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Metabolizable energy and digestible lysine requirements of growing Japanese quail (*Coturnix coturnix japonica*)

Exigência de energia metabolizável e lisina digestível para codornas japonesas (*Coturnix coturnix japonica*) em crescimento

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Highlights

Metabolizable energy (ME) and digestible lysine (DL) requirements were estimated.
Starter diets for Japanese quail should contain 3030 kcal ME and 1.221% DL.
In the grower phase, the recommended levels are 3055 kcal ME and 1.202% DL.
The maximum bone mineral density is reached with 2942 kcal ME.

Abstract

This study aimed to estimate metabolizable energy (ME) and digestible lysine (DL) requirements of Japanese quail in the starter (1 to 14 days) and grower (15 to 42 days) phases and investigate the influence of these dietary factors on body chemical composition, relative organ weights, and blood and bone parameters. The design was completely randomized with a 4 × 4 factorial arrangement (ME = 2830, 2970, 3110, and 3250 kcal × DL = 0.90%, 1.07%, 1.24%, and 1.41%), totaling 16 treatments, 3 replications per treatment, 50 birds per experimental unit in the starter phase (n = 2400 birds), and 35 birds per experimental unit in the grower phase (n = 1680 birds). In the starter phase, there was no interaction between factors on bird performance. Body weight, body weight gain, feed intake, and feed conversion ratio had a quadratic response to ME and DL levels in starter diets. In this phase, there was

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an interaction effect on carcass ether extract content, femur Seedor index, and tibia Seedor index and a linear main effect of DL on the relative weight of the liver. In the grower phase, factors had a significant interaction effect on body weight gain and feed intake. Feed conversion ratio responded quadratically to both factors, and body weight, femur Seedor index, tibia Seedor index, and femur density were quadratically influenced by ME. Estimates derived from overlaid contour plots showed that Japanese quail require 3030 kcal ME and 1.221% DL in the starter phase and 3055 kcal ME and 1.202% DL in the grower phase.
Key words: Body development. Residual effect. Overlaid contour plots.

Resumo

Este trabalho objetivou estimar as exigências nutricionais de energia metabolizável (EM) e lisina digestível (LD) para codornas Japonesas nas fases de cria (1 a 14 dias) e recria (15 a 42 dias) e verificar suas implicações na composição química corporal, peso relativo dos órgãos, parâmetros sanguíneos e ósseos. O delineamento adotado foi o inteiramente casualizado em esquema fatorial 4 x 4 (EM = 2.830, 2.970, 3.110 e 3.250 kcal x LD = 0,90; 1,07; 1,24 e 1,41%), perfazendo 16 tratamentos com 3 repetições cada, contendo 50 codornas por unidade experimental na fase de cria (totalizando 2.400 aves) e 35 codornas por unidade experimental na fase de recria (totalizando 1.680 aves). Não foi verificada interação entre os fatores sobre o desempenho de codornas Japonesas na fase de cria, sendo que as variáveis peso médio, ganho de peso, consumo de ração e conversão alimentar apresentaram efeito quadrático tanto para EM quanto para LD. Nesta fase, o extrato etéreo da carcaça e o índice de Seedor do fêmur e da tíbia exibiram interação significativa, e o peso relativo do fígado apresentou efeito linear da LD. Na fase de recria houve interação dos fatores para ganho de peso e consumo de ração. A conversão alimentar apresentou efeito quadrático de ambos os fatores e o peso médio foi influenciado de modo quadrático pela EM, que também influenciou de modo quadrático o índice de Seedor nos dois ossos e a densidade óssea do fêmur. Considerando as estimativas obtidas por meio dos gráficos de contornos sobrepostos, os níveis de 3.030 kcal de EM e 1,221% de LD foram estimados para a fase de cria e os níveis de 3.055 kcal de EM e 1,202% de LD foram estimados para a fase de recria.
Palavras-chave: Desenvolvimento corporal. Efeito residual. Gráfico de contornos sobrepostos.

Introduction

In a balanced diet, metabolizable energy (ME) levels should be aligned with essential nutrient levels, as these two dietary parameters are closely related. Feed volume and total nutrient concentrations are inversely related to energy contents (Classen, 2017). Thus, high energy levels result in decreased nutrient availability and increased production costs, impairing animal performance during growth.

In view of the relationship between feed ME and nutrient levels, Kaur et al. (2008) studied the performance and immunity responses of growing quail to diets containing different levels of essential amino acids and ME. The authors concluded that an optimal diet should contain 2700 kcal ME, 25.83% crude protein, 1.49% total lysine, 0.58% total methionine, and 1.17% total threonine for improved feed conversion from the first to the fifth week of age.

Lysine is used as a reference amino acid for designing diets according to the ideal protein concept. Therefore, it is of utmost importance to accurately determine the lysine requirements of birds at each developmental phase, given that this parameter will be used to determine the required levels of other amino acids (Bailey, 2020). Lysine has important physiological functions in the animal body, participating in protein deposition (Siqueira et al., 2013), bone matrix formation in young animals (Ribeiro et al., 2003), and synthesis of carnitine, which plays a role in transporting fatty acids into mitochondria for β-oxidation (Golzar Adabi et al., 2011).

Recent studies assessed the lysine requirements of growing Japanese quail (*Coturnix coturnix japonica*), such as Mehri et al. (2013, 2015), who estimated that quail aged 7 to 21 days and 21 to 35 days had digestible lysine (DL) requirements of 1.34% and 1.36%, respectively. However, the vast majority of studies determine amino acid requirements based on total amino acid contents, following the National Research Council [NRC] (1994) method. It is essential to specifically consider DL levels in diet formulation.

When determining nutritional requirements for maximization of animal responses, it is possible to use overlaid contour plots to simultaneously analyze several variables. This approach is widely used in industrial research but somewhat new in animal nutrition research (Zainal et al., 2014). In view of these observations, this study aimed to estimate the ME and DL requirements of Japanese quail in the starter (1 to 14 days) and grower (14 to 42 days) phases and identify the optimal conditions for animal performance using overlaid contour plots.

Material and Methods

All experimental procedures were previously approved by the Animal Ethics Committee at the State University of Maringá (CEUA protocol No. 4066080715/2015).

This study was conducted between July and August 2014. The experiment was designed to determine the ME and DL requirements of Japanese quail in the starter (1 to 14 days) and grower (15 to 42 days) phases. The temperature and relative humidity of the housing shed were measured daily by using a thermohygrometer. The maximum and minimum temperatures were 24.5 and 14.2 °C, respectively, and the maximum and minimum relative humidity values were 78.2% and 65.4%, respectively.

Animals, facilities, and management

The experiment was conducted in the Quail Production Sector of the Iguatemi Experimental Farm, State University of Maringá, Brazil, and was divided into two phases (starter and grower). One-day-old female Japanese quail (C. coturnix japonica) were purchased from a commercial breeding farm (Vicami® line, Assis, SP, Brazil) and raised in 2.5 m2 cages in a conventional shed with French tile roof, earthen floor covered with rice straw, 0.50 m high masonry walls covered with wire mesh up to the roof and equipped with movable side curtains.

The birds used for determination of nutritional requirements in the starter phase were housed according to the experimental design, whereas those used in the grower phase were housed and raised separately in order to avoid possible residual effects of treatments from the starter phase.

Water and feed were available *ad libitum* throughout the experimental period. Feed was provided in tray feeders and water in chick troughs up to 10 days of age. From 10 days of age onward, these were replaced by tubular feeders and automatic pendular troughs. In the starter phase (up to 14 days of age), protective circles were placed around the cages to minimize temperature fluctuations and wind exposure. Heating was provided by electric hoods with infrared drying lamps (250 W), which were kept on 24 h a day until the 10th day of age.

Experimental design and diets

The experimental design was completely randomized with a 4 × 4 factorial arrangement (ME = 2830, 2970, 3110, and 3250 kcal × DL = 0.90%, 1.07%, 1.24%, and 1.41%), totaling 16 treatments, 3 replications per treatment, 50 birds per experimental unit in the starter phase (n = 2400 birds, initial mean weight = 7.05 ± 0.08 g), and 35 birds per experimental unit in the grower phase (n = 1680 birds, initial mean weight = 21.47 ± 1.29 g).

Diets were formulated based on the recommendations and chemical composition values of ingredients described by Rostagno et al. (2011), except for the amino acid profile of corn, soybean meal, and corn gluten meal, which were previously determined by high-performance liquid chromatography (HPLC) by Ajinomoto Animal Nutrition (São Paulo, Brazil).

Birds received the same diets in both phases (Table 1). However, birds used for determination of nutritional requirements in the grower phase were fed a diet based on corn and soybean meal (2900 kcal ME, 22.0% crude protein, 1.12% DL, 0.76% digestible methionine + cysteine, 0.90% Ca, 0.37% P, and 0.18% Na) up to 14 days of age, following the recommendations of Rostagno et al. (2011).

Table 1
Ingredient composition and calculated nutrient levels of experimental diets containing different metabolizable energy and digestible lysine contents for Japanese quail in the starter and grower phases

ME, kcal	2830				2970				3110				3250			
DL, %	0.90	1.07	1.24	1.41	0.90	1.07	1.24	1.41	0.90	1.07	1.24	1.41	0.90	1.07	1.24	1.41
Corn	59.56	59.56	59.56	59.56	58.90	58.90	58.90	58.90	58.24	58.24	58.24	58.24	57.59	57.59	57.59	57.59
Soybean meal, 46% CP	28.65	28.65	28.65	28.65	28.69	28.69	28.69	28.69	28.73	28.73	28.73	28.73	28.77	28.77	28.77	28.77
Corn gluten meal, 67% CP	4.26	3.94	3.61	3.28	4.31	3.98	3.65	3.32	4.36	4.03	3.70	3.37	4.41	4.08	3.75	3.42
Soybean oil	0.00	0.04	0.07	0.11	1.81	1.85	1.89	1.93	3.63	3.67	3.70	3.74	5.44	5.48	5.52	5.56
Limestone	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19
Dicalcium phosphate	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48
DL-Methionine, 99%	0.17	0.17	0.18	0.19	0.17	0.17	0.18	0.19	0.17	0.17	0.18	0.19	0.17	0.17	0.18	0.19
L-Lysine HCl, 78%	0.00	0.22	0.44	0.66	0.00	0.22	0.44	0.66	0.00	0.22	0.44	0.66	0.00	0.22	0.44	0.66
L-Threonine, 99%	0.14	0.14	0.15	0.15	0.14	0.14	0.15	0.15	0.14	0.14	0.15	0.15	0.14	0.14	0.15	0.15
L-Tryptophan, 99%	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Common salt	0.40	0.31	0.22	0.13	0.40	0.31	0.21	0.12	0.40	0.30	0.21	0.12	0.40	0.30	0.21	0.12
Sodium bicarbonate	0.00	0.09	0.19	0.20	0.00	0.09	0.18	0.27	0.00	0.09	0.19	0.28	0.00	0.10	0.19	0.28
Mineral and vitamin premix1	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Inert (kaolin)	3.74	3.80	3.85	3.91	2.49	2.55	2.61	2.66	1.25	1.30	1.36	1.41	0.00	0.06	0.11	0.17
Calculated values, %																
ME, kcal kg^{-1}	2.830	2.830	2.830	2.830	2.970	2.970	2.970	2.970	3.110	3.110	3.110	3.110	3.250	3.250	3.250	3.250
CP	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
DL	0.90	1.07	1.24	1.41	0.90	1.07	1.24	1.41	0.90	1.07	1.24	1.41	0.90	1.07	1.24	1.41
Digestible methionine + cysteine	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76	0.76
Digestible threonine	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Digestible tryptophan	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Calcium	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Available phosphorus	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Sodium	0.18	0.16	0.15	0.14	0.18	0.16	0.15	0.14	0.18	0.16	0.15	0.14	0.18	0.16	0.15	0.14

Abbreviations: ME, metabolizable energy; DL, digestible lysine; CP, crude protein.
1Provided per kg of feed: manganese, 155 mg; zinc, 126 mg; iron, 98 mg; copper, 30.62 mg; iodine, 1.94 mg; selenium, 0.51 mg; cobalt, 0.40 mg; vitamin A, 18,000 IU; vitamin D3, 5,000 IU; vitamin E, 16 mg; menadione, 4.03 mg; thiamine, 1.11 mg; riboflavin, 8 mg; pyridoxine, 2.10 mg; vitamin B12, 20 µg; pantothenic acid, 16 mg; niacin, 40 mg; choline, 560 mg.

Animal performance

Quail and feed were weighed at the beginning of the experiment and at the end of each experimental phase (starter and grower). These data were used to calculate the following performance parameters: final body weight (g), body weight gain (g), feed intake (g), and feed conversion ratio (g g^{-1}). Dead birds were counted daily for correction of feed intake, as recommended by Sakomura and Rostagno (2016).

Carcass parameters

Body chemical composition was determined at the end of each experimental phase. At 14 and 42 days of age, 10 and 3 birds per experimental unit, respectively, were selected based on mean weight (±5%), fasted for 6 h, and sacrificed. Carcasses were frozen whole (with feathers, viscera, feet, and head) and subsequently thawed, weighed, pre-ground in an industrial mill, homogenized, and pre-dried in a forced-ventilation oven at 55 °C for 72 h. Then, samples were ground in a knife mill and taken to the Laboratory of Food Analysis and Animal Nutrition for chemical composition analyses.

Dry matter (method 925-09), mineral matter (method 923-03), crude protein (method 920-87), and ether extract (method n. 920-85) contents were determined according to Association of Official Analytical Chemistry [AOAC] (2005) methods. Body composition data of 14- and 42-day-old birds and additional data on 30 chicks slaughtered at hatch were used to calculate protein (PDR) and fat (FDR) deposition rates (g day−1), following a method adapted from Fraga et al. (2008). PDR and FDR values were then used to estimate carcass energy retention (CER) by the equation proposed by Sakomura (2004).

Blood biochemical parameters

In each experimental unit, four and two birds were selected on the basis of mean body weight (±5%) at 14 and 42 days of age, respectively, and fasted for 6 h. Subsequently, blood collection was performed for quantification of serum levels of albumin, total protein, uric acid, cholesterol, and alkaline phosphatase. Furthermore, globulin concentration was estimated as the difference between total protein and albumin, and the albumin/globulin ratio was determined.

Blood was collected from the ulnar vein, and samples were placed in test tubes and immediately centrifuged at 3,000 rpm for 15 min. After separation, the serum was placed in labeled Eppendorf tubes and stored at −10 °C until analysis. Samples were analyzed on a spectrophotometer (BioPlus Produtos para Laboratórios Ltda, modelo Bio-2000) using commercial kits (Gold Analisa Diagnóstica Ltda) specific for each parameter.

Relative organ weights

At 14 and 42 days of age, two birds per experimental unit were sacrificed and eviscerated for extraction of the heart, liver, gizzard, and small intestine. The ovary and oviduct were sampled only at 42 days of age. Organs were weighed on a precision scale for estimation of relative weights, calculated using bird body weight.

Bone parameters

From birds sampled for blood and organs at 14 and 42 days of age, the tibia and femur of the left leg were harvested for determination of Seedor index, radiographic optical density, bone strength, and ash content. After harvesting, bones were frozen (−18 °C), subjected to removal of muscle tissue, weighed on a precision scale, and measured for length (mm) using a digital caliper (Digimess, precision of 0.02 mm). From these data, the Seedor index was calculated, as proposed by Seedor et al. (1996). For determination of the other parameters, bones were degreased by immersion in petroleum ether for 24 h and pre-dried in a forced-air oven at 55 °C for 72 h. For measurement of radiographic optical density (mm Al eq), images were obtained at the Dental Clinic of the University Hospital of Maringá. Bones were placed on periapical film (Kodak® Intraoral E-Speed film) along with metal numbering for identification and an aluminum wedge (10 steps, 1 mm increments). Then, samples were X-rayed (Spectro 70X Eletronic, Dabi Atlante®, operating at 70 kVp, 7 mA) using an exposure time of 0.2 s and a focus-film distance of 6 cm, as previously determined.

Radiographic films were processed using an automatic processor (Revel Indústria e Comércio De Equipamentos Ltda), with Kodak RP X-OMAT solutions, scanned by a scanner, and saved as .jpeg files. Images were further processed using the histogram tool of Adobe Photoshop® CS6 software, which is based on a 256 grayscale, in which 0 represents black and 256 represents white. Radiographic optical density was determined by comparing the area of three central points of the bone (10 × 10 pixels) with that of the third step of the aluminum wedge.

Bone strength (kgf) analysis was conducted at the Soil Mechanics Laboratory, Department of Civil Engineering, State University of Maringá, Brazil. Samples were subjected to simple compressive strength tests. For this, bones were placed in the anteroposterior position, supported in the region of the epiphyses. A force was applied to the central region via a probe with a speed of 5 mm s^{-1} and a load of 500 N. The force at rupture was recorded. After determining bone strength, we assessed the dry matter (method 925-09) and mineral matter (method 923-03) contents of the tibia and femur together, according to AOAC (2005).

Statistical analysis

Statistical analysis of the data was performed in the R statistical environment (R Core Team [R], 2013). When the effects of factors were significant ($P<0.05$), DL and ME data were subjected to polynomial regression. The best polynomial model fit was determined according to Montgomery (2013). The nutritional requirement for each significant variable was determined based on a quadratic model, as proposed by Sakomura and Rostagno (2016).

After regression models were determined and ME and DL requirements were estimated, weight gain and feed conversion data were used to construct contour plots (or contour lines) superimposed on curves of the regression models. The analysis was conducted in the R statistical environment (R, 2013).

Results and Discussion

Animal performance

There were no interaction effects of ME and DL on Japanese quail performance in the starter phase. However, ME and DL levels influenced body weight, body weight gain, feed intake, and feed conversion ratio quadratically. The estimated optimal levels of ME and DL were 3064 kcal ME and 1.13% DL for body weight; 3085 kcal ME and 1.13% DL for body weight gain; 3094 kcal ME and 1.27% DL for feed intake; and 2941 kcal ME and 1.19% DL for feed conversion ratio (Table 2).

In the grower phase (Table 2), there were significant ME and DL interaction effects on body weight gain (2986 kcal ME and 1.27% DL) and feed intake (2958 kcal ME). ME and DL quadratically influenced feed conversion ratio, and the best results were estimated to be achieved with 2941 kcal ME and 1.19% DL. ME had a quadratic effect on body weight, with an estimated optimal level of 3069 kcal ME.

Overlaid contour plots were generated to facilitate interpretation of the results for the dependent variables through combined analysis of multiple responses. For the starter phase, ME and DL requirements were estimated at 3030 kcal and 1.221%, respectively (Figure 1). For the grower phase, the estimated requirements were 3055 kcal ME and 1.202% DL (Figure 2).

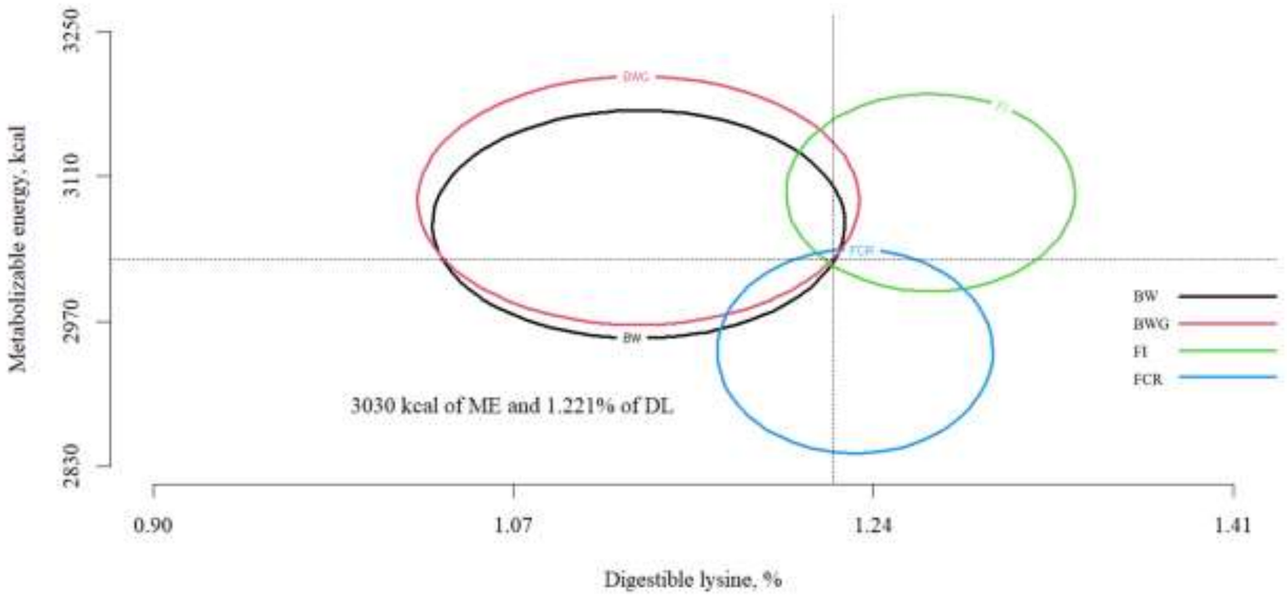

Figure 1. Overlaid contour plots for body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) according to metabolizable energy (ME) and digestible lysine (DL) intakes of Japanese quail from 1 to 14 days of age.

ME is a regulator of feed intake, and DL is the reference amino acid in the ideal protein concept. These variables may be considered the most important when formulating economically efficient diets for Japanese quail in starter and grower phases. Here, the influence of ME and DL on bird performance was evident, as all performance parameters were affected by ME and DL levels.

Feed intake, influenced quadratically by the main and interaction effects of ME and DL in the grower phase, tended to decrease with increasing ME levels, thereby improving feed conversion ratio. Classen (2017) and Jesus et al. (2023) observed that, by increasing ME levels in grower diets for Japanese quail, there was a significant decrease in feed intake and an improvement in feed conversion ratio throughout the experimental period. These findings suggest that, during growth, feed energy content is a crucial parameter. In situations of low energy intake, birds consume a greater amount of feed but do not show improvements in body weight gain, possibly resulting in distension of digestive organs and underdevelopment of the reproductive system. According to Abdollahi et al. (2018), birds adjust their feed intake according to dietary energy levels: quail fed low-energy diets tend to compensate for insufficient energy levels by ingesting a greater amount of feed, as a strategy to maintain physiological functions. This behavior confirms the existence of a consumption regulation mechanism.

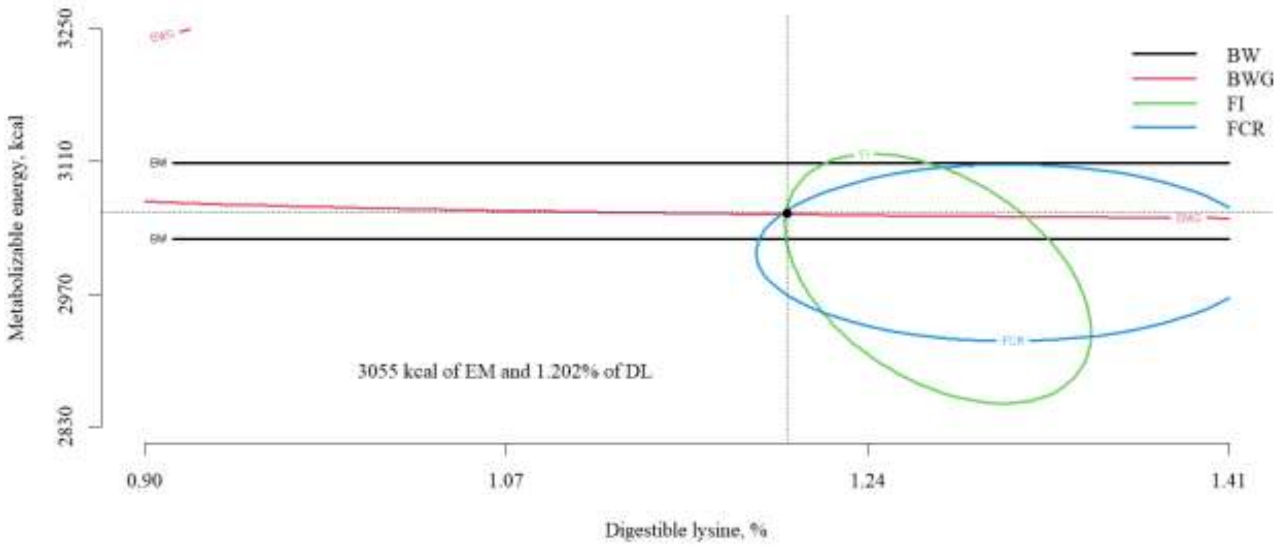

Figure 2. Overlaid contour plots for body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) according to metabolizable energy (ME) and digestible lysine (DL) intakes of Japanese quail from 15 to 42 days of age.

The results show that both body weight and body weight gain increased with increasing dietary ME up to a certain limit. After a certain level, as evidenced by the inflection point of the curve, Japanese quail stop responding to energy increments, demonstrating limited body growth. ME levels above such a threshold may compromise performance, as birds reduce feed intake in the case of excessively high ME levels, possibly resulting in insufficient nutrient intake.

The optimal DL levels for body weight and body weight gain were the same in the starter phase (1.13%), in agreement with Hajkhodadadi et al. (2013), who found that DL requirements for these variables tend to be aligned. The authors, in analyzing the effect of dietary lysine on Japanese quail performance and immunity, observed that DL had a significant effect on performance at various ages and that feed conversion ratio improved significantly with the increase in DL. Similarly, Lima et al. (2016), in estimating the optimal DL level of grower Japanese quail, found that the amino acid had an effect on performance variables. Mehri et al. (2013, 2015) underscored that the total lysine levels required to improve feed conversion ratio may be higher than those required for growth. Bouyeh (2013) stated that increased total lysine levels reduce feed conversion ratio, possibly translating into greater feed efficiency as a result of improved energy and protein metabolism.

Overlaid contour plots allowed identification of the intersection area of variables, visually indicating the viable ME and DL levels for a combination of variables. The viable region of the plots represents the combination of factors that lead to the best responses of dependent variables. All combinations were compatible with the desired responses. Therefore, the lowest ME and DL levels that satisfied the conditions of variables within the viable region of the graph were selected. ME and DL requirements for the starter phase were estimated at 3030 kcal ME and 1.221% DL, corresponding to a daily intake of 26.46 kcal ME and 106.84 mg of DL. For the grower phase, ME and DL requirements were 3055 kcal ME and 1.202% DL, corresponding to daily intakes of 50.09 kcal ME and 193.86 mg of DL.

The energy levels determined here for starter and grower phases were higher than those reported by Silva and Costa (2009), of 2990 and 3050 kcal ME, respectively. These findings show that current quail lineages have greater energy requirements. It is also evident that energy requirements increase with increasing age in quail, in contrast to the recommendations of NRC (1994), Reda et al. (2015), and Rostagno et al. (2017), who proposed the same ME level for both growth phases (2900 kcal ME).

Our DL estimates were higher than those estimated by Silva and Costa (2009), which were of 1.19% during the starter phase and 1.05% during the grower phase. Lima et al. (2016), in determining the DL requirements of grower Japanese quail, estimated a DL level of 1.18% for birds aged 0 to 40 days. By contrast, literature data show higher DL requirements for the starter phase, such as 1.276% DL in the study of Hajkhodadadi et al. (2013), Shivazad et al. (2013), Attia (2014), and Reda et al. (2015). For the grower phase, the reported requirements were closer to those estimated here, such as 1.21%, 1.20%, 1.14%, and 1.05% DL reported by Hajkhodadadi et al. (2014), Mehri et al. (2015), Reda et al. (2015), and Lima et al. (2020), respectively.

Such differences in requirements might be related to the genetic improvement of Japanese quail lineages over the past years. With improvements in performance, quail may demand more nutrients. As underscored by Hajkhodadadi et al. (2014), the nutritional recommendations of the NRC (1994) were based on studies conducted in the 5 to 6 years prior to their publication.

Carcass parameters, blood biochemical parameters, and relative organ weights

Among carcass parameters, only ether extract content at 14 days of age was influenced by DL, ME, and their interaction (Table 3). A decreasing linear effect of DL was observed on the relative liver weight of birds aged 14 days (Table 4). Other relative organ weights and blood biochemical parameters were not influenced by factors at either age (Table 5). The body weight of birds must be adequately distributed among water, protein, fat, and mineral fractions for good performance. Higher weight does not always translate into better productive characteristics, as it may be related to body fat accumulation, which, in excess, negatively affects laying, decreasing egg production (Zhou et al., 2006; Zhang et al., 2018).

The fact that ME and DL levels only influenced ether extract content indicates that high-ME diets favor fat deposition. Excess carcass fat resulting from an energy imbalance might be related to fat accumulation in organs. Increased fat in the liver and ovary, in particular, leads to reductions in egg production (Barron et al., 1999; Webster, 2003). However, although the liver was the only organ affected by treatments at 14 days (in terms of relative weight), it showed a decreasing linear relationship with increasing DL level.

Table 2
Performance of Japanese quail from 1 to 14 and 15 to 42 days of age as a function of metabolizable energy and digestible lysine contents in experimental diets

Variable	ME, kcal				DL, %				SEM	P-value		
	2830	2970	3110	3250	0.90	1.07	1.24	1.41		ME	DL	ME × DL
1-14 days												
BW[1], g	42.08	42.64	43.62	42.58	42.26	42.64	43.19	41.69	0.186	0.004(Q)	<0.001(Q)	0.144
BWG[2], g	35.07	35.60	36.52	35.52	35.21	35.59	36.13	34.65	0.185	0.006(Q)	<0.001(Q)	0.202
FI[3], g bird^{-1}	115.66	114.38	113.38	114.50	118.97	118.86	109.63	113.45	0.547	0.053(Q)	<0.001(Q)	0.774
FCR[4], g g^{-1}	3.30	3.22	3.11	3.23	3.38	3.34	3.04	3.28	0.024	0.007(Q)	<0.001(Q)	0.144
15-42 days												
BW[5], g	141.01	142.77	145.20	140.66	141.95	142.64	142.96	142.09	0.409	<0.001(Q)	0.412	0.088
BWG[6], g	101.40	100.27	101.58	98.10	102.2	98.868	99.7725	100.51	0.359	0.003(Q)	0.014(L)	0.012
FI[7], g bird^{-1}	458.63	459.84	453.85	457.53	479.32	453.35	443.6675	449.52	2.171	0.001(Q)	<0.001(Q)	<0.001
FCR[8], g g^{-1}	4.64	4.56	4.47	4.67	4.8125	4.5875	4.45	4.4775	0.027	0.048(Q)	<0.001(Q)	0.075

Abbreviations: ME, metabolizable energy; DL, digestible lysine; SEM, standard error of the mean; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; L, linear effect; Q, quadratic effect.
[1] $BW = 182.47 + 58.76DL - 26.02DL^2 + 0.13ME - 0.00002ME^2$; $R^2 = 0.68$.
[2] $BWG = - 182.35 + 58.69DL - 25.99DL^2 + 0.12ME - 0.00002ME^2$; $R^2 = 0.67$.
[3] $FI = 500.37 - 151.81DL + 59.91DL^2 - 0.19ME + 0.00003ME^2$; $R^2 = 0.94$
[4] $FCR = 32.91 - 14.45DL + 5.87DL^2 - 0.02ME + 0.000003ME^2$; $R^2 = 0.84$.
[5] $BW = - 603.95 + 0.49ME - 0.00008ME^2$; $R^2 = 0.77$.
[6] $BWG = -362.54 + 78.93DL + 0.36ME - 0.0006ME^2 + 0.03DL \times ME$; $R^2 = 0.75$.
[7] $FI = 2104.94 - 1086.08DL + 275.25DL^2 - 0.65ME + 0.00008ME^2 + 0.129DL \times ME$; $R^2 = 0.95$.
[8] $FCR = 41.11 - 5.73DL + 2.19DL^2 - 0.02ME + 0.000004ME^2$; $R^2 = 0.88$.

Table 3
Chemical composition (organic matter basis), protein deposition rate, fat deposition rate, and energy retention as determined by carcass analysis of 14- and 42-day-old Japanese quail fed diets containing different levels of metabolizable energy and digestible lysine

Variable	ME, kcal				DL, %				SEM	P-value		
	2830	2970	3110	3250	0.90	1.07	1.24	1.41		ME	DL	ME × DL
14 days												
CP, %	61.55	63.40	58.96	60.42	61.41	60.56	60.28	62.08	0.648	0.152	0.781	0.930
EE[1], %	15.82	16.03	17.69	17.75	17.37	17.23	16.36	16.32	0.297	<0.001(L)	0.140	0.004
MM, %	12.51	12.17	11.77	11.64	12.03	11.80	11.96	12.29	0.101	0.775	0.723	0.432
PDR, g day^{-1}	0.43	0.44	0.40	0.38	0.40	0.42	0.40	0.44	0.007	0.051	0.375	0.961
FDR, g day^{-1}	0.10	0.11	0.12	0.11	0.11	0.12	0.10	0.11	0.003	0.276	0.247	0.279
CER, kcal g^{-1}	3.36	3.52	3.35	3.26	3.32	3.48	3.23	3.45	0.050	0.378	0.313	0.872
42 days												
CP, %	61.29	60.40	62.56	62.61	60.93	61.63	62.97	61.32	0.603	0.476	0.629	0.147
EE, %	14.61	16.16	14.95	14.93	15.65	14.20	14.35	16.45	0.490	0.712	0.350	0.590
MM, %	10.99	10.84	11.19	10.75	10.73	10.82	11.26	10.96	0.134	0.718	0.581	0.766
PDR, g day^{-1}	0.52	0.52	0.56	0.55	0.54	0.53	0.54	0.54	0.007	0.216	0.851	0.444
FDR, g day^{-1}	0.12	0.15	0.12	0.12	0.13	0.11	0.12	0.15	0.008	0.609	0.264	0.379
CER, kcal g^{-1}	4.01	4.32	4.28	4.09	4.25	4.11	4.17	4.17	0.065	0.674	0.281	0.333

Abbreviations: ME, metabolizable energy; DL, digestible lysine; SEM, standard error of the mean; CP, crude protein; EE, ether extract; MM, mineral matter; PDR, protein deposition rate; FDR, fat deposition rate; CER, carcass energy retention; L, linear effect.
[1] $EE_{14\ days} = -4.95 + 0.009ME - 0.002DL \times ME$; $R^2 = 0.55$.

Table 4
Relative organ weights of 14- and 42-day-old Japanese quail fed diets containing different levels of metabolizable energy and digestible lysine

Variable	ME, kcal				DL, %				SEM	*P*-value		
	2830	2970	3110	3250	0.90	1.07	1.24	1.41		ME	DL	ME × DL
14 days												
Heart, %	0.99	1.06	1.06	1.17	0.83	0.89	0.86	0.84	0.021	0.560	0.637	0.983
Liver[1], %	2.78	3.27	3.13	3.18	2.80	2.47	2.35	2.27	0.073	0.070	0.006(L)	0.590
Gizzard, %	3.35	3.17	3.27	3.30	2.58	2.70	2.56	2.63	0.049	0.661	0.632	0.744
Intestine, %	4.88	5.25	4.90	5.00	4.12	3.88	4.05	3.96	0.077	0.359	0.577	0.728
42 days												
Heart, %	1.04	1.08	0.94	1.50	0.87	0.83	0.83	1.13	0.122	0.383	0.645	0.301
Liver, %	1.89	1.84	1.73	1.81	1.47	1.37	1.58	1.38	0.211	0.416	0.518	0.432
Gizzard, %	2.70	2.71	2.51	2.65	2.13	2.06	2.17	2.09	0.301	0.427	0.469	0.544
Intestine, %	3.88	3.42	3.32	3.52	2.81	2.80	2.88	2.81	0.423	0.396	0.439	0.498
Ovary, %	0.15	0.13	0.12	0.14	0.08	0.12	0.12	0.12	0.010	0.707	0.240	0.710
Oviduct, %	0.06	0.02	0.03	0.04	0.01	0.04	0.02	0.04	0.007	0.188	0.330	0.641

Abbreviations: ME, metabolizable energy; DL, digestible lysine; SEM, standard error of the mean; L, linear effect.
[1] $Liver_{14\ days} = 4.54 - 1.26DL$; $R^2 = 0.61$.

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Table 5
Blood biochemical parameters of 14- and 42-day-old Japanese quail fed diets containing different levels of metabolizable energy and digestible lysine

Variable	ME, kcal				DL, %				SEM	*P*-value		
	2830	2970	3110	3250	0.90	1.07	1.24	1.41		ME	DL	ME × DL
14 days												
TP, g dL^{-1}	3.01	3.28	3.15	3.39	3.28	3.25	3.21	3.09	0.037	0.204	0.352	0.807
ALB, g dL^{-1}	1.21	1.20	1.21	1.25	1.16	1.26	1.30	1.16	0.016	0.742	0.076	0.092
GLO, g dL^{-1}	1.86	2.11	1.96	2.12	2.17	2.02	1.94	1.91	0.048	0.212	0.244	0.958
ALB/GLO ratio	0.68	0.59	0.63	0.62	0.56	0.63	0.69	0.63	0.020	0.504	0.192	0.712
UA, mg dL^{-1}	5.53	6.10	5.55	5.07	6.27	5.34	5.54	5.09	0.121	0.684	0.241	0.548
TC, mg dL^{-1}	139.98	146.43	150.59	158.73	155.30	150.78	137.45	152.20	1.870	0.064	0.124	0.266
ALP, × 10^3 U L^{-1}	2.261	2.046	2.108	1.973	2.354	1.951	2.061	2.023	9.190	0.545	0.448	0.615
42 days												
TP, g dL^{-1}	2.85	2.94	2.94	2.99	2.83	3.00	2.97	2.92	0.028	0.939	0.557	0.596
ALB, g dL^{-1}	1.10	1.13	1.16	1.08	1.09	1.10	1.10	1.18	0.012	0.064	0.213	0.331
GLO, g dL^{-1}	1.89	1.79	1.76	1.92	1.79	1.90	1.86	1.80	0.033	0.324	0.600	0.802
ALB/GLO ratio	0.59	0.63	0.67	0.56	0.61	0.58	0.61	0.65	0.014	0.682	0.294	0.788
UA, mg dL^{-1}	4.32	4.89	4.94	4.76	4.55	4.41	4.68	5.27	0.119	0.910	0.202	0.133
TC, mg dL^{-1}	157.36	158.68	150.08	163.93	161.95	138.19	164.87	165.05	2.668	0.941	0.315	0.573
ALP, × 10^3 U L^{-1}	1.466	1.134	1.135	1.139	1.600	1.092	1.097	1.085	4.431	0.270	0.522	0.370

Abbreviations: ME, metabolizable energy; DL, digestible lysine; SEM, standard error of the mean; TP, total proteins; ALB, albumin; GLO, globulin; UA, uric acid; TC, total cholesterol; ALP, alkaline phosphatase.

One of the main indicators of metabolic changes in the liver is serum albumin level, which is related to alterations in the systemic use of proteins. The values observed here in both phases suggest that ME and DL did not influence albumin or other blood biochemical parameters. As such, blood biochemical parameters did not depend on the diet (Hussein et al., 2010; Ukashatu et al., 2014).

Bone parameters

In the starter phase (Table 6), DL and ME exerted significant interaction effects on the Seedor index of the tibia (linear effect) and femur (quadratic effect). The estimated optimal levels of DL and ME for femur Seedor index were 0.86% and 3267 kcal, respectively. Femoral strength was influenced by DL level, in an increasing linear manner. Bone mineral matter, on the other hand, had a quadratic relationship with ME level, with the optimal level being estimated at 2942 kcal ME. In the grower phase (Table 7), only ME influenced the Seedor index of the tibia and femur, albeit in a quadratic manner. The estimated optimal levels were 2973 kcal ME for the tibia and 2944 kcal ME for the femur. Femoral bone density also showed a quadratic relationship with ME, with an estimated optimal ME level of 2721 kcal.

The Seedor index, which estimates bone volume and serves as an indicator of bone density, was influenced by the ME × DL interaction effect at 14 days and by the main effect of ME at 42 days, suggesting higher lysine activity in the initial period of bird development. With advancing age, the effect of ME level was more pronounced.

Femoral bone strength at 14 days was the only variable influenced by the main effect of DL. This characteristic is also related to bone volume and is modulated mainly by collagen, found in the bone matrix. Collagen confers elasticity and minerals, promoting sturdiness to bone tissues (Lima et al., 2016). Lysine actively participates in the synthesis of collagen, in the form of hydroxylysine, being important for structure formation. Together with hydroxyproline, lysine aligns and stabilizes the triple helix of amino acids that form collagen (Salo & Myllyharju, 2020), which provides oriented support during bone mineralization.

Lysine degradation pathways include the lysyl oxidase pathway, responsible for cross-linking collagen and elastin. This enzyme, at high concentrations, mistakenly stimulates bone resorption, predisposing birds to various bone problems, such as tibial dyschondroplasia, decreased mineralization and, consequently, low bone strength (Gaar et al., 2020). Therefore, lysine participates in collagen formation, which, in turn, contributes to bone and eggshell composition. For this reason, DL levels must be sufficient to meet the demands of protein synthesis and other compound synthesis pathways. ME levels should be chosen so as to ensure that the amount of feed consumed contains the necessary volume of nutrients for other body functions.

Table 6
Bone parameters of 14-day-old Japanese quail fed diets containing different levels of metabolizable energy and digestible lysine

Variable	ME, kcal				DL, %				SEM	*P*-value		
	2830	2970	3110	3250	0.90	1.07	1.24	1.41		ME	DL	ME × DL
$MM_{tibia+femur}$[1], %	45.57	45.72	45.14	41.66	44.74	43.79	44.38	45.17	0.399	0.008(Q)	0.189	0.691
Tibia												
SI[2], mg mm^{-1}	137.47	136.03	141.97	148.39	149.59	143.72	139.60	130.94	1.817	<0.001(L)	0.002(L)	<0.001
BD, mm Al eq	1.78	1.77	1.75	1.74	1.75	1.77	1.78	1.72	0.014	0.676	0.528	0.365
BS, kgf	19.16	19.52	19.90	17.99	18.86	19.04	19.70	18.96	0.384	0.093	0.727	0.656
Femur												
SI[3], mg mm^{-1}	131.25	138.41	148.42	143.09	139.72	142.64	141.12	137.71	1.214	<0.001(Q)	0.013(Q)	<0.001
BD, mm Al eq	1.62	1.57	1.60	1.65	1.62	1.61	1.62	1.58	0.014	0.278	0.451	0.389
BS[4], kgf	20.91	19.01	21.24	19.04	17.90	19.90	20.51	21.88	0.641	0.252	0.007(L)	0.283

Abbreviations: ME, metabolizable energy; DL, digestible lysine; SEM, standard error of the mean; MM, mineral matter; SI, Seedor index; BD, bone density; BS, bone strength; L, linear effect; Q, quadratic effect.

[1] $MM_{tibia+femur} = -356.09 + 0.27ME - 0.00005ME^2$; $R^2 = 0.78$.

[2] $SI = -520.42 + 499.85DL + 0.23ME - 0.18DL \times ME$; $R^2 = 0.58$.

[3] $SI = -1.941.51 + 509.86DL - 54.78DL^2 + 1.15ME - 0.0002ME^2 - 0.13DL \times ME$; $R^2 = 0.89$.

[4] $BS = 9.20 + 8.85DL$; $R^2 = 0.91$.

Table 7
Bone parameters of 42-day-old Japanese quail fed diets containing different levels of metabolizable energy and digestible lysine

Variable	ME, kcal				DL, %				SEM	*P*-value		
	2830	2970	3110	3250	0.90	1.07	1.24	1.41		ME	DL	ME × DL
$MM_{tibia+femur}$, %	52.44	52.44	51.72	49.47	53.01	51.56	52.45	49.05	0.746	0.328	0.340	0.440
Tibia												
SI[1], mg mm^{-1}	87.76	87.75	86.96	92.25	89.21	90.42	88.33	86.76	0.580	0.014(Q)	0.169	0.503
BD, mm Al eq	2.30	2.32	2.35	2.29	2.35	2.26	2.34	2.31	0.011	0.155	0.109	0.431
BS, kgf	35.52	33.45	39.18	36.55	37.16	35.06	35.29	37.18	0.713	0.261	0.082	0.887
Femur												
SI[2], mg mm^{-1}	79.67	80.62	79.69	86.21	81.56	83.48	79.77	81.38	0.739	0.039(Q)	0.101	0.544
BD[3], mm Al eq	2.20	2.25	2.22	2.15	2.21	2.16	2.24	2.22	0.012	0.009(Q)	0.339	0.377
BS, kgf	35.60	38.52	40.68	39.04	39.71	35.59	43.97	34.57	0.791	0.628	0.886	0.843

Abbreviations: ME, metabolizable energy; DL, digestible lysine; SEM, standard error of the mean; MM, mineral matter; SI, Seedor index; BD, bone density; BS, bone strength; Q, quadratic effect.

[1] $SI = 684.43 - 0.40ME + 0.00007ME^2$; $R^2 = 0.70$.

[2] $SI = 695.54 - 0.42ME + 0.00007ME^2$; $R^2 = 0.78$.

[3] $BT = -10.98 + 0.008ME - 0.000001ME^2$; $R^2 = 0.74$.

Feeding programs should focus on maximizing growth rate and body development, allowing quail to reach the ideal weight at sexual maturity, thereby promoting batch uniformity and ensuring normality during the production phase (Mehri et al., 2013). Therefore, the nutritional conditions of the growth phase influence laying performance (Lima et al., 2016). Bird growth influences not only early production parameters but also sustained production and egg quality. Various studies aimed at bone parameters focused more on mineral content and composition than on the organic matrix. However, manipulation of DL and ME levels may generate a higher-quality organic matrix predisposed to enhanced mineral aggregation, contributing to better external egg quality indices.

Conclusion

Overlaid contour plots of ME and DL effects on performance parameters showed that 3030 kcal ME and 1.221% DL are optimal for the starter phase, corresponding to a daily intake of 26.46 kcal ME, 0.107 g of DL, and an ME/DL ratio of 247. For the grower phase, the estimated optimal values are 3055 kcal ME and 1.202% DL, corresponding to a daily intake of 50.09 kcal ME, 0.194 g of DL, and an ME/DL ratio of 258.

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