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Requirement of digestible methionine + cystine for growing Japanese quail and its subsequent effects on laying phase

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ABSTRACT - The objective of this study was to estimate the nutritional requirement of digestible methionine + cystine for Japanese quail during the growth phase and its residual effect on the laying phase. One experiment was conducted, including three phases: starter, from 1 to 14 days of age; grower, from 15 to 42 days of age; and laying, from 43 to 168 days of age. The experimental design was entirely randomized with five treatments (0.52, 0.64, 0.76, 0.88, and 1.00% of digestible methionine + cystine) and five replicates. In starter phase, 48 quail/experimental unit (box) were used; in grower phase, 31 quail/experimental unit (box) were used, which were reared until 14 days old, receiving conventional feed and, in laying phase, 12 quail/experimental unit were selected from grower phase to evaluate the residual effect. To evaluate animal performance, feed intake (g/bird), body weight (g), weight gain (g), feed conversion (g/g), and viability (%) were analyzed. At 14 and 42 days of age, the relative weight (%) of liver, spleen, and cloacal pouch organs, the birds' warping (%) and body chemical composition (%) were evaluated. During laying, performance and egg quality were evaluated. In the starter and grower stages, quadratic effects were observed for all performance variables. Laying performance results confirmed the estimates obtained in the grower phase; however, there was no effect on egg quality. Considering the best feed conversion, the nutritional recommendation of digestible methionine + cystine for Japanese quail in the starter phase is 0.85% and in the grower phase, it is 0.77%, corresponding to daily intake of 50.43 and 158.5 mg of digestible methionine + cystine/day, respectively.

Keywords: egg quality, feed conversion, residual effect, sulfur amino acids

1. Introduction

Adequate knowledge of nutritional requirements allows the formulation of diets with the correct balance of nutrients for each animal species based on their developmental stage. According to Toledo et al. (2007), dietary requirements of birds for aminoacids are high during the first weeks of age and decrease with increasing age.

Protein is the most expensive component of animal feed and its quality in one type of feed can be compared to another by its aminoacid composition, especially the proportions of the nine essential aminoacids (Silva et al., 2014).

From previous studies regarding animal nutrition and metabolism as well as production of industrial aminoacids at compatible prices, it has become possible to formulate diets with aminoacid levels close to the requirements of the animals (Murakami and Garcia, 2014), which favours the use of aminoacids for protein synthesis rather than simply being targeted as a source of energy (Corrêa et al., 2006).

In diets based on corn and soybean meal, it is not possible to fully meet requirements of quails for maintenance and growth; therefore, their diets must be supplemented with industrial aminoacids. Methionine is the main aminoacid limiting bird growth and is present in feeds in only small amounts; therefore, daily requirements of this aminoacid should be elevated.

The two aminoacids with sulfur in their structure are methionine and cysteine. Methionine is an essential aminoacid for all higher animal species (NRC, 1994; Lewis, 2003; NRC, 2012); however, cysteine can be synthesised from methionine and is classified as a non-essential aminoacid (Rodwell, 2006; Nelson and Cox, 2009). Depending on animal species, cysteine may account for up to 50% of methionine requirement in the diet, with this proportion being lower in higher-performance animals (Dalibard et al., 2014). In general, cysteine molecules are very unstable in solution and can be easily oxidised to their dimer, cystine. When the feed protein is hydrolysed, cystine is produced and, therefore, the requirement for sulfur aminoacids in feed is normally expressed as methionine + cystine (Lewis, 2003).

One of the great challenges regarding nutrition is to provide protein in the quantity and quality that is ideally suited for ensuring growth, development, and future egg production. Nutritional conditions established during the growth period influence the performance of birds during production phase (Lima et al., 2016).

One experiment was performed in the present study to determine the nutritional requirements of digestible methionine + cystine in Japanese quail (*Coturnix coturnix japonica*) during the starter (1 to 14 days old) and grower (15 to 42 days old) phases to obtain maximum animal performance and evaluate the residual effect of these treatments during the laying phase (43 to 168 days of age).

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (Protocol Number 9195040417).

2.1. Animals, diets, and experimental design

The experiments were performed in Maringá, Paraná, Brazil (Latitude: 23º 25' 38" South, Longitude: 51º 56' 15" West) from July to December 2015.

The one-day-old birds, of a commercial strain (purchased from Vicami Codornas), were housed in a conventional shed in 2.5 m^2 boxes with a French tile roof, beaten clay floor, and masonry sidewalls with wire screens to the roof, fitted with side curtains, and with a rice straw bed on the floor.

One experiment was performed to determine nutritional requirements of digestible methionine + cystine during starter and grower phases. Starter phase was from 1 to 14 days of age, grower phase was from 15 to 42 days of age, and laying phase was from 43 to 168 days of age to evaluate the residual effect of treatments received. The quail used to determine the nutritional requirements of digestible methionine + cystine in starter phase were housed following the experimental design. The quail used in grower phase were conventionally housed until the beginning of the experimental period, at 14 days of age, according to the requirements proposed by Rostagno et al. (2017).

The experimental design was completely randomised and included five treatments (0.52, 0.64, 0.76, 0.88, and 1.00% digestible methionine + cystine) and five replicates for starter and grower phases. In starter phase, 48 female quail were used per experimental unit (box), totalling 1200 birds, and in grower phase, 31 female quail were used per experimental unit (box), totalling 775 birds.

At 43 days, 12 birds per experimental unit from grower phase were rehomed in posture cages to evaluate the residual effect of treatments received during the grower phase on the laying stage (43 to 168 days of age), during which time the birds received conventional feed according to the requirements proposed by Rostagno et al. (2017).

Up to the 10th day of age, protective circles and infrared drying lamps (250 W) were used for 24 h as the heating source. From 1 to 42 days of age, the lighting programme used was natural light. From the 43rd day, the light programme was 14 h of light and each week 30 min was added until 17 h of natural + artificial light was reached, which was controlled with the use of a timer.

Minimum and maximum temperatures (starter phase: 22.55 and 30.00 °C; grower phase: 20.95 and 26.11 °C; and laying phase: 18.61 and 28.92 °C, respectively) and relative humidity (starter phase: 31.07 and 76.00%; grower phase: 51.38 and 86.76%; and laying phase: 61.18 and 88.01%, respectively) were recorded early in the morning and in the late afternoon using dry bulb thermometers located at the same height as the birds and three points in the shed.

The aminoacid composition of corn and soybean meal was determined at Evonik Industries. Digestible aminoacids present in feed and in the industrial aminoacids were calculated using digestibility coefficients proposed by Rostagno et al. (2017) (Table 1).

Table 1 - Percentage composition of experimental diets for Japanese quail in the starter (1 to 14 days old) and growth (15 to 42 days old) phases

Ingredient (%)	Digestible methionine + cystine (%)				
	0.52	0.64	0.76	0.88	1.00
Corn grain	59.00	59.00	59.00	59.00	59.00
Soybean meal (45%)	30.84	30.84	30.84	30.84	30.84
Glutamic acid	3.88	3.75	3.62	3.49	3.36
Soybean oil	0.92	0.89	0.86	0.83	0.80
Inert[1]	1.26	1.3	1.34	1.38	1.42
Monocalcium phosphate	1.43	1.43	1.43	1.43	1.43
Limestone	1.38	1.38	1.38	1.38	1.38
Vit/min supplement[2]	0.40	0.40	0.40	0.40	0.40
Common salt	0.40	0.40	0.40	0.40	0.40
L-lysine	0.28	0.28	0.28	0.28	0.28
DL-methionine	0.00	0.12	0.24	0.36	0.48
L-threonine	0.19	0.19	0.19	0.19	0.19
L-tryptophan	0.01	0.01	0.01	0.01	0.01
Antioxidant[3]	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00

[1] Sand.
[2] Mineral/vitamin supplement (guaranteed levels per kg product): vitamin A, 2,500,000 IU; vitamin D3, 750,000 IU; vitamin E, 5,000 IU; vitamin B1, 625 mg; vitamin B2, 1,500 mg; vitamin B6, 1,250 mg; vitamin B12, 5,000 mcg; vitamin K3, 750 mg; Ca pantothenate, 3,000 mg; niacin, 6,000 mg; folic acid, 250 mg; biotin, 50.0 mg; choline, 75 g; antioxidant, 4,360 mg; Zn, 12.5 g; Fe, 12.5 g; Mn, 15.0 g; Cu, 3,000 mg; Co, 50 mg; I, 250 mg; Se, 62.5 mg; vehicle q.s.p., 1,000 g.
[3] BHT (butyl hydroxy toluene).

Diets were formulated to meet requirements proposed by Rostagno et al. (2017) for Japanese quail in the first and second growth stages, except for methionine + cystine (Table 2).

Feeds were formulated with corn and soybean meal to be isocalphic, isophosphoric, isoenergetic, and isoaminoacidic, except for the digestible methionine + cystine. The different digestible methionine + cystine levels of the feed were adjusted by varying the amounts of DL-methionine (99%), glutamic acid, soybean oil, and inert sand.

Table 2 - Nutritional composition of experimental diets for Japanese quail (calculated values) in the starter (1 to 14 days old) and growth (15 to 42 days old) phases

Nutrient	Digestible methionine + cystine (%)				
	0.52	0.64	0.76	0.88	1.00
ME (kcal/kg)	2900	2900	2900	2900	2900
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
Calcium (%)	0.90	0.90	0.90	0.90	0.90
Available phosphorus (%)	0.37	0.37	0.37	0.37	0.37
Digestible lysine (%)	1.12	1.12	1.12	1.12	1.12
Digestible threonine (%)	0.79	0.79	0.79	0.79	0.79
Digestible tryptophan (%)	0.21	0.21	0.21	0.21	0.21
Sodium (%)	0.18	0.18	0.18	0.18	0.18
Chlorine (%)	0.34	0.34	0.34	0.34	0.34
Potassium (%)	0.74	0.74	0.74	0.74	0.74
EB (mEq kg^{-1})	170.02	170.02	170.02	170.02	170.02

ME - metabolisable energy; EB - electrolyte balance.

2.2. Animal performance

To evaluate the animal performance (starter and grower phases), the quail were weighed weekly, and experimental diets were simultaneously weighed to determine the feed intake (g/bird), body weight (g), gain (g), feed conversion (g/g), and viability (%).

The laying phase was subdivided into five production cycles of 21 days each, with the birds evaluated from their 64th day of life when their egg production was homogeneous. Birds and feeds were weighed at the end of each cycle to determine daily feed intake (g/bird/day), body weight (g), feed conversion per egg mass (g/g of eggs), feed conversion per dozen eggs (g/dz eggs), and viability (%).

The eggs were collected daily in the morning to calculate the egg laying rate (%) and egg mass production (g eggs/bird/day), counting all eggs that were produced.

2.3. Body chemical composition

For the determination of the body chemical composition, four birds at 14 days of age and two birds at 42 days of age were selected by their mean weight (±5%) using the methodology described by Sakomura and Rostagno (2007). The selected birds were fasted for 5 h and were then sacrificed using intravenous desensitisation with barbiturate thiopental (100 mg/kg), followed by cervical dislocation.

After slaughter, quail were frozen and then ground using an industrial meat grinder. The samples were homogenised, weighed, and placed in a forced ventilation oven at 55 °C for pre-drying. After 72 h, they were removed and reweighed. After pre-drying, the samples were milled in a knife-type mill and taken to the laboratory for determination of the dry matter (procedure no. 925.09), mineral matter (procedure no. 923.03), crude protein (procedure no. 920.87), and fat (procedure no. 920.85) based on the methodologies described by the AOAC (2005).

To determine the protein deposition rate and body fat (g/d), 20 one-day-old quail were slaughtered for comparative analysis of body chemical composition with birds of 14 and 42 days old. Amounts of protein and fat (g) of the final carcass were determined by subtracting the respective amounts at the beginning of the experiment and dividing by the experimental period (days) as described by Fraga (2008).

To obtain the energy retained in the carcass, the equation described by Sakomura (2004) was used based on energy values of the protein (5.66 kcal/g) and fat (9.37 kcal/g).

2.4. Relative weight of organs and warping

At the conclusion of starter and grower phases (14 and 42 days of age), two birds per experimental unit were selected, within the mean weight (±5%), to measure warping and relative body weight. These birds were fasted for 5 h to completely empty their gastrointestinal tracts and were weighed (weight 1) before being sacrificed. After slaughter, the quail were scalded (at 55 °C for 10 s), plucked, and reweighed (weight 2) to quantify the weight of feathers (weight 1 – weight 2) (Cooper and Washburn, 1998).

The same birds, after quantification of warping, were eviscerated by abdominal cutting that was performed with surgical scissors to extract the following organs: spleen, liver, and cloacal sac at 14 and 42 days of age as well as ovaries at 42 days of age only. These organs were individually weighed on a precision scale to obtain the relative weight (%) in relation to the weight of the bird (weight 1).

2.5. Quality of eggs

On the last three days of each cycle in laying phase, all eggs were identified, weighed individually on a digital precision scale (0.01 g), and three eggs were selected from the mean weight of all eggs (±10%) of the experimental unit to perform internal and external quality analyses.

Specific gravity analysis was performed with all collected eggs by immersing them in different concentrations of saline solution and adjusting the density using a Baumé densimeter (range of 0.005 g/mL) from 1.060 to 1.085 g/mL according to the methodology described by Hamilton (1982).

For subsequent analyses, we used the three eggs per repetition that were previously selected. These eggs were sectioned in the equatorial portion with surgical scissors, and the internal contents were arranged on a glass surface to measure the height (mm) and diameter (mm) of yolk and albumen (density) using a Digimess digital calliper with an accuracy of 0.02 mm. From the density of the albumen and the weight of the egg, Haugh unit was obtained according to the methodology described by Haugh (1937). After measurements, yolk and albumen were separated and weighed on a precision scale. The weight of albumen was obtained by subtracting the weight of the egg and weights of yolk and shell. The weight of shell per unit area was determined using the formula adapted by Rodrigues et al. (1996).

2.6. Statistical analysis

Statistical analysis of data was performed using the statistical environment R (R Core Team, 2013). For the effects test ($P<0.05$), the model described below was adopted, and then the assumption of the normality of the residues was verified.

$$Y_{ij} = \beta 0 + \beta 1 A_i + \beta 2 A_i^2 + e_{ij},$$

in which Y_{ij} = variable measured in experimental unit j, fed a diet containing level i of methionine + cystine; A_i = methionine + cystine level (A_i1 = 0.52; A_i2 = 0.64; A_i3 = 0.76; A_i4 = 0.88; A_i5 = 1.00%); β0 = general constant; β1 = simple linear regression coefficient as a function of methionine + cystine level; β2 = quadratic linear regression coefficient as a function of methionine + cystine level; and e_{ij} = random error associated with each observation.

A significant effect of the factors ($P<0.05$) was obtained by polynomial regression analysis for digestible methionine + cystine levels to estimate the best-fit model of the data according to methodology described by Montgomery (2012). Estimates of the best methionine + cystine levels for each significant variable were obtained using the quadratic model as proposed by Sakomura and Rostagno (2016).

3. Results

The performance variables from 1 to 14 days old presented a quadratic effect ($P<0.05$) for the digestible methionine + cystine levels in the diet. Optimal digestible methionine + cystine levels were 0.73, 0.89, 0.85, 0.84, and 0.87% for feed intake, weight gain, feed conversion, viability, and mean weight at 14 days of age, respectively (Table 3).

When evaluating the influence of methionine + cystine levels tested on poultry warping and relative weights of lymphoid organs and liver, there was no significant difference (P>0.05) in feather and spleen weights. However, the lower relative weight of liver had a quadratic effect (P<0.05), with an optimal digestible methionine + cystine diet level of 0.89%.

Table 3 - Average performance and relative weight of organs of Japanese quail in the starter phase (1 to 14 days old), depending on the digestible methionine + cystine levels

MC (%)	0.52	0.64	0.76	0.88	1.00	SEM
			Performance			
FI (g bird^{-1})	85.13	81.46	83.22	83.49	86.23	0.476
WG (g)	24.65	32.37	35.62	37.42	36.44	0.958
FC (g g^{-1})	3.45	2.52	2.34	2.23	2.37	0.093
V (%)	88.05	95.00	97.38	94.23	95.83	0.823
BW (g)	31.59	38.90	42.33	44.70	42.30	0.954
			Relative weight			
Warping (%)	7.65	8.06	7.90	7.63	7.96	0.056
Liver (%)	4.65	4.13	3.67	3.60	3.70	0.091
Cloacal sac (%)	0.18	0.14	0.14	0.14	0.14	0.007
Spleen (%)	0.05	0.04	0.05	0.04	0.05	0.002

Regression equation	P-value	R^2	Estimate MC (%)
$FI = 112.0959 - 81.95984\ MC + 56.23016\ MC^2$	0.002 (Q)	0.40	0.73
$WG = -36.1169\ 0 + 165.9043\ MC - 93.4524\ MC^2$	<0.001 (Q)	0.97	0.89
$FC = 10.22818 - 18.90198\ MC + 11.08135\ MC^2$	<0.001 (Q)	0.94	0.85
$V = 40.45107 + 134.97214\ MC - 80.68452\ MC^2$	0.004 (Q)	0.50	0.84
$BW = -32.9958 + 177.0257\ MC - 101.5476\ MC^2$	<0.001 (Q)	0.95	0.87
$Liver = 9.63862 - 13.5511\ MC + 7.63889\ MC^2$	0.001 (Q)	0.72	0.89

MC - digestible methionine + cystine; FI - feed intake; WG - weight gain; FC - feed conversion; V - viability; BW - body weight at 14 days old; SEM - standard error of mean; R^2 - coefficient of determination; Q - quadratic effect.

In the grower phase (15 to 42 days of age), quadratic effects (P<0.05) were observed for feed intake, weight gain, feed conversion, and mean weight at 42 days of age, estimated at optimal levels of 0.77, 0.77, 0.77, and 0.80%, respectively (Table 4).

At 42 days of age, no significant effects (P>0.05) regarding methionine + cystine levels were observed for relative weight of feathers, cloacal sac, spleen, and ovaries. However, the relative weight of liver was influenced by the evaluated levels, presenting a quadratic effect (P<0.05) and the optimal level was 0.76% of methionine + cystine in the diet.

Among the chemical composition variables, deposition rates of protein, fat, and energy retained in the carcass during the starter and grower phases showed no significant effects (P>0.05) regarding the methionine + cystine levels in the diet (Table 5).

The birds that received digestible methionine + cystine (0.52% to 1.00%) during grower phase presented different responses during the laying phase. These responses indicate the repercussions of the experimental treatments of growth on the performance of birds in posture cages.

Variables daily feed intake, feed conversion by egg mass, feed conversion per dozen eggs, and viability showed a quadratic effect (P<0.05), with optimal levels of 0.87, 0.82, 0.76, and 0.77% methionine + cystine, respectively, indicating the best response to methionine + cystine levels studied during the growth phase (Table 6). No significant differences (P>0.05) were observed for mean weights of birds, egg masses, posture rates, or age of the first egg.

Regarding internal and external qualities of eggs, the different methionine + cystine treatments offered during the growth phase did not present a significant effect (P>0.05) on the quality of eggs produced (Table 7).

Table 4 - Average performance and relative weight of organs of Japanese quail in the growth phase (15 to 42 days old), depending on the digestible methionine + cystine levels

MC (%)	0.52	0.64	0.76	0.88	1.00	SEM
			Performance			
FI (g $bird^{-1}$)	608.57	580.67	573.76	589.47	598.65	3.507
WG (g)	102.59	108.18	109.55	108.35	103.81	0.642
FC (g g^{-1})	5.93	5.37	5.24	5.44	5.77	0.061
V (%)	93.08	93.82	98.37	99.35	97.42	0.747
BW (g)	145.73	148.35	149.79	149.54	147.64	0.462
			Relative weight			
Warping (%)	7.72	8.39	7.70	8.80	7.80	0.112
Liver (%)	2.56	2.31	2.25	2.18	2.60	0.039
Cloacal sac (%)	0.19	0.18	0.15	0.20	0.17	0.008
Spleen (%)	0.04	0.03	0.04	0.04	0.04	0.002
Ovaries (%)	0.03	0.02	0.02	0.04	0.03	0.002

Regression equation	P-value	R^2	Estimate MC (%)
$FI = 860.6537 - 738.8402\ MC + 480.0298\ MC^2$	0.002 (Q)	0.46	0.77
$WG = 42.66782 + 174.3998\ MC - 113.3135\ MC^2$	<0.001 (Q)	0.77	0.77
$FC = 11.45729 - 16.12373\ MC + 10.46627\ MC^2$	<0.001 (Q)	0.72	0.77
$BW = 115.79022 + 85.17294\ MC - 53.2837\ MC^2$	0.002 (Q)	0.42	0.80
$Liver = 5.99622 - 9.975635\ MC + 6.5377\ MC^2$	<0.001 (Q)	0.70	0.76

MC - digestible methionine + cystine; FI - feed intake; WG - weight gain; FC - feed conversion; V - viability; BW - body weight at 42 days old; SEM - standard error of mean; R^2 - coefficient of determination; Q - quadratic effect; L - linear effect.

Table 5 - Body chemical composition[1] and protein/fat deposition in Japanese quail in the starter (1 to 14 days old) and growth (15 to 42 days old) phases as a function of the digestible methionine + cystine levels in the feed

MC (%)	0.52	0.64	0.76	0.88	1.00	SEM
			14 days old (starter phase)			
Crude protein (%)	56.63	58.09	60.13	58.19	56.80	0.737
Fat (%)	13.99	13.67	12.70	14.04	13.67	0.394
Mineral matter (%)	11.44	12.35	11.89	11.78	11.46	0.155
PDR (g d^{-1})	0.39	0.39	0.40	0.40	0.35	0.007
FDR (g d^{-1})	0.09	0.09	0.08	0.09	0.09	0.003
ERC (kcal g^{-1})	3.06	3.02	3.05	3.15	2.80	0.045
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	
			42 days old (growth phase)			
Crude protein (%)	59.01	65.64	62.76	62.09	61.54	0.741
Fat (%)	17.10	15.36	18.23	17.30	17.06	0.370
Mineral matter (%)	11.68	11.59	11.08	10.01	10.38	0.191
PDR (g d^{-1})	0.71	0.79	0.78	0.77	0.75	0.012
FDR (g d^{-1})	0.22	0.24	0.25	0.25	0.24	0.005
ERC (kcal g^{-1})	6.14	6.75	6.78	6.65	6.47	0.109
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	

MC - digestible methionine + cystine; PDR - protein deposition rate; FDR - fat deposition rate; ERC - energy retained in the carcass; SEM - standard error of mean.
[1] Data presented in dry matter values.

Table 6 - Average performance of Japanese quail in the laying phase as a function of the digestible methionine + cystine levels offered during the growth phase

MC (%)	0.52	0.64	0.76	0.88	1.00	SEM
BW (g)	174.02	173.88	174.12	173.20	172.69	0.80
DFI (g bird d^{-1})	31.08	29.62	27.92	26.88	28.30	0.33
EM (g bird d^{-1})	9.90	9.92	9.86	9.80	9.57	0.08
ELR (%)	89.49	88.47	89.01	88.29	89.00	0.59
FCM (g g of $eggs^{-1}$)	3.14	2.99	2.83	2.75	2.96	0.04
FCD (g dz of $eggs^{-1}$)	0.41	0.41	0.39	0.39	0.42	0.00
AFE (d)	56.20	56.00	56.20	55.00	55.60	0.37
V (%)	80.39	99.33	97.00	96.67	84.93	1.68

Regression equation	P-value	R^2	Estimate MC (%)
$DFI = 51.4748 - 55.2544\ MC + 31.7956\ MC^2$	<0.001 (Q)	0.75	0.87
$FCM = 5.5052 - 6.5652\ MC + 3.9881\ MC^2$	0.001 (Q)	0.49	0.82
$FCD = 0.5921 - 0.5261\ MC + 0.3472\ MC^2$	0.043 (Q)	0.17	0.76
$V = -73.9994 + 452.9223\ MC - 294.4544\ MC^2$	<0.001 (Q)	0.75	0.77

MC - digestible methionine + cystine; BW - body weight; DFI - daily feed intake; EM - egg mass; ELR - egg laying rate; FCM - feed conversion per egg mass; FCD - feed conversion per dozen eggs; AFE - age at first egg; V - viability; SEM - standard error of mean; R^2 - coefficient of determination; Q - quadratic effect.

Table 7 - Quality of Japanese quail eggs in the laying phase depending on the digestible methionine + cystine levels offered during the growth phase

MC (%)	0.52	0.64	0.76	0.88	1.00	SEM
Albumen (%)	62.69	62.52	62.49	62.61	62.57	0.11
Shell (%)	7.31	7.32	7.38	7.40	7.31	0.04
Yolk (%)	29.99	30.15	30.12	29.98	30.19	0.10
YI	0.47	0.47	0.47	0.48	0.48	0.01
WSA	3.73	3.75	3.77	3.78	3.70	0.01
SG (g mL^{-1})	1.05	1.05	1.05	1.07	1.06	0.00
EW (g)	11.06	11.21	11.08	11.10	10.76	0.05
Haugh unit	93.41	93.97	93.70	93.54	93.67	0.18
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	

MC - digestible methionine + cystine; YI - yolk index; WSA - weight of the shell per unit area; SG - specific gravity; EW - egg weight; SEM - standard error of mean.

4. Discussion

Results of the present study showed that there was a physiological need for these aminoacids for birds to express growth, development, and productivity responses. This was shown at all stages from the digestible methionine + cystine estimates that were very close when compared among the performance variables.

The percentage requirement of digestible methionine + cystine was higher during the starter phase compared with the grower phase owing to the higher protein deposition during the first days of life. This phase division for the determination of nutritional requirements is of great importance because the nutrients present in the diet must meet the physiological needs of the different proportions of proteins, fats, minerals, vitamins, and water depending on the developmental stage of the animal.

Finco et al. (2016) calculated growth curves for quail of the Vicami strain that were used in the present study to characterise their body developmental stages and chemical components. These authors emphasised the importance of this division mainly during the first days of life when growth and protein deposition were the more intense.

Both the NRC (1994) and Brazilian Tables for Poultry and Swine created by Rostagno et al. (2011) present a single requirement of methionine + cystine for growth. However, the most recent data showed the growth phase subdivided into starter and grower, e.g. Silva and Costa (2009): 1 to 21 days and 22 to 42 days and Rostagno et al. (2017): 1 to 14 days and 15 to 35 days.

Among the aminoacids used for animal nutrition, methionine is the one that initiates the process of translation of the synthesis of eukaryotic proteins (Nelson and Cox, 2014). It promotes the formation of specialised products that are of great importance for metabolism.

Previous studies regarding digestible methionine + cystine in Japanese quail diets are currently limited, and the experimental conditions and strains used are variable, perhaps because it is a species that does not have a well-defined genetic pattern. The NRC (1994) recommendations are generally used, which are old and outdated, as modern lineages are more demanding. The latest published tables, by Silva and Costa (2009) and Rostagno et al. (2011, 2017), present values that are closer to the Brazilian reality; however, both studies involve different genetic materials, leading to different estimates.

According to the NRC (1994), Japanese quail should receive 0.75% total methionine + cystine in their diets during the growth phase (0.68% digestible methionine + cystine) according to the values estimated by Shrivastav and Panda (1987). However, other studies have suggested that methionine levels above the NRC (1994) recommendations may result in better performance (Kaur et al., 2008; Parvin et al., 2010; Khosravi et al., 2016; Rostagno et al., 2017) because over the past 30 years, there have been genetic improvements in this species, which have transformed it into a more productive bird and, consequently, it has higher nutritional needs to direct to the deposition of tissues to improve its efficiency.

For maximum weight gain, 0.89 and 0.77% digestible methionine + cystine for the starter and grower phases, respectively, were required. We observed a higher requirement during the first phase owing to the higher growth peak. Parvin et al. (2010) observed that methionine levels influenced weight gain only during the 0 to 21 days phase, with no significant effect during the 21 to 35 days.

In a study examining the determination of lysine requirement, using the ideal protein concept, Rostagno et al. (2017) recommended the requirement of 0.74 and 0.69% digestible methionine + cysteine in diets with 24.36 and 23.01% crude protein for Japanese quail during the starter and grower phases, respectively.

Previous studies by Kaur et al. (2008), Parvin et al. (2010), and Khosravi et al. (2016) presented methionine + cystine requirements for Japanese quail during the growth phase in the form of total aminoacids of 0.94, 0.90, and 0.91%, respectively. Kaur et al. (2008) used maize, soybean meal, and rice; Parvin et al. (2010) formulated diets from maize, soybean meal, rice, and fishmeal; and Khosravi et al. (2016) used corn, wheat, and soybean meal.

Rostagno et al. (2017) recommended that the digestibility of aminoacids for birds is, on average, 90% in diets based on corn and soybean meal. However, other studies normally express the results based on total aminoacids and commonly used alternative feeds, making it difficult to compare the results. When analysing the results of previous studies, special attention should be paid to crude protein level of the diets. This should be considered to prevent birds from utilising nitrogen of the essential aminoacids for synthesis of non-essential aminoacids, and thus impair animal performance. As protein content is reduced, non-essential nitrogen can become a limiting factor in diets (Rostagno et al., 2017). The synthesis of non-essential aminoacids has an energy demand and the correct balance between essential and non-essential aminoacids can provide greater efficiency for the use of dietary nitrogen (van Milgen and Dourmad, 2015).

For feed conversion, requirements of 0.85 and 0.77% of digestible methionine + cystine levels were obtained for starter and grower phases, respectively, which were very close to those estimated for weight gain. According to Ren et al. (2013), aminoacids have several metabolic functions; however, their main function is related to the formation of body protein (muscle mass), making their supplementation favour the genetic expression for weight gain and feed conversion.

When formulating the experimental diets, the lowest possible level of digestible methionine + cystine (0.52%) was used in the diets based on corn and soybean meal to estimate requirements during both phases. In previous studies by Kaur et al. (2008) and Parvin et al. (2010), it was not possible to estimate the best level of feed conversion from 21 to 35 days of age because the crude protein level of the diet was above 23% and the lowest level tested was 0.83 and 0.75% total methionine + cystine, respectively.

In birds, the liver is the most relevant organ for the evaluation of performance because it centralises the general metabolism, alters the weight of the animal, and controls metabolic activities (Barbosa et al., 2010). The results from the present study showed that the liver weight had a quadratic effect, decreasing to the inflection point of the curve and with optimal values of 0.89 and 0.76% digestible methionine + cystine during the starter and grower phases, respectively. These levels favoured lower catabolic activity because the relative weight was lower than that of the other treatments.

Feathers have a high concentration of sulfurous aminoacids in their structure, with approximately 2% of methionine and 25% of cystine directed from the diet of the birds (Leeson and Summers, 2001; Oliveira Neto, 2014). However, in the present study, the sulfur aminoacids levels in the diet did not significantly influence the production of feathers in the birds, corroborating the results obtained by Lima et al. (2016).

When assessing the relative weight of lymphoid organs, spleen, and cloacal sac, which have an important immunological function of producing antibodies, the levels evaluated did not influence their weights, which was in agreement with the studies of Kaur et al. (2008) and Parvin et al. (2010).

The methionine + cystine levels tested in the present study did not significantly influence the chemical composition of the carcass, rate of deposition of protein/fat, and energy retained. This was possibly due to the biological importance of these aminoacids, which are first acquired and then used to produce molecules that are essential for metabolism.

According to Brosnan et al. (2007) and Williams and Schalinske (2007), SAM, an active form of methionine, is the most potent methylating cofactor in the body and is essential for the biosynthesis of several cellular components, such as carnitine, phosphatidylcholine, creatine, epinephrine, melatonin, proteins, DNA, and RNA. Similar to methionine, cysteine is involved in the production of proteins and is responsible for the formation of several important body components, such as mucins, GSH, taurine, and hydrogen sulfate (Stipanuk, 2004; Nelson and Cox, 2014).

All these compounds formed from the metabolism of methionine play fundamental roles in lipid metabolism, integrity and maintenance of intestinal functions, protection of damage caused by oxidising agents, and pro-inflammatory response.

Based on the performance results observed during the laying phase, it was possible to confirm the estimates obtained during the grower phase. Variables such as feed conversion by egg mass, feed conversion per dozen eggs, viability, and feed intake showed a quadratic effect, with levels of 0.82, 0.76, 0.77, and 0.87% of digestible methionine + cystine used during the grower phase, respectively, being adequate for influencing these productive parameters in the posture.

Lima et al. (2016) also observed a residual effect of methionine + cystine levels on growth over the laying phase, which influenced feed intake and feed conversion per dozen eggs in an increasing linear fashion.

These results indicate that diets with 0.77% digestible methionine + cystine (Table 4) could increase productivity via the number of eggs produced, which was directly related to feed conversion per dozen eggs (0.76%). However, to increase egg weight, supplementation of 0.82% of methionine + cystine was required during the grower phase because the feed conversion by egg mass was correlated to intake, posture rate, and egg weight.

According to Harms et al. (1999), hens consume energy to support the number of eggs they produce; however, the weight of eggs depends on the aminoacids levels in the diet, especially sulfides, with methionine being an important factor in controlling egg size. Studies have shown an increase in total solids of egg components when using higher sulfur aminoacids levels, which directly influences egg weight (Keshavarz, 1995; Brumano et al., 2010; Schmidt et al., 2011). However, the chemical composition of eggs is quite stable and difficult to modify nutritionally because its components are secreted by the oviduct epithelial cells (Leeson and Summers, 2001).

A key factor for good performance during the egg production phase is that birds have a balanced body development during the growth phase and maintain adequate protein and body fat ratios, so that they reach sexual maturity at an ideal and uniform weight (Macari and Mendes, 2005). Shim and Chen (1989), when testing different sources of methionine and its effects on the sexual maturity of growing Japanese quail, concluded that quail fed a higher methionine diet had higher ovary weights and egg production.

The levels evaluated during the grower phase influenced the mean weight of birds at 42 days of age in a quadratic manner (Table 4); however, this variation was not enough to influence the age of the first egg (Table 6), maintained between 55 and 56 days of age, regardless of the treatment received during the grower phase. This can be attributed to compensatory growth that, according to the study by Pacheco et al. (2007), is defined by having feed restrictions (quantitative or qualitative) followed by a feeding period at will, with the animals expressing an accelerated growth rate after the restriction period compared with the animals receiving adequate diets.

Lerman and Bie (1975) argued that the lack of a perfect model for estimating the nutritional needs of animals is the most important problem for nutritionists. Therefore, we decided to choose the parameter feed conversion for the estimation of digestible methionine + cystine owing to its economic importance during growth and production phases, as it is involved in the intake of diets (i.e. cost) and weight gain (i.e. production).

5. Conclusions

The requirement for digestible methionine + cystine for Japanese quail is 0.85% during the starter phase (1 to 14 days of age) and 0.77% during the grower phase (15 to 42 days of age) to achieve a better response in feed conversion, which corresponds to a digestible methionine + cystine:digestible lysine ratio of 0.76 and 0.69% and daily intake of 50.43 and 158.5 mg of digestible methionine + cystine per day, respectively.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: T.P. Perine and S.M. Marcato. Data curation: T.P. Perine, D.O. Grieser and T.M. Oliveira-Bruxel. Formal analysis: T.P. Perine, D.O. Grieser, P.C. Pozza and T.M. Oliveira-Bruxel. Investigation: T.P. Perine, C.E. Stanquevis, E.M. Finco, M.I. Benites and T.M. Oliveira-Bruxel. Methodology: T.P. Perine, D.O. Grieser, C.E. Stanquevis, E.M. Finco, M.I. Benites, T.M. Oliveira-Bruxel and S.M. Marcato. Project administration: T.P. Perine and S.M. Marcato. Resources: S.M. Marcato. Software: D.O. Grieser. Supervision: S.M. Marcato. Validation: D.O. Grieser, P.C. Pozza and S.M. Marcato. Visualization: D.O. Grieser, P.C. Pozza and S.M. Marcato. Writing – original draft: T.P. Perine. Writing – review & editing: T.P. Perine.

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