineage, heredity, room temperature, bird age, live weight, diet, and sanity. Similarly, Roll et al. (2009) evaluated the performance of brown layers in two rearing systems (floor and enriched cages) and did not detect any influence on egg weight. By contrast, while studying the welfare of commercial laying hens in different production systems and environmental conditions, Barbosa Filho (2006) observed lower egg weights in the cage rearing system. These authors associated the aforementioned to higher stress levels and difficulty in losing heat when birds are housed in cages. That could also explain our results.

Unlike what we observed in this study, other authors have reported that dietary protein level affects egg weight. Silva et al. (2010) concluded that egg weight of commercial laying hens increased linearly with increasing protein levels (120, 140, 160, and 180 g kg^{-1}). Pavan et al. (2005) assessed the performance of brown layers fed different protein levels (140, 150, 160, and 170 g kg^{-1}) and observed lower egg weight for diets with 170 g kg^{-1} of crude protein at 52 weeks of age. According to Silva et al. (2010), higher protein levels in diets affect egg weight since laying hens are unable to reserve protein efficiently for maintenance, therefore, varying with daily intake.

The feed conversion ratio (kg/dz) varies with feed intake and egg production, being considered an index of efficiency. Layers raised in a floor system fed more and had more room to move and spend their energy, which might have contributed to the worst feed conversion observed. The same was not observed for feed conversion expressed as kg/egg mass. This outcome is similar to that observed by Valkonen et al. (2006), who assessed the performance of white and brown laying hens raised in conventional or enriched cages (access to nest, perch, litter box, and 750 cm^2 available area/bird) and fed diets with reduced (147 g kg^{-1}) or high (190 g kg^{-1}) protein levels; yet, they did not observe any effect of rearing systems on feed conversion ratio.

Our findings show that it is possible to reduce dietary protein levels from 180 to 140 g kg^{-1}, maintaining levels of the main essential amino acids without affecting laying performance. Reduced protein levels in laying hen diets decrease nitrogen excretion (Roberts et al., 2007) and improve environmental quality (Meluzzi et al., 2001), besides reducing heat increase and feed costs.

Layers raised on the floor had a higher percentage of dirty eggs (15.10%) compared with those raised in cages (5.67%). Throughout the experimental period, eggs were rarely found on litter, which denotes the preference of birds to lay eggs in the nests. Likewise, Alves et al. (2007) and Roll et al. (2009) also verified a higher occurrence of dirty eggs for layers in a floor rearing system. Nonetheless, these results are contrary to the observations of Barbosa Filho et al. (2006), in which the number of dirty eggs tends to decrease when the space allowance per bird was satisfactory. Becker et al. (2011) highlighted that a higher rate of dirty eggs is related to the rearing environment. Birds raised in litter systems have a large area for locomotion and nesting, which may favor direct contact of eggs with excreta.

Dirty eggs may represent losses and egg contamination. Hannah et al. (2011) studied bacterial contamination of washed and non-washed eggshells from birds raised in conventional cages and on the floor; they observed that contamination level was similar in both systems, after washing with a commercial solution. However, compared with eggs produced on the floor, eggs from cages with excreta removal were less contaminated, regardless of washing.

If egg laying is performed in nests, the number of dirty eggs may be reduced. Zupan et al. (2008) studied laying hen preferences for nesting in individual boxes and verified that 17 out of the 24 birds preferred nests, and the other seven the floor (wood shavings). According to the authors, birds that laid eggs on the floor remained scratching and turning the substrate shortly before egg laying, while the others sought the nests. The authors also suggested that commercial laying hens have at least two behavioral patterns in terms of egg-laying site preferences, even for groups of the same age and genetic pattern.

Cracked and broken egg rates were higher in the cage system, which may be due to notches and slopes on the bottom of most cages (Barbosa Filho et al., 2006). These bottlenecks also represent losses and should be avoided.

5. Conclusions

A floor rearing system is an option for Hisex brown laying hens in the production phase. Performance of layers is not affected by reductions in dietary protein level from 180 to 140 g kg^{-1}.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.A. Costa. Data curation: E. Arnhold. Formal analysis: E.F. Viana and E. Arnhold. Funding acquisition: E.F. Viana, W.J. Souza and J.H. Stringhini. Investigation: E.F. Viana, F.B. Carvalho, H.H.C. Mello and J.H. Stringhini. Methodology: E.F. Viana, M.A. Costa, F.B. Carvalho, H.H.C. Mello, M.B. Café and J.H. Stringhini. Project administration: E.F. Viana, M.B. Café and J.H. Stringhini. Resources: J.H.

Stringhini. Supervision: W.J. Souza, E. Arnhold, F.B. Carvalho and J.H. Stringhini. Validation: M.A. Costa, E. Arnhold, H.H.C. Mello and M.B. Café. Writing-original draft: E.F. Viana, M.A. Costa and E. Arnhold. Writing-review & editing: M.A. Costa.

Acknowledgments

This work was supported by Instituto Federal Goiano/Campus Urutaí, Olvego Óleos Vegetais Ltda, MCassab, and Ajinomoto®.

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