Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v38i2.29533

Performance of Japanese quails fed diets with low-protein and isoleucine

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ABSTRACT. Aiming to assess isoleucine levels in low protein diets for laying Japanese quails, 648 quails of 182 days of age were distributed in experimental block design with six treatments and six replicates of 18 birds each. Treatments consisted of a basal level corresponding to 0.672% isoleucine and supplemented with isoleucine to the levels of 0.816, 0.960; 1.104; 1.248%. The experimental diets were compared to a control diet containing 20% CP. The parameters studied were: performance, egg quality, total solids and nitrogen in the excreta. There was a linear increase only for isoleucine intake with increasing levels of isoleucine in the diets. The comparison of mean values of each combination of isoleucine levels for diets with 16% CP with the control with 20% CP showed that the intake of CP and isoleucine, egg weight, nitrogen excretion and yolk color were significantly affected. For Japanese quail fed diets with 16% CP, with isoleucine level at 0.672%, meets the requirements for obtaining satisfactory performance and egg quality and promoted a reduction in nitrogen excretion.

Keywords: branched-chain amino acid, performance, nitrogen excretion, egg quality.

Niveis de isoleucina em dietas para codornas japonesas

RESUMO. Com o objetivo de avaliar níveis de isoleucina em rações para codornas japonesas em postura, foram utilizadas 648 codornas, distribuídas em delineamento em blocos ao acaso com seis tratamentos e seis repetições de 18 aves cada. Os tratamentos consistiram de ração basal com 16% de PB correspondente em nível de isoleucina de 0,672% e suplementada com isoleucina, em substituição ao ácido glutâmico, correspondendo aos níveis de isoleucina de 0,816; 0,960; 1,104; 1,248%. As dietas experimentais foram comparadas a uma dieta controle contendo 20% PB. Foram estudados o desempenho, a qualidade do ovo, os sólidos totais dos ovos e nitrogênio nas excretas das aves. Observou-se aumento linear apenas para consumo de isoleucina com elevação dos níveis de isoleucina das dietas. Pela comparação das médias de cada combinação de níveis de isoleucina para dietas com 16% PB com o tratamento controle 20% PB, verificou-se que o consumo de PB, consumo de isoleucina, peso do ovo, excreção de nitrogênio e cor da gema foram significativamente influenciados. Para codornas japonesas submetidas a dietas com 16% de PB, o nível de isoleucina de 0,672%, atende as exigências para obtenção de resultados satisfatórios de desempenho e qualidade de ovos e proporcionou uma redução na excreção de nitrogênio.

Palavras-chave: aminoácido ramificado, desempenho, excreção de nitrogênio, qualidade de ovos.

Introduction

Coturniculture is an important activity that is currently highly relevant in the Brazilian agriculture sector, but despite the increasing production, much is still unknown about Japanese quail nutrition. To make rational production viable, researches aimed at implementing appropriate feeding programs is needed during both the initial and production stages, where there is scarce research on the subject.

Among the studies available, those referring to protein levels are noteworthy, since excessive protein levels in feed are costly, in addition to increasing the excretion of nitrogen and environmental pollution. Moreover, the simple reduction of protein levels in feed without the correct supplementation of essential amino acids causes a reduction in feed intake and egg production, as well as altering the social behavior of the birds, resulting in cannibalism (Peganova & Eder, 2003).

Because of the availability of synthetic amino acids in the market, an alternative for cost reduction and for diet optimization is their incorporation to the feeds, allowing the formulation of diets containing crude protein levels lower than those nutritionally required.

Usually, studies conducted to evaluate the reduction in crude protein by means of amino acid supplementation use only L-Lysine, DL-methionine, L-threonine and L-tryptophan, but, progressive CP reduction in diets may lead to a situation in which other amino acids, such as arginine, valine and isoleucine, act as limiting factors for improved performance (Peganova & Eder, 2002).

Isoleucine is an essential amino acid of the family of aliphatic hydrophobic amino acids mainly found mainly in proteins and enzymes. After ingestion, L-isoleucine is absorbed by the small intestine and transported in the blood stream to the liver, where a part is used for the production of proteins, and the other is catabolized, in the presence of vitamin $B_{12,}$ into derivatives essential for energy production.

In general, isoleucine ranks fifth and sixth places, respectively, as the most limiting amino acid for broiler chickens and pigs. However, information on requirements with respect to quails is limited. In this way, the objective of this study was to evaluate levels of isoleucine in low-protein diets for Japanese quails, so as to obtain a level that optimizes the production and quality of eggs.

Material and methods

The experiment was conducted at UNESP – Univ. Estadual Paulista, Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, in the poultry farming section, between January and February 2012, during 63 days divided into three 21-day cycles.

Japanese quails (n = 648) with average initial laying rate of 89.90% and 182 days of age were housed in cages, with eighteen birds each. Experimental feeds were supplied freely.

Birds were distributed in a randomized block design, with six 18-bird repetition per experimental unit and six treatments.

Feeds were formulated based on the composition of the ingredients presented by Rostagno et al. (2005). Feeds were isocaloric (2800 kcal ME kg^{-1} feed) and isoproteic (16 % CP) with varying isoleucine levels, being isonutritious for the other nutrients, which were set in accordance with the recommendations of Silva (2009). A basal diet corresponding to a total level of isoleucine of 0.672% was supplemented with isoleucine (0.267; 0.411; 0.555; 0.699%), in substitution of glutamic acid, in protein equivalent, corresponding to isoleucine levels 0.816; 0.960; 1.104; 1.248% in the feed. The differences from adjusting for protein equivalents of isoleucine and glutamic acid were compensated by inert substance. Experimental diets were compared to a control diet containing 20% CP, totaling six treatments; the levels of the other nutrients were also in accordance with Silva (2009). The nutritional levels of the experimental diets are listed in Table 1.

Table 1. Estimated percentage and nutritional composition of experimental diets.

	Isoleucine levels (%)					
Ingredients	0.672	0.816	0.960	1.104	1.248	Control
Corn	65.748	65.748	65.748	65.748	65.748	57.468
Soybean meal (45%)	15.328	15.328	15.328	15.328	15.328	19.822
Wheat meal	7.516	7.516	7.516	7.516	7.516	6.675
Protenose ® (60%)	-	-	-	-	-	4.910
Meat and bone meal (41%)	-	-	-	-	-	2.628
Calcitic limestone	7.165	7.165	7.165	7.165	7.165	6.823
Dicalcium phosphate	0.946	0.946	0.946	0.946	0.946	-
L-Lysine	0.661	0.661	0.661	0.661	0.661	0.318
DL-Methionine	0.308	0.308	0.308	0.308	0.308	0.137
L-Threonine	0.271	0.271	0.271	0.271	0.271	0.082
L-Tryptophan	0.042	0.042	0.042	0.042	0.042	-
L-Isoleucine	0.123	0.267	0.411	0.555	0.699	0.189
L-Arginine	0.508	0.508	0.508	0.508	0.508	0.211
L-Valine	0.038	0.038	0.038	0.038	0.038	0.088
Glutamic Acid	0.697	0.523	0.349	0.174	0.000	-
Salt	0.350	0.350	0.350	0.350	0.350	0.350
Vitamin-mineral[1] supplement	0.300	0.300	0.300	0.300	0.300	0.300
Inert (Kaolitine)	0.000	0.030	0.060	0.090	0.120	-
Total (kg)	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition:						
Crude Protein (%)	16.00	16.00	16.00	16.00	16.00	20.00
Metabolizable Energy (kcal kg^{-1})	2,800	2,800	2,800	2,800	2,800	2,800
Calcium (%)	3.050	3.050	3.050	3.050	3.050	3.050
Available Phosphorus (%)	0.280	0.280	0.280	0.280	0.280	0.280
Total Lysine (%)	1.150	1.150	1.150	1.150	1.150	1.150
Total methionine + Cystine (%)	0.780	0.780	0.780	0.780	0.780	0.780
Total threonine (%)	0.790	0.790	0.790	0.790	0.790	0.790
Total tryptophan (%)	0.200	0.200	0.200	0.200	0.200	0.200
Total isoleucine (%)	0.672	0.816	0.960	1.104	1.246	0.960
Total arginine (%)	1.350	1.350	1.350	1.35	1.350	1.350
Total valine (%)[2]	0.686	0.686	0.686	0.686	0.686	0.980

[1]Content kg^{-1} of feed: Vit. A: 7,000 IU; Vit. D3: 2,000 IU; Vit. E: 50.00 IU; Vit. K3: 1.6 mg; Vit. B2: 3 mg; Vit. B12: 8 μg; Niacin: 20 mg; Pantothenic Acid: 5 mg; Choline: 234.36 mg; Selenium: 0.2 mg; manganese: 70 mg; Iron: 50 mg; Copper: 8 mg; Zinc: 50 mg; Iodine: 1.2 mg; Zinc bacitracin: 20 mg; Qsp vehicle: 1,000g. [2] level of valine determined by valine level experiments of the doctoral thesis of the first author.

Source: the authors.

The light schedule consisted of 17 hours of light per day, and during the entire experiment, the birds were exposed to identical feed management. Feeding was carried out twice daily.

Performance characteristics evaluated were: intake of feed, crude protein and isoleucine, laying percentage, percentage of whole eggs, average egg weight, egg mass, feed conversion per dozen eggs and per kilogram eggs produced and feasibility.

The quality analysis of the eggs was carried out every 21 days for three consecutive days. A sample of two eggs per repetition was collected every day, adding up to a total number of 108 eggs analyzed per treatment at the end of the three periods.

Quality characteristics evaluated were: internal quality: percentage of yolk, albumen, yolk color and total solids of whole eggs; external quality: specific gravity, shell percentage, shell thickness, shell resistance to breakage.

In the last day of each 21-day period, the total amount of solids in the eggs was evaluated using the methodology of Silva & Queiroz (2002)

At the end of the experimental period (63 days), nitrogen levels in the excretions were evaluated, expressed in 100% dry matter. Two samples were collected per treatment every 24 hours and during three consecutive days. Fecal collection trays were placed under the cages and protected by a plastic covering. Every sample was standardized and weighed for pre-drying in a forced ventilation oven at 55º for 48 hours. After pre-drying, samples were exposed to air to reach equilibrium with room temperature and humidity. They were immediately weighed, ground and stored in containers for analysis of nitrogen content according to the methodology of Empresa Brasileira de Pesquisa Agropecuária [Embrapa] (2005).

The statistical analysis of the results was run with the software SISVAR, in accordance with Ferreira (1998). Information referring to the characteristics evaluated, within the treatments used, was subjected to the analysis of variance (5% significance), and the effects of the levels of isoleucine underwent regression analysis; the degrees of freedom of the levels were broken down into linear, quadratic and cubic effects, for selecting the model that better fits the data. The control treatment (20% CP) was compared with the others (16% CP) by the Dunnett's test at 5% probability, using the software Action, developed in the software R.

Results and discussion

According to Table 2, there was an influence of the levels of isoleucine only on the consumption of this amino acid.

Feed intake and crude protein were not influenced by the levels of isoleucine in the diets. Mean values for these characteristics were 28.81 g feed bird^{-1} day^{-1} and 4.61 g crude protein bird^{-1} day^{-1}. The lack of significant results may be because alterations in the voluntary feed intake may take place according to the level of energy in the diet, or if the birds reduce the voluntary feed intake in conjunction with increased energy density, with the intention of maintaining constant energy intake. In this experiment, feeds were isocaloric and isoproteic, with varying levels of isoleucine, and it was not necessary to adjust feed intake to maintain constant the intake of energy and nutrients related to its consumption.

Harms and Russell (2000) failed to find any significant differences in feed intake in laying birds aged 36 weeks and fed corn and soybean meal-based feed supplemented with L-isoleucine in five levels (0.49 to 0.61%).

Table 2. Effects of isoleucine levels on feed intake (CR), crude protein intake (CPB), isoleucine intake (CISO), laying percentage (POST), egg weight (PO), percentage of whole eggs (OINT), egg mass (MO), feed conversion per egg mass (AC/Kg), feed conversion per dozen eggs (CA/dz), feasibility (VIAB) and nitrogen excretion (N).

Parameter			Isoleucine level 16% CP			Control 20% CP	
	0.672	0.816	0.960	1.104	1.248	0.960	CV (%)
CR (g)	28.39	29.22	28.23	28.73	29.46	28.91	4.60
CPB (g)	4.54*	4.68*	4.52*	4.60*	4.72*	5.78	4.56
CISO (mg)[1]	190.80*	238.48*	271.02	317.15*	367.65*	277.54	5.01
POST (%)	84.49	90.21	87.16	90.30	84.80	88.86	8.45
PO (g)	10.15	10.10*	10.05*	10.32	10.19	10.66	3.47
OINT (%)	99.68	99.79	99.55	99.63	99.28	99.65	0.40
MO (g)	8.61	9.11	8.78	9.32	8.63	9.47	10.00
AC Kg^{-1}	3.46	3.29	3.37	3.13	3.53	3.11	15.41
CA dz^{-1}	0.41	0.39	0.39	0.38	0.42	0.39	13.26
N (%)[2]	6.02*	5.64*	5.94*	5.66*	5.83*	7.36	6.12

Mean values followed by *in the same row are significantly different from the control treatment ($p < 0.05$) according to the Dunnett's test; [1]Regression for levels between 0.672 and 1.248 of isoleucine: linear effect: Y= -11. 23 + 300.26x; $R^2 = 0.99$; [2]nitrogen content expressed in dry matter of excreta.

On the other hand, Shivazad et al. (2002) observed differences in feed intake for commercial laying birds aged 35 weeks when supplying isoleucine levels varying between 0.39 and 0.60%. The authors verified a significant reduction both in feed and energy intake when the birds were fed diets containing isoleucine levels below 0.48%, which were not tested in this study.

Peganova and Eder (2002) also observed significant differences in feed intake for commercial laying birds aged between 25 and 32 weeks when testing eight isoleucine levels (0.39 to 0.81%), with the lowest consumption observed in birds fed 0.81% isoleucine. These authors highlighted that the margin between the demand and excess of isoleucine is very slight. The depressant effect of excess intake of isoleucine may be caused by the antagonism between this and another two branched chain amino acids, such as valine and leucine. All three are structurally similar, with branched chain and having the same transport systems, through cellular membranes, as well as using the same degradation enzymes (Harper, Miller, & Block, 1984). It may be inferred that in this experiment, the levels used, which were of up to 30% above the recommended levels, were insufficient to provoke the antagonism between the branched chain amino acids.

As for the comparison of values for each combination of isoleucine levels for 16% CP diets with the 20% CP control diet, it was verified that the feed intake presented no significant influence; as for the crude protein intake, it was significantly ($p < 0.05$) higher for birds fed higher protein level diets.

According to Costa, Souza and Gomes (2004), feed intake is not necessarily controlled by protein levels in the diet, but, Baker and Han (1994) reported an increase in feed intake among birds fed low levels of crude protein level, which was not verified during this study.

Lower crude protein intake was registered for all the treatments when compared to the control diet. This is due to the lack of difference in feed intake between the treatments with isoleucine levels and control feed, and because the control diet contained higher percentages of crude protein. These results coincide with those observed by Freitas, Fuentes, Freitas, Sucupira and Oliveira (2005) and Silva et al. (2010), which further observed that diets with higher crude protein content promoted a greater consumption of the nutrient.

As expected, increasing isoleucine levels in the diets resulted in a linear effect on isoleucine intake. The lowest isoleucine level (0.672%) meets the requirements of birds, therefore, there was no effect on performance. The graphic representation and the linear regression equation are illustrated in Figure 1.

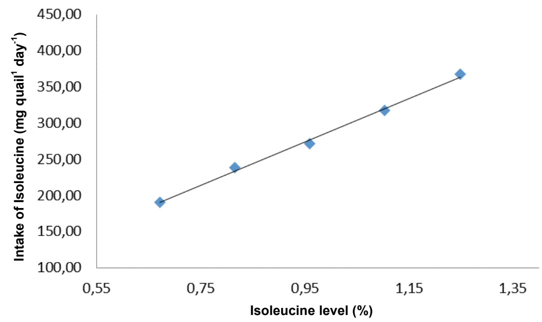

Figure 1. Estimated isoleucine intake among Japanese quails based on the levels of isoleucine in the diet.

The results obtained for the intake of isoleucine confirmed those demonstrated by Shivazad et al. (2002) and Peganova and Eder (2002), who worked with commercial laying birds and digestible levels of isoleucine of 0.39 to 0.81% in the feed, and observed a linear increase in isoleucine intake with increasing levels of isoleucine in the diet.

For the intake of isoleucine, the diet with 0.960% isoleucine did not differ from the control diet by the Dunnett's test, which was expected, as the control diet contained a level of 0.960%, the level recommended by Silva (2009). Diets with levels above and below those of the control diet present higher and lower isoleucine intakes, respectively, than those of the control diet.

The laying percentage was not affected by isoleucine levels in the diet, averaging 87.39%. Egg production is influenced by the availability of amino acids in the diet, as they will constitute the proteins present in the egg. Amino acids valine, isoleucine and leucine are known as BCAA (Branched Chain Amino Acids), and they compete with each other for absorption sites, however, we may presume that amino acid imbalance stemming from the levels of isoleucine used did not occur, and all the concentration levels evaluated met the requirements of the birds in respects to egg production.

Studies on laying hens aged 35 weeks conducted by Shivazad et al. (2002) showed that whenever they received diets with levels below 0.51% isoleucine, egg production was negatively affected.

No significant difference in laying percentage was revealed by Dunnett's test. The lack of effects of the treatments on the egg laying rate coincides with the data obtained by Freitas et al. (2005), who evaluated four levels of crude protein in diets for quails during the production phase (16 to 22%). Garcia et al. (2005)

recorded changes in the laying percentage of Japanese quails when assessing feeds containing three levels of crude protein (16, 18 and 20%). The authors obtained lower egg production with 16% of the nutrient in the diets, which probably occurred due to inadequate levels of amino acids in the diet.

Egg weight was not influenced by different levels of isoleucine added to the diets, with an average of 10.16 g. Egg weight is affected by the daily protein intake of the laying bird. Considering that there was no statistical difference in intake of feed and protein containing the levels of isoleucine evaluated, it may be inferred that our results for average egg weight suggest that the amino acid isoleucine has a weak influence on this variable.

Significant differences were found by Shivazad et al. (2002), who observed a reduction in egg weight in studies with laying birds aged 35 weeks, when supplying diets containing isoleucine levels below 0.48%. A reduction in the weight of eggs was not registered during this study, which could be because the levels used were over 0.48% isoleucine.

Dunnett's test evidenced lower egg weight for diets containing 0.686 and 0.960% isoleucine, when compared to the control diet, and birds fed the control diet (20% CP and 0.960% isoleucine) presented egg weight similar to those fed 16% CP and 0.672, 1.104 and 1.248% isoleucine. A significant difference in the percentage of whole eggs was not detected by Dunnett's test.

The egg mass was not significantly different between treatments, averaging 8.89g. Regarding that egg mass relates indices of egg laying percentage and egg weight, and that these characteristics were not influenced in this study, a lack of effects of isoleucine levels on egg mass was expected.

The Dunnett's test for egg mass showed no significant differences. Similar results to those found in this study were verified elsewhere (Mattos Filho, Pedroso, Moraes, & Ariki, 1999; Freitas et al., 2005; Silva et al., 2010).

Taking into account that no significant differences were observed in relation to feed intake, laying percentage and egg mass, the lack of effects of the treatments on the feed conversion per dozen or per kg eggs is justified, as these variables depend on each other. Analyzing different levels of isoleucine (0.39 to 0.81%) for commercial laying birds, Peganova and Eder (2002) also failed to find any significant differences for the feed conversion per egg mass and per dozen eggs.

No significant differences were found for feed conversion per dozen eggs and per egg mass between the control treatment and the others. According to Silva et al. (2010), no alterations were observed in feed conversion per egg mass, when evaluated four protein levels (12 to 18%) in diets for commercial laying birds. Likewise, Freitas et al. (2005) examined protein levels in quail diets (16 to 22%) and detected no changes in AC kg^{-1} eggs. When assessing three levels of crude protein for commercial laying birds (15, 16 and 17%), Garcia et al. (2005) verified improved feed conversion per dozen eggs for the level of 17% crude protein and a linear reduction on feed conversion per egg mass with increasing levels of protein in the diet.

According to the results in Table 2, there was no influence of isoleucine levels on nitrogen excretion. Considering that quails consumed equivalent amounts of crude protein between the treatments and similarly used the protein for egg production and composition, the inexistence of significant alterations in the nitrogen excreted is justified. Peganova and Eder (2002) also observed no negative effects on nitrogen excretion when studied isoleucine levels between 0.37 and 0.57% in the diet for commercial laying hens.

Nitrogen excretion in this study was on average 21% lower for 16% CP diets in relation to 20% CP diet. When studying the reduction in nitrogen excretion by laying birds receiving low protein diets (14, 15.5 and 17%), supplemented with sulphur-containing amino acids, Pavan, Mori, Garcia, Scherer and Pizzolante (2005) concluded that nitrogen excretion was 27% lower for 14% CP diets in relation to the diet with 17% CP. By evaluating CP levels (24, 20 and 18%) in Japanese quail diets, Minoguchi, Ohguchi, Yamamoto and Hanaki (2010) reached a 28% reduction in nitrogen excretion when the diet was reduced from 24 to 18%, which corresponds to a decrease of 4.66% in relation to each CP unit.

Information referring to the internal quality of eggs is found in Table 3.

Table 3. Effect of isoleucine levels on albumen percentage (ALB), yolk percentage (GEM), yolk color (CORGE) and total solid percentage (ST) of quail eggs.

Parameter	Isoleucine level					Control	
	16% CP					20% CP	
	0.672	0.816	0.960	1.104	1.248	0.960	CV (%)
ALB (%)	60.47	60.68	61.04	60.41	60.63	61.02	2.52
GEM (%)	31.32	31.25	30.76	31.62	31.33	31.26	3.82
CORGE	5.12*	5.13*	5.17*	5.02*	5.00*	6.43	7.30
ST (%)	26.34	26.49	26.63	26.29	26.05	26.59	2.41

Mean values followed by * in the same row are significantly different from the control treatment ($p < 0.05$) by Dunnett's test

There were no significant changes of isoleucine levels on egg internal quality parameters (Table 3). The values observed were 60.65, 31.26, 5.08 and 26.26%, respectively, for percentage of albumen and yolk, yolk color and total solids content. Divergent results were demonstrated by Harms and Russel (2000), who argued that when laying hens aged between 36 and 44 weeks received diets with isoleucine levels below 0.49% in the diet, the internal content of the eggs was reduced. The authors recommended an intake of 601.4 mg day^{-1} isoleucine for an optimum level of egg content.

The Dunnett's test evidenced no significant differences for albumen and yolk percentages and total solid content. The results are similar to those presented by Abdel-Azeem (2011), who detected no influence of protein levels of the diets (14 to 20%) on the percentage of albumen and yolk in Japanese quail eggs, however, found differences in yolk color. In the same way, Garcia et al. (2005) did not observe any effects caused by different protein levels in the feed, ranging from 16 to 22%, on the percentage of albumen and yolk in quail eggs.

Also, Novak, Yakout and Scheideler (2006) reported no effects of dietary protein content (18.9, 17.0 and 14.4%) on the total solids content in eggs, as observed in this study. According to Coon (2002), the albumen solids are almost entirely protein-based, and the demand for protein and amino acids for its composition is high, i.e., the lack of protein or amino acids would result in a reduced amount of albumin, and as a consequence, in the egg size.

The crude protein intake by quails of the control treatment was greater, but had no influence on protein deposition in the albumen and egg yolk, given the lack of effects on the percentage of albumen and yolk. In this sense, 16% crude protein level and 0.672% isoleucine level (intake of 190.80 mg bird^{-1} day^{-1}) promoted a protein supply sufficient for the formation of albumen and yolk.

Egg yolk color was not influenced by the levels of isoleucine evaluated. This result may be attributed to the nutritional composition of the experimental diets, which involved an equivalent inclusion of corn amongst the treatments. Corn is rich in pigments, which directly affect the coloring of yolks.

The comparison of values of each combination of isoleucine level and 16% CP with the 20% CP control showed that only the yolk color was significantly affected ($p < 0.05$), in which the worst yolk color was verified in eggs of the treatments of all isoleucine levels when compared to those of the control diet. This result is probably associated with the nutritional composition of the control diet, which contained 60% corn gluten. It is known that this ingredient is rich in pigmenting agents, like xanthophyll and canthaxanthin, with direct influence on the color of the yolk.

According to the results in Table 4, there was no influence of isoleucine levels on the external quality characteristics of eggs evaluated. The mean values were 8.06%; 0.19mm; 1.0800 g cm^{-3}; 1.3064 Kgf, respectively, for percentage of shell, shell thickness, specific gravity and resistance to breakage.

There is a high positive correlation between the eggshell percentage, eggshell thickness, specific gravity and eggshell resistance to breakage. Nonetheless, studies evaluating this parameter are relatively new in the literature, which does not allow for a broad comparison of results.

The Dunnett's test revealed no significant differences for the eggshell percentage, eggshell thickness, specific gravity and eggshell resistance to breakage.

The lack of effects of the protein levels on eggshell quality was also observed in other studies.

Conclusion

For Japanese quails given 16% CP diets, the 0.672% isoleucine level, equating to a daily intake of 190.80 mg isoleucine and/or 22.16 mg g^{-1} egg^{-1} mass day^{-1}, met the expectations for achieving satisfactory performance and egg quality.

The level of crude protein in the feed for laying Japanese quails may be reduced to 16% crude protein without affecting the performance of birds or the quality of eggs, provided the diet is properly supplemented with the limiting essential amino acids. A reduction in CP also caused a reduction by 21% in nitrogen excretion, on average.

Table 4. Effect of isoleucine levels on the eggshell percentage (ESHELL), eggshell thickness (ESP), specific gravity (GE) and eggshell resistance to breakage (RQ) of quail eggs.

Parameter	Isoleucine level 16% CP					Control 20% CP	
	0.672	0.816	0.960	1.104	1.248	0.960	CV (%)
ESHELL(%)	8.22	7.93	8.00	8.02	8.11	8.00	3.44
ESP (mm)	0.19	0.19	0.19	0.19	0.19	0.19	3.29
GE (g cm^{-3})	1.078	1.077	1.077	1.082	1.084	1.085	1.44
RQ (kgf)	1.3421	1.2866	1.2895	1.2898	1.3241	1.3225	6.81

Mean values followed by * in the same row are significantly different from the control treatment ($p < 0.05$) by Dunnett's test.

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Received on October 18, 2015.
Accepted on November 27, 2015.