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Dietary energy-density and digestible lysine formulation strategies for Japanese quails in the late laying phase under hot and humid tropical conditions

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ABSTRACT - This study evaluated the laying performance, egg quality, and economic responses of Japanese quails in the late laying phase fed diets differing in energy density and digestible lysine levels under hot and humid tropical conditions. Six hundred 175-day-old laying Japanese quails were distributed in a completely randomized design with a 3 × 4 factorial arrangement, totaling 12 treatments with five replicates and 10 birds per experimental unit. Dietary treatments were formulated to contain three energy densities (2600, 2875, or 3150 kcal/kg metabolizable energy; ME) combined with four digestible lysine levels (0.90, 1.05, 1.20, or 1.35%; DLys). During the 112-day feeding period, birds had *ad libitum* access to feed and water and were kept at an average temperature of 27.00 ± 2.35 °C and a relative humidity of 81.70 ± 5.86%. No ME × DLys interaction was detected (P>0.05). Compared with the low-energy diet, diets formulated with 2875 or 3150 kcal/kg ME increased feed intake, DLys intake, egg production, and egg mass, and improved economic responses and feed conversion (P<0.05). The 3150 kcal/kg diet increased ME intake, body weight, and egg specific gravity (P<0.05). Increasing DLys levels linearly increased DLys intake (P<0.05), but did not improve productive performance, egg quality, or economic responses. Therefore, under a practical feed formulation approach, diets formulated with moderate or high energy density supported better productive and economic responses than the low-energy diet, whereas increasing DLys above 0.90% provided no additional benefits. These responses reflect the comprehensive outcome of dietary energy-density strategies driven by ingredient availability or feed-cost constraints, encompassing the physical and nutritional properties of fiber-rich or oil-rich feedstuffs.

Keywords: *Coturnix coturnix japonica*, dietary energy density, egg production, feed formulation, tropical environment

1. Introduction

Japanese quail (*Coturnix coturnix japonica*) farming has gained economic interest because of its high egg production potential, rapid production cycle, and reduced space needs (Jansson et al., 2025).

To optimize productivity and profitability, nutritional management is critical during the laying phase, when birds require an adequate nutrient supply for body maintenance and egg production (Sakomura and Rostagno, 2016).

Dietary metabolizable energy (ME) density and digestible lysine (DLys) are central components of cost-effective feed formulation for laying quails. Dietary energy density influences voluntary feed intake, nutrient intake, feed conversion, and feed cost, whereas DLys is closely related to protein synthesis, egg formation, and proper balance of essential amino acids (Kim et al., 2020). However, because feed intake may change according to dietary energy density, DLys should be evaluated not only as a dietary concentration but also in relation to energy intake and productive performance (Ton et al., 2011; Ribeiro et al., 2013).

Nutritional recommendations for Japanese quails vary according to age, laying performance, feed intake, environmental conditions, and genetic background (Barros Júnior et al., 2024). Current recommendations for laying quails indicate approximately 2850 kcal/kg ME and DLys levels ranging from 1.048 to 0.993% as the laying phase advances (Rostagno et al., 2024). However, responses may differ under tropical production conditions, particularly when dietary energy density and amino acid supply are adjusted through practical feed formulation strategies (Jongbo et al., 2026).

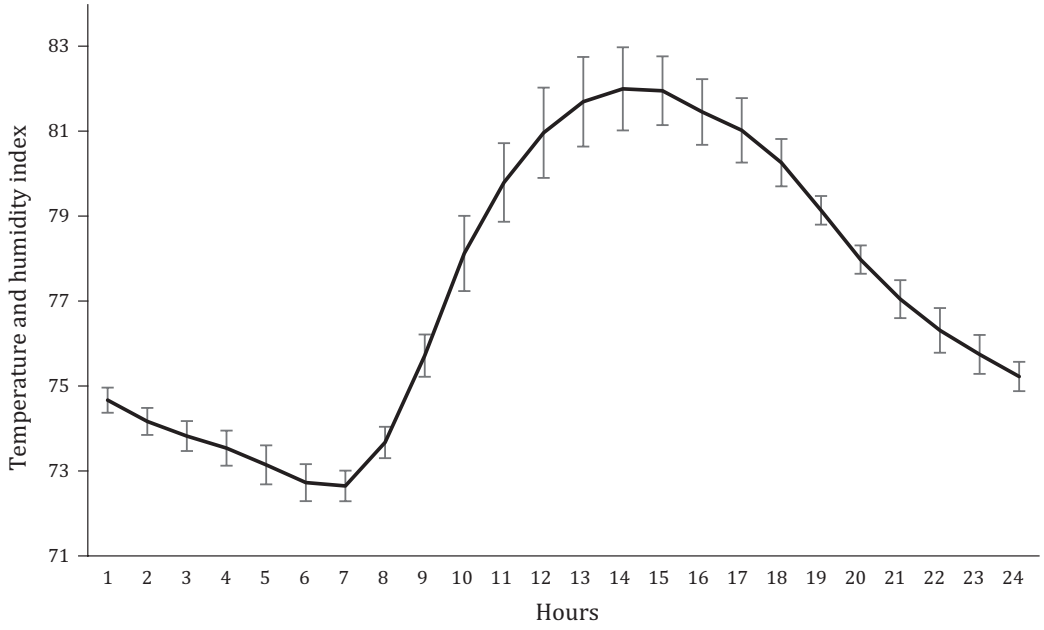
In practical feed formulation, dietary energy density is adjusted according to nutrient requirements, ingredient availability, feedstuff prices, and expected feed intake (Pomar et al., 2021). Lower-energy diets often include fiber-rich ingredients, whereas higher-energy diets commonly require oil or other energy-dense ingredients (Hervik and Svihus, 2019). These formulation choices may alter dietary nutrient density, physical characteristics, and the energy-to-amino acid relationship, with potential consequences for voluntary feed intake, laying performance, egg quality, and economic returns (Mohebbifar et al., 2025). Therefore, this study evaluated laying performance, egg quality, and economic responses of Japanese quails in the late laying phase fed diets differing in energy density and DLys levels under hot and humid tropical conditions, focusing on practical formulation strategies in which changes in dietary energy density inherently involve changes in ingredient composition (such as soybean hulls and oil inclusion).

2. Material and methods

The experiment was carried out in the experimental warehouse of the Nonruminant Nutrition Research sector of the Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso, Sinop, Mato Grosso, Brazil (379 m altitude, 11°51'48.024" South latitude 55°29'8.160" West longitude of Greenwich). The research was previously approved by the Ethics Committee in the Use of Farming Animals at the Universidade Federal de Mato Grosso (protocol number 23108.71669/2015-12).

Six hundred laying Japanese quails (*Coturnix coturnix japonica*) from the Fujikura lineage, 175 days of age and weighing 188 ± 10 g, were distributed in a completely randomized design with 12 dietary treatments in a 3 × 4 factorial arrangement (dietary ME density × DLys levels), with five replicates per treatment and 10 quails per experimental unit. Birds were housed in galvanized wire cages (25 cm wide × 35 cm deep × 18 cm high) in an open-sided shed with wire-mesh sidewalls and a fiber-cement tile roof. The shed was equipped with two fans operating during the day for air renewal. Birds were exposed to 17 h of light per day, including natural and artificial lighting, throughout the 112-day experimental period, divided into four consecutive 28-day cycles. Ambient temperature and relative humidity were recorded hourly using a digital data logger. The temperature and humidity index (THI; Figure 1) was calculated according to Thom (1959).

Dietary treatments were formulated to contain three ME densities (2600, 2875, or 3150 kcal/kg) combined with four DLys levels (0.90, 1.05, 1.20, or 1.35%). The experimental diets (Table 1) were formulated on an as-fed basis using the chemical composition of feedstuffs and nutritional recommendations described by Rostagno et al. (2011). Dietary energy densities were achieved



Values above 78 indicate more challenging thermal conditions for laying quails, occurring between 11:00 and 20:00 h.

Figure 1 - Hourly temperature–humidity index (THI) recorded during the experimental period.

Table 1 - Composition of experimental diets (as-fed basis)

	Dietary metabolizable energy (ME) density (kcal/kg)											
	2600				2875				3150			
Dietary digestible lysine (DLys) level (%)	0.90	1.05	1.20	1.35	0.90	1.05	1.20	1.35	0.90	1.05	1.20	1.35
Ingredient												
Corn	55.6	55.4	55.3	55.3	58.8	59.5	60.2	60.9	52.4	53.0	53.6	54.4
Soybean meal (45% CP)	28.7	27.9	27.0	26.0	30.3	29.5	28.7	27.7	31.4	30.7	29.9	28.9
Soybean hulls	7.10	7.65	8.20	8.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soybean oil	0.00	0.00	0.00	0.00	2.15	1.90	1.65	1.40	7.45	7.21	6.97	6.73
Dicalcium phosphate	1.08	1.09	1.10	1.10	1.09	1.10	1.11	1.12	1.10	1.11	1.11	1.12
Limestone	6.60	6.60	6.59	6.59	6.68	6.68	6.67	6.68	6.67	6.67	6.67	6.67
DL-Methionine	0.24	0.37	0.50	0.65	0.23	0.36	0.49	0.63	0.24	0.36	0.49	0.63
L-Lysine HCl	0.02	0.24	0.46	0.68	0.01	0.23	0.45	0.67	0.00	0.00	0.11	0.21
L-Threonine	0.00	0.02	0.13	0.24	0.00	0.01	0.11	0.21	0.00	0.00	0.11	0.21
Salt	0.33	0.33	0.33	0.33	0.32	0.32	0.32	0.32	0.33	0.33	0.33	0.33
Trace mineral and vitamin premix[1]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100	100	100	100	100	100	100
Calculated composition												
Crude protein (%)	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5
Crude fiber (%)	4.82	4.94	5.07	5.20	2.63	2.60	2.56	2.53	2.58	2.55	2.51	2.48
Ether extract (%)	2.73	2.73	2.72	2.72	4.80	4.56	4.33	4.09	9.86	9.64	9.41	9.18
Digestible methionine + cystine (%)	0.74	0.86	0.98	1.11	0.74	0.86	0.98	1.11	0.74	0.86	0.98	1.11
Digestible threonine (%)	0.54	0.63	0.72	0.81	0.54	0.63	0.72	0.81	0.54	0.63	0.72	0.81
Calcium (%)	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92
Available phosphorus (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Linoleic acid (%)	1.43	1.43	1.43	1.43	2.52	2.40	2.27	2.15	5.20	5.07	4.95	4.83

[1] Trace mineral and vitamin guaranteed levels (per kg of premix): vit. A - 2,500,000 IU; vit. D3 - 500,000 IU; tocopherol - 6,250 mg; thiamine - 375 mg; riboflavin - 1,500 mg; pyridoxine - 600 mg; vit. B12 - 3,000 mcg; vit. K3 - 500 mg; folic acid - 175 mg; biotin - 17.5 mg; calcium pantothenate - 3,000 mg; niacin - 8,750 mg; choline chloride - 87.5 g; zinc oxide - 12.5 g; iron sulphate - 12.5 g; manganese sulphate - 14.5 g; copper sulphate - 3,000 mg; cobalt sulfate - 50 mg; iodine - 250 mg; selenium - 80 mg.

through practical adjustments in soybean hull and soybean oil inclusion levels. Synthetic L-lysine HCl, DL-methionine, and L-threonine were added to obtain the planned DLys levels while maintaining dietary crude protein concentration and the methionine + cystine-to-lysine and threonine-to-lysine ratios. Diets were provided in mash form, and birds had *ad libitum* access to feed and water.

Eggs were collected daily, counted, weighed, and classified as marketable, defective or broken. At the end of each 28-day cycle, total egg production, marketable egg production, and total egg weight were recorded for each experimental unit. Feed offered, feed wastage and refusals were weighed to calculate feed intake. Birds weighted by experimental unit at the end of each cycle.

Feed intake (FI), body weight (BW), egg production (EP), egg mass (EM), viable eggs for marketing (VE), and feed conversion ratio (FCR) were calculated for each experimental unit. Egg production (EP, %) was obtained by dividing the daily average number of eggs laid by the number of birds in each experimental unit, multiplied by 100. Egg mass (EM, g/day) was calculated by multiplying egg production by average egg weight and expressed as g/bird/day. Viable eggs for marketing (VE, %) were calculated by dividing the number of marketable eggs by the total number of eggs produced and multiplying by 100. Feed conversion rate (FCR) was calculated by dividing total FI by the number of dozens of eggs produced or by total egg mass and expressed as kg/dz and g/g, respectively. Daily ME intake (MEI) and DLys intake (DLysI) were calculated from FI and dietary ME and DLys concentrations and expressed as kcal/bird/day and mg/bird/day, respectively.

For egg quality analysis, three eggs per experimental unit were sampled during the last four days of each 28-day cycle, according to the average egg weight of each experimental unit (±10%). Thus, 12 eggs per per experimental unit were evaluated per cycle, totaling 60 eggs per treatment per cycle.

Specific gravity (SG) was determined using saline solutions (NaCl) with densities ranging from 1.066 to 1.102 g mL^{-1}, according to Bennett (1993). Each egg was sequentially immersed in saline solutions of increasing density until flotation, and the density of the solution in which the egg floated was recorded as its specific gravity (Feddern et al., 2017).

After SG, eggs were broken on a flat glass surface, and albumen height was measured using a digital caliper. Haugh units (HU) were calculated according to Stadelman et al. (1995):

$$HU = 100 \text{ Log } (H + 7.57 - 1.7\ W^{0.37}),$$

in which H is the height of the albumen (HA) (mm); W is the weight of the egg (g); 7.57 is the correction factor for albumen height; and 1.7 is the correction factor for egg weight.

Albumen (PA), yolk (PY), and shell (PS) percentages and albumen pH were determined according to Freitas et al. (2011). Shell thickness (TS) was measured with a digital micrometer (Mitutoyo®) by averaging three measurements in the equatorial region of the egg (Fonseca et al., 2021).

Economic analysis was performed by calculating gross income (GI), margin over feed (MOF), and profitability index (PI), according to Martin et al. (1998). Feed cost was calculated from the ingredient composition of each diet, feed intake, and ingredient prices. Egg selling price was based on the local market price of quail eggs. Gross income was calculated as GI = Q × SP, where Q is the number of eggs produced per experimental unit per day and SP is the egg selling price. Margin over feed was calculated as MOF = GI − FC, where FC is the daily feed cost per experimental unit. Profitability index was calculated as PI = (MOF/GI) × 100. Gross income and margin over feed were expressed as R$/day/experimental unit, and profitability index was expressed as percentage. Ingredient and egg prices were obtained from local market quotations in Sinop, Mato Grosso, Brazil.

Data was checked for normality and homogeneity of variances. When assumptions were not met, Box-Cox or square-root transformation [$\sqrt{(Y+1)}$] was applied. Data were analyzed using SISVAR software (Ferreira, 2011), considering the cage as the experimental unit. Variables measured over the four consecutive 28-day cycles were averaged for each experimental unit over the 112-day experimental period. Thus, the final analysis of variance was performed using one overall mean per experimental unit, according to the following model:

$$Y_{ijk} = \mu + ME_i + DLys_j + ME \times DLys_{ij} + e_{ijk},$$

in which Y_{ijk} is the dependent variable measured in experimental unit k fed a diet containing the ME density i and DLys level j; μ is the overall mean; ME_i is the fixed effect of dietary ME density (i = 2600, 2875, or 3150 kcal/kg); $DLys_j$ is the fixed effect of dietary DLys level (j = 0.90, 1.05, 1.20, or 1.35%); $ME \times DLys_{ij}$ is the fixed effect of the interaction between ME density and DLys level; and e_{ijk} is the residual error.

When the ME × DLys interaction was significant (P<0.05), simple effects were evaluated. Dietary ME density means were compared using the Scott-Knott test. Linear and quadratic contrasts were used to evaluate the dose-dependent effects of dietary DLys levels.

3. Results

The average room temperature and relative humidity during the experimental period were 27.00 ± 2.35 °C and 81.70 ± 5.86%, respectively. No ME × DLys interaction (P>0.05) was detected for laying performance (Table 2), egg quality (Table 3), or economic variables (Table 4).

Compared with the low-energy diet, diets formulated with 2875 or 3150 kcal/kg ME increased FI, digestible lysine intake (DLysI) EP, EM, and VE, and improved FCR (P<0.05; Table 2). The diet formulated with 3150 kcal/kg ME increased metabolizable energy intake (MEI) and body weight (BW) compared with the 2600 and 2875 kcal/kg ME diets (P<0.05). Increasing dietary DLys levels linearly increased DLysI (P<0.01), but did not affect FI, MEI, BW, EP, EM, VE, or FCR.

Egg weight (EW) and albumen pH were greater in birds fed diets formulated with 2875 and 3150 kcal/kg ME than in birds fed low-energy diet (P<0.05; Table 3). Egg specific gravity was greater in birds fed the 3150 kcal/kg ME diet than in those fed the 2600 or 2875 kcal/kg ME diets (P<0.05). Yolk percentage, Haugh unit, albumen height, albumen percentage, shell percentage, and shell thickness were not affected by dietary ME density or DLys levels (P>0.05).

Compared with low-energy diet, diets formulated with 2875 and 3150 kcal/kg ME improved gross income (GI), margin of feed (MOF) and profitability index (PI) (P<0.01; Table 4).

Table 2 - Laying performance of Japanese quails fed diets differing in metabolizable energy (ME) density and digestible lysine (DLys) levels

Variable	Dietary ME density (kcal/kg)			Dietary DLys level (%)				CV (%)	P-value		
	2600	2875	3150	0.90	1.05	1.20	1.35		ME[1]	DLys[2]	ME × DLys[3]
Feed intake (g/day)	25.37b	27.11a	28.25a	26.37	27.50	26.81	26.96	12.41	0.0304	0.8284	0.2508
DLys intake (mg/bird/day)	287b	305a	317a	237	289	322	364	11.54	0.0283	<0.0001	0.2867
ME intake (kcal/bird/day)	65.98c	77.93b	89.00a	76.31	79.19	77.39	77.65	12.25	<0.0001	0.8719	0.3032
Body weight (g)	188b	188b	199a	191	191	190	195	5.57	0.0013	0.6711	0.1981
Egg production (%)	72.62b	87.98a	90.72a	83.88	82.96	84.15	84.10	12.15	<0.0001	0.9877	0.9322
Egg mass (g/day)	10.87b	11.39a	11.50a	11.08	11.30	11.30	11.33	3.42	<0.0001	0.2470	0.0717
Marketable egg (%)	97.47b	98.40a	98.47a	97.70	98.27	98.19	98.30	1.13	0.0101	0.4188	0.4613
FCRg	3.21b	2.84a	2.74a	2.91	3.10	2.82	2.90	12.25	0.0003	0.2005	0.7578
FCRdz	0.42b	0.38a	0.38a	0.39	0.42	0.38	0.39	10.55	0.0059	0.1429	0.2508

FCRg - feed conversion ratio per egg mass; FCRdz - feed conversion ratio per dozen eggs produced; CV - coefficient of variation.
[1] Main effect of dietary ME density.
[2] Main effect of dietary DLys level.
[3] Interaction between dietary ME density and DLys level.
Data are reported as means. For main effects, n = 20 for ME density and n = 15 for DLys levels.
Within each row, means followed by different letters differ by the Scott-Knott test (P<0.05).
Regression equation:
Linear effect of dietary DLys levels on DLysI (DLysI = −6.81068 + 275.358658 × DLys, R^2 = 99.30).

Table 3 - Physicochemical quality of eggs from Japanese quails fed diets differing in metabolizable energy (ME) density and digestible lysine (DLys) levels

Variable	Dietary ME density (kcal/kg)			Dietary DLys level (%)				CV (%)	P-value		
	2600	2875	3150	0.90	1.05	1.20	1.35		ME[1]	DLys[2]	ME × DLys[3]
Egg weight (g)	11.15b	11.45a	11.54a	11.33	11.39	11.33	11.48	2.90	0.0014	0.5862	0.5737
Yolk percentage (%)	30.58	30.26	30.26	29.85	30.70	30.51	30.41	4.09	0.6408	0.2819	0.2142
Haugh unit	95.26	93.88	94.43	93.37	94.68	94.64	95.41	2.32	0.1479	0.0965	0.4833
Albumen height (mm)	5.41	5.37	5.31	5.28	5.42	5.35	5.40	3.44	0.2681	0.1819	0.1653
Albumen percentage (%)	61.99	62.26	62.31	62.62	61.92	62.01	62.18	2.01	0.6794	0.4294	0.3567
Albumen pH	9.17b	9.28a	9.26a	9.21	9.22	9.25	9.27	1.18	0.0077	0.4914	0.7318
Specific gravity (g/cm^3)	1.077b	1.077b	1.078a	1.078	1.077	1.077	1.077	0.13	0.0131	0.2326	0.3904
Shell percentage (%)	7.60	7.60	7.61	7.67	7.62	7.59	7.55	4.06	0.9843	0.7359	0.8008
Shell thickness (mm)	0.218	0.219	0.223	0.220	0.220	0.220	0.219	3.28	0.0821	0.9197	0.5778

CV - coefficient of variation.
[1] Main effect of dietary ME density.
[2] Main effect of dietary DLys level.
[3] Interaction between dietary ME density and DLys level.
Data are reported as means. For main effects, n = 20 for ME density and n = 15 for DLys levels.
Within each row, means followed by different letters differ by the Scott-Knott test (P<0.05).

Table 4 - Economic responses of Japanese quails fed diets differing in metabolizable energy (ME) density and digestible lysine (DLys) levels

Variable	Dietary ME density (kcal/kg)			Dietary DLys level (%)				CV (%)	P-value		
	2600	2875	3150	0.90	1.05	1.20	1.35		ME[1]	DLys[2]	ME × DLys[3]
Gross income (R$/experimental unit/day)	1.02b	1.25a	1.22a	1.14	1.10	1.20	1.19	9.69	<0.0001	0.0638	0.5099
Margin over feed (R$/experimental unit/day)	0.54b	0.73a	0.70a	0.65	0.61	0.70	0.67	19.13	<0.0001	0.2027	0.5912
Profitability index (%)	51.59b	58.22a	57.28a	53.03	53.57	57.67	55.52	11.27	0.0031	0.3651	0.5151

CV - coefficient of variation.
[1] Main effect of dietary ME density.
[2] Main effect of dietary DLys level.
[3] Interaction between dietary ME density and DLys level.
Data are reported as means. For main effects, n = 20 for ME density and n = 15 for DLys levels.
Within each row, means followed by different letters differ by the Scott-Knott test (P<0.05).

4. Discussion

The experimental period was characterized by hot and humid conditions, with average room temperature and relative humidity of 27.00 ± 2.35 °C and 81.70 ± 5.86%, respectively. These values were above the thermal comfort range of 18 to 22 °C reported for birds (Rosa et al., 2011). The THI pattern (Figure 1) indicated more challenging thermal conditions, with values above 78 from 11:00 to 20:00 h (NUPEA, 2010; Macari and Maiorka, 2017). High ambient temperatures can directly affect poultry performance (Pereira et al., 2010, 2022), and relative humidity above 75% may further impair heat dissipation because birds depend mainly on evaporative respiratory heat loss under warm conditions (Lima et al., 2014). However, bird responses to thermal challenge vary according to genetics, sex, age, body size, diet, physiological state, acclimatization, air temperature, and humidity (Pereira and Nääs, 2008; Lara and Rostagno, 2013; Cândido et al., 2020). Although bird-based physiological indicators were not measured to confirm the clinical heat stress status, the environmental data indicate a challenging thermal environment during most of the day. Therefore, evaluating energy-density and DLys strategies is relevant for laying quails under hot and humid tropical conditions. In tropical climate zones, restrictions in feed intake and reductions in laying performance, egg mass, and egg quality may affect the profitability of quail farming (Fonseca et al., 2021).

In the present study, dietary ME density was adjusted through practical modifications in ingredient composition, specifically by varying the inclusion of soybean hulls and soybean oil. This approach reflects common commercial feed formulation practices, where dietary energy is manipulated according to ingredient availability and feed-cost constraints. However, from a strictly biological standpoint, this formulation strategy introduces a confounding dietary composition, as treatments differed not only in ME levels but also in crude fiber and fat contents. Consequently, the superior productive and economic responses observed in the 2875 and 3150 kcal/kg ME groups cannot be attributed exclusively to metabolizable energy alone. Instead, these findings must be interpreted as integrated outcomes of energy-density formulation strategies, reflecting changes in dietary palatability, reduced crude fiber content resulting from soybean hull removal, and the potential benefits of soybean oil inclusion on nutrient density and digesta passage rate. The 2600 kcal/kg ME diets included soybean hulls and had greater crude fiber content, whereas the 2875 and 3150 kcal/kg ME diets were formulated without soybean hulls and with soybean oil inclusion. This formulation approach reflects common feed formulation practice, in which energy density is adjusted not only to meet energy supply but also according to ingredient availability, feed cost, and expected nutrient intake. Therefore, the observed responses should be interpreted as outcomes of dietary energy-density strategies accounting for the physical and nutritional properties of fiber-rich or oil-rich feedstuffs.

Birds fed the 2600 kcal/kg ME diets had lower feed intake than those fed 2875 or 3150 kcal/kg ME diets (25.37 vs 27.11 or 28.25 g/day, respectively; $P<0.05$). Although birds can regulate feed intake according to dietary energy density to meet energy requirements for maintenance, body weight regulation, and egg production (Barreto et al., 2007), this regulatory capacity may be limited by the physical characteristics of the diet and by environmental conditions that challenge heat dissipation (Özbey et al., 2004; Freitas et al., 2017; Soares et al., 2020). In the present study, the higher inclusion of soybean hulls and greater crude fiber content in the 2600 kcal/kg ME diets may have increased dietary bulk and limited voluntary feed intake, preventing birds from compensating for the lower dietary energy density. Although soybean hulls can be included up to 20% in diets for Japanese quails without impairing productive performance, egg quality, or serum metabolic traits under some conditions (Duarte et al., 2013), the present results suggest that the response to fiber-rich ingredients depends on the overall energy-density strategy, environmental conditions, and productive stage.

Conversely, the 2875 and 3150 kcal/kg ME diets, formulated without soybean hulls and with soybean oil inclusion, supported greater feed intake and ME intake. The inclusion of oil may increase dietary energy density with less heat increment and may also affect palatability, digesta passage, and nutrient utilization (Attia et al., 2020), which may have contributed to greater egg production, egg mass, and improved feed conversion. Ribeiro et al. (2014) reported that increasing dietary ME from 2700 to 3000 kcal/kg reduced feed intake in laying hens, indicating that voluntary intake responses to energy density may vary according to species, diet composition, and formulation strategy. Thus, the higher feed intake observed in birds fed the 2875 and 3150 kcal/kg ME diets in the present study should be interpreted within the combined context of energy density, soybean hull withdrawal, soybean oil inclusion, and diet physical characteristics.

The 2875 and 3150 kcal/kg ME diets resulted in similar FI, DLysI, EP, EM, VE, FCdz and FCg, whereas the 3150 kcal/kg ME diet had greater MEI and BW. Fonseca et al. (2021) also reported improved performance responses in laying quails fed diets containing 3150 kcal/kg ME, and Pinto et al. (2002) reported better FCR values with diets containing between 2950 and 3050 kcal/kg for Japanese quail in the late laying phase. In the present study, however, increasing dietary energy density from 2875 to 3150 kcal/kg ME did not further improve most productive responses, suggesting that Japanese quails in the late laying phase did not require the highest energy-density strategy to maintain egg production and egg mass. Thus, the moderate-energy formulation strategy was sufficient to sustain laying performance while limiting unnecessary increases in energy intake and body weight.

Dietary factors affecting shell resistance include energy, minerals, and vitamins (Vercese et al., 2012). In this study, egg specific gravity was greater in birds fed the 3150 kcal/kg diet, although the observed value (1.078 g/cm^3) remained slightly below the reference values of 1.080 to 1.084 g/cm^3 reported for

egg quality and self-life (Oliveira and Oliveira, 2013). This response may be associated with the greater feed intake and nutrient intake observed in birds fed the high-energy diet, which may have increased the supply of nutrients involved in eggshell formation. However, because shell percentage and shell thickness were not affected, the biological relevance of this small difference in specific gravity should be interpreted with caution. Egg quality responses should also be interpreted within the physiological context of the late laying phase. As birds age, egg size may increase without a proportional increase in calcium carbonate deposition in the shell, which can reduce shell quality and specific gravity (Carvalho and Fernandes, 2013; Reis et al., 2021).

The greatest economic responses were observed in birds fed diets with 2875 or 3150 kcal/kg ME, mainly because of improvements in egg production, egg mass, and feed conversion compared with the low-energy, higher-fiber diet. However, because productive responses were similar between 2875 and 3150 kcal/kg ME, the decision to increase dietary energy density within this range should consider feedstuff price fluctuations, soybean oil cost, productive output, feed conversion, and body weight control.

Increasing dietary Dlys levels from 0.90 to 1.35% increased DLys intake but did not improve laying performance, egg quality, or economic responses. Therefore, under the dietary energy-density strategies and environmental conditions evaluated in the present study, a dietary DLys level of 0.90% was enough to support laying performance. Amino acids supplied above the requirement may be deaminated, with the carbon skeleton used for energy production or fat deposition and surplus nitrogen excreted as uric acid, or transaminated for non-essential amino acid synthesis, both of which are energy-consuming pathways (Ribeiro et al., 2003; Bernardino et al., 2013). Thus, excessive DLys supply may reduce nutrient-use efficiency without improving productive or economic outcomes.

5. Conclusions

In conclusion, diets formulated with moderate (2875 kcal/kg) or high (3150 kcal/kg) ME density supported better laying performance, feed conversion, and economic responses in Japanese quails during the late laying phase under hot and humid conditions compared with a low-energy, fiber-rich diet. Increasing DLys above 0.90% provided no additional productive or economic benefits. Rather than establishing a definitive, isolated ME requirement, these findings highlight that energy-density strategies driven by ingredient prices and availability must carefully balance the physical and nutritional properties of fiber-rich or oil-rich feedstuffs to optimize voluntary feed intake and productive performance.

Data availability

The entire dataset supporting the results of this study is available upon request to the corresponding author.

Author contributions

Conceptualization: Ton, A. P. S. **Data curation:** Rodrigues, C. P. **Formal analysis:** Andrade, E. A. **Investigation:** Ton, A. P. S. and Fonseca, T. S. **Methodology:** Rodrigues, C. P.; Sbardella, M. and Grieser, D. O. **Project administration:** Ton, A. P. S. **Supervision:** Ton, A. P. S. **Visualization:** Freitas, L. W. **Writing – original draft:** Ton, A. P. S.; Rodrigues, C. P.; Fonseca, T. S.; Sbardella, M.; Corassa, A.; Andrade, E. A. and Grieser, D. O. **Writing – review & editing:** Ton, A. P. S.; Sbardella, M.; Corassa, A. and Freitas, L. W.

Conflict of interest

The authors declare no conflict of interest.

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