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Original Article

# Digestible Threonine to Lysine Ratios for Meat-Type Quails

**Author(s)**

Castro MR[I] https://orcid.org/0000-0001.8509.8497
Pinheiro SRF[I] https://orcid.org/0000-0002.6631.6417
Oliveira RG[II] https://orcid.org/0000-0001.8070.0768
Abreu LRA[II] https://orcid.org/0000-0001.9926.6359
Mota LFM[III] https://orcid.org/0000-0001.9983.1784
Miranda JA[I] https://orcid.org/0000-0002.9616.0740

[I] Departamento de Zootecnia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil.
[II] Departamento de Zootecnia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
[III] Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brazil.

**Mail Address**

Corresponding author e-mail address
Sandra Regina Freitas Pinheiro
Universidade Federal dos Vales do Jequitinhonha e Mucuri, Campus JK, Rodovia MGT 367 km 583, no 5000, Diamantina, MG, Brazil
Email: sandrafreitaspinheiro@gmail.com

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## ABSTRACT

Three experiments were conducted to estimate the optimal digestible threonine to lysine ratios (Thr:Lys) of meat-type quails during the pre-starter (1 to 7 days), starter (8 to 14 days) and grower I (15 to 21 days) phases. In each experiment, 600 birds were distributed according to a completely randomized experimental design into five treatments, consisting of five dietary Thr:Lys ratios (0.66, 0.71, 0.76, 0.81 and 0.86%), with eight replicates of 15 quails each. Performance parameters and intestinal morphology were evaluated. During the pre-starter phase, feed intake, threonine intake, weight gain, and ileal villus height linearly increased ($p<0.01$) as dietary digestible Thr:Lys increased. During the starter phase, increasing digestible Thr:Lys ratios had a linear effect ($p<0.01$) on threonine intake and livability, and linear and quadratic effects ($p<0.01$) on feed intake. During the grower I phase, increasing digestible Thr:Lys ratios promoted linear increase in threonine intake and feed conversion ratio ($p<0.05$), and a linear decrease in weight gain. The estimated digestible Thr:Lys ratios recommended for meat-type quails are 0.85, 0.73 and 0.66%, for the pre-starter, starter and grower I phases, respectively.

## INTRODUCTION

Although threonine is the third limiting amino acid, after methionine and lysine, in poultry feeds based on corn and soybean meal, it is currently considered a key amino acid in least-cost formulations as it may allow reducing dietary crude protein level (Fernandez *et al.*, 1994; Umigi *et al.*, 2007). Threonine is directly involved in the immune response as it is essential for the maintenance of immune cells (immunoglobulins) and for the gastrointestinal production of mucin (Mao *et al.*, 2011), contributing for the maintenance and development of intestinal mucosa integrity. In addition, threonine is essential for feather growth, enzyme synthesis, and lean meat accretion (Kidd, 2000; Wang *et al.*, 2009).

A significant fraction of dietary threonine is absorbed in the small intestine, especially in the ileum, which is the main site of amino acid uptake due to the presence large numbers of carriers (Le Bellego *et al.*, 2002). The development of the lumen surface area and villus height determine the efficiency of nutrient digestion and absorption, and are essential for optimal animal performance (Cera *et al.*, 1988; Wang *et al.*, 2007). There is limited information on the threonine: lysine ratio (Thr:Lys) requirements for meat-type quails compared with other poultry. Therefore, the objective of this study was to estimate optimal digestible threonine: lysine ratios of meat-type quails for the pre-starter (1 to 7 days), starter (8 to 14 days) and grower I (15 to 21 days) phases.

# MATERIAL AND METHODS

## Animal care

The experimental procedures applied in the present study were approved by the Ethics Committee on Animal Use of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), state of Minas Gerais, Brazil (protocol n. 032/2012).

## Birds, housing and experimental design

Three experiments were conducted at the Laboratory for Research on Non-Ruminant Animals of the Department of Animal Science of UFVJM between January and June 2013.

In each experiment, 600 non-sexed meat-type quails (Coturnix coturnix) were evaluated during the pre-starter (1 to 7 days of age; Experiment 1), starter (8 to 14 days of age; Experiment 2) and grower I (15 to 21 days of age; Experiment 3). Initial average body weights were determined as 9.41 ± 0.1 g; 36.5 ± 0.1 g; 86.2 ± 0.5 g, respectively.

Quails were housed in galvanized-wire cages (60 cm long x 60 cm wide x 35 cm-high) in three 4-tiered batteries. Each cage was equipped with a trough drinker and a trough feeder placed in the front of the cage. Cages were heated with incandescent 100, 60, and 40 watt lamps. Room temperature was monitored twice a day (08:00 and 15:00 h) by a digital thermometer placed each battery. Bird behavior was daily observed to evaluate their thermal comfort. The bulbs were changed as the birds grew to prevent overheating.

**Table 1 –** Ingredients and calculated composition of the pre-starter experimental diets (1 to 7 days of age).

| Ingredients | Threonine:Lysine Ratio | | | | |
|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 |
| Corn | 53.7524 | 53.7524 | 53.7524 | 53.7524 | 53.7524 |
| Soybean meal (45%) | 35.968 | 35.968 | 35.968 | 35.968 | 35.968 |
| Corn gluten (60%) | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Corn starch | 0.5000 | 0.5244 | 0.5482 | 0.5721 | 0.5963 |
| Soybean oil | 0.1583 | 0.1583 | 0.1583 | 0.1583 | 0.1583 |
| Limestone | 1.2779 | 1.2779 | 1.2779 | 1.2779 | 1.2779 |
| Dicalcium Phosphate | 1.0263 | 1.0263 | 1.0263 | 1.0263 | 1.0263 |
| Salt | 0.3818 | 0.3818 | 0.3818 | 0.3818 | 0.3818 |
| Choline chloride (60%) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| L-Lysine HCl (78%) | 0.1784 | 0.1784 | 0.1784 | 0.1784 | 0.1784 |
| DL-Methionine (99%) | 0.3124 | 0.3124 | 0.3124 | 0.3124 | 0.3124 |
| L-Threonine (96%) | 0.0195 | 0.0827 | 0.1470 | 0.2102 | 0.2744 |
| L-Isoleucine (99%) | 0.1319 | 0.1319 | 0.1319 | 0.1319 | 0.1319 |
| L-Arginine (99%) | 0.3831 | 0.3831 | 0.3831 | 0.3831 | 0.3831 |
| L-Glutamic (99.4%) | 0.5000 | 0.4129 | 0.3243 | 0.2372 | 0.1488 |
| Vitamin-mineral premix[1-2] | 0.4000 | 0.4000 | 0.4000 | 0.4000 | 0.4000 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Calculated nutritional composition | | | | | |
| Metabolizable energy (kcal/kg) | 2,900 | 2,900 | 2,900 | 2,900 | 2,900 |
| Crude protein (%) | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Calcium (%) | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Available phosphorus (%) | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| Sodium (%) | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Crude fiber (%) | 3.36 | 3.36 | 3.36 | 3.36 | 3.36 |
| Digestible amino acids (%) | | | | | |
| Lysine | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Methionine + Cysteine | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
| Tryptophan | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Threonine | 0.82 | 0.88 | 0.95 | 1.01 | 1.07 |
| Arginine | 1.82 | 1.80 | 1.80 | 1.80 | 1.80 |
| Isoleucine | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Valine | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 |

[1]Nutrients per kilogram of diet: Cobalt 2g, Copper 20g, Iron 100g, Iodine 2g; Manganese 160g; Zinc 100g, Vehicle q.s.p. 1,000g.

[2]Nutrients per kilogram of diet: folic acid 750 mg; pantothenic acid 12,000 mg; BHT 1,000 mg; biotin 25 mg; niacin 35 mg; Vit. A 8,000,000 IU; Vit B1 1,500 mg; Vit. B12 12,000mcg; Vit B2 5,000 mg; Vit B6 2,800 mg; Vit D3 2,000,000 IU; Vit. E 15,000 IU; Vit. K 1,8000 mg; Vehicle q.s.p. 1,000g.

In all three experiments, birds were distributed in a completely randomized design, consisting of five treatments (dietary dig. Thr:Lys ratios), with eight replicates of 15 birds each.

### Experimental diets

The experimental standard diet was based on corn and soybean meal, and formulated to contain 0.82% digestible threonine in order to obtain a Thr:Lys ratio of 0.66. The digestible Thr:Lys ratios of 0.66, 0.71, 0.76, 0.81 and 0.86% of the five experimental diets were obtained by the supplementation of five L-threonine (99%) levels (Tables 1, 2, and 3). L-threonine was included in replacement of glutamic acid in protein equivalents, and of starch in energy equivalents, in order to obtain equal protein and energy levels in all experimental diets.

The experimental diets were balanced for digestible amino acid contents, according the nutritional requirements established by Rostagno *et al.* (2011), and the nutritional recommendations of Silva & Costa (2009), except for digestible lysine, which level was 8.5% lower than the recommendations. Dietary digestible methionine+cystine ratios were estimated according to Castro (2014). The amino acid to lysine ratios proposed by Silva & Costa (2009) were applied, except for digestible threonine (Tables 1, 2 and 3). All amino acids, except for digestible lysine and threonine, were included at 3% higher levels than those recommended in order to ensure the supply of their requirements.

**Table 2 –** Ingredients and calculated composition of the starter experimental diets (8 to 14 days of age).

| Ingredient | Threonine:Lysine Ratio | | | | |
|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 |
| Corn | 53.7346 | 53.7346 | 53.7346 | 53.7346 | 53.7346 |
| Soybean meal (45%) | 35.9917 | 35.9917 | 35.9917 | 35.9917 | 35.9917 |
| Corn gluten (60%) | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Corn starch | 0.50 | 0.5239 | 0.5652 | 0.5720 | 0.5963 |
| Soybean oil | 0.1664 | 0.1664 | 0.1564 | 0.1664 | 0.1664 |
| Limestone | 1.2779 | 1.2779 | 1.2779 | 1.2779 | 1.2779 |
| Dicalcium phosphate | 1.0261 | 1.0261 | 1.0261 | 1.0261 | 1.0261 |
| Salt | 0.3817 | 0.3818 | 0.3818 | 0.3818 | 0.3818 |
| Choline chloride (60%) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| L-Lysine HCl (78%) | 0.1778 | 0.1778 | 0.1778 | 0.1778 | 0.1778 |
| DL-Methionine (99%) | 0.3007 | 0.3007 | 0.3007 | 0.3007 | 0.3007 |
| L-Threonine (96%) | 0.0192 | 0.0824 | 0.1466 | 0.2098 | 0.2741 |
| L-Isoleucine (99%) | 0.1315 | 0.1315 | 0.1315 | 0.1315 | 0.1315 |
| L-Arginine (99%) | 0.3824 | 0.3824 | 0.3824 | 0.3824 | 0.3824 |
| L-Glutamic (99.4%) | 0.5000 | 0.4127 | 0.3173 | 0.2372 | 0.1488 |
| Vitamin-mineral premix [1-2] | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated nutritional composition | | | | | |
| Metabolizable energy (kcal/kg) | 2,900 | 2,900 | 2,900 | 2,900 | 2,900 |
| Crude protein (%) | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Calcium (%) | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Available phosphorus (%) | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| Sodium (%) | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Crude fiber (%) | 3.36 | 3.36 | 3.36 | 3.36 | 3.36 |
| Digestible amino acids (%) | | | | | |
| Lysine | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Methionine + Cysteine | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 |
| Tryptophan | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Threonine | 0.82 | 0.88 | 0.95 | 1.01 | 1.047 |
| Arginine | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 |
| Isoleucine | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Valine | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 |

[1]Nutrients per kilogram of diet: cobalt 2 g; copper 20 g; iron 100 g; iodine 2 g; manganese 160 g; zinc 100 g; vehicle q.s.p. 1,000 g.

[2]Nutrients per kilogram of diet: folic acid 750 mg; pantothenic acid 12,000 mg; BHT 1,000 mg; biotin 25 mg; niacin 35 mg; Vit. A 8,000,000 IU; Vit B1 1,500 mg; Vit. B12 12,000 mcg; Vit B2 5,000 mg; Vit B6 2,800 mg; Vit D3 2,000,000 IU; Vit. E 15,000 IU; Vit. K 1,8000 mg; Vehicle q.s.p. 1,000 g.

**Table 3 –** Ingredients and calculated composition of the starter experimental diets (15 to 21 days of age).

| Ingredient | Threonine:LysineRatio | | | | |
|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 |
| Corn | 53.7672 | 53.7672 | 53.7672 | 53.7672 | 53.7672 |
| Soybean meal (45%) | 35.9484 | 35.9484 | 35.9484 | 35.9484 | 35.9484 |
| Corn gluten (60%) | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Cornstarch | 0.50 | 0.52 | 0.54 | 0.57 | 0.59 |
| Soybean oil | 0.1516 | 0.1516 | 0.1516 | 0.1516 | 0.1516 |
| Limestone | 1.2779 | 1.27 | 1.27 | 1.27 | 1.27 |
| Dicalcium phosphate | 1.02 | 1.02 | 1.02 | 1.02 | 1.02 |
| Salt | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| Choline chloride (60%) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| L-Lysine HCl (78%) | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| DL-Methionine (99%) | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| L-Threonine (96%) | 0.01 | 0.08 | 0.14 | 0.21 | 0.27 |
| L-Isoleucine (99%) | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 |
| L-Arginine (99%) | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| L-Glutamic (99.4%) | 0.50 | 0.41 | 0.32 | 0.23 | 0.14 |
| Vitamin-mineral premix [1-2] | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Calculated nutritional composition | | | | | |
| Metabolizable energy (kcal/kg) | 2,900 | 2,900 | 2,900 | 2,900 | 2,900 |
| Crude protein (%) | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Calcium (%) | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| Available phosphorus (%) | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 |
| Sodium (%) | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Crude fiber (%) | 3.35 | 3.35 | 3.35 | 3.35 | 3.35 |
| Digestible amino acids (%) | | | | | |
| Lysine | 1.25 | 1.25 | 1.25 | 1.25 | 1.25 |
| Methionine + Cysteine | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 |
| Tryptophan | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |
| Threonine | 0.82 | 0.88 | 0.95 | 1.01 | 1.07 |
| Arginine | 1.80 | 1.80 | 1.80 | 1.80 | 1.80 |
| Isoleucine | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Valine | 1.01 | 1.01 | 1.01 | 1.01 | 1.01 |

[1]Nutrients per kilogram of diet: cobalt 2 g; copper 20 g; iron 100 g; iodine 2 g; manganese 160 g; zinc 100 g; vehicle q.s.p. 1,000 g.

[2]Nutrients per kilogram of diet: folic acid 750 mg; pantothenic acid 12,000 mg; BHT 1,000 mg; biotin 25 mg; niacin 35 mg; Vit. A 8,000,000 IU; Vit B1 1,500 mg; Vit. B12 12,000 mcg; Vit B2 5,000 mg; Vit B6 2,800 mg; Vit D3 2,000,000 IU; Vit. E 15,000 IU; Vit. K 1,8000 mg; Vehicle q.s.p. 1,000 g.

### Performance parameters and intestinal morphometry

The performance parameters evaluated were feed intake (g/bird), threonine intake (mg/bird), weight gain (g/bird), feed conversion ratio (g/g) and livability (%).

Small intestine morphological parameters were evaluated in 8-d-old birds. Eight birds per treatment were selected according to the average weight of each replicate and sacrificed. The duodenum, jejunum and ileum were opened in the mesenteric region and fragments of approximately 2.0 cm in length were stretched by the serous tunic and fixed in commercial formaldehyde at 4%. Samples were dehydrated in graded ethanol series, cleared in xylol, embedded in paraffin and cut into 5-μm sections (Prophet *et al.*, 1992). Three slides were prepared using three semi-serial sections of each intestinal fragment (duodenum, jejunum, ileum). Two slides were stained with hematoxylin-eosin, while the third was stained with Alcian blue and hematoxylin-eosin. Images (5x magnitude) were obtained using an optical microscope and a Leica Qwin camera. Straight-line lengths of 30 villi of each intestinal segment were randomly measured using the software ImageJ, and are expressed in μm.

### Statistical analysis

The obtained data were tested for normality and homoscedasticity, and then subjected to analysis of variance, and then to polynomial linear, quadratic and linear response plateau regression models. The software package Sistemas de Análises Estatísticas e Genéticas (SAEG, 2007) was used for statistical analyses.

## RESULTS AND DISCUSSION

Average environmental temperatures recorded during the experimental periods were 35° C±1.26 (minimum) and 38° C±0.88 (maximum) during the pre-starter phase (1 to 7 days age); 34° C±1.39 (minimum) and 37 °C±0.95 (maximum) during the starter phase (8 to 14 days age); and 31° C±1.30 (minimum) and 34° C±1.15 (maximum) during grower I phase (15 to 21 days age). According to Sousa (2013), the thermal comfort range for meat-type quails is 36-39ºC during the first week, 27-30ºC during the second week, and 24ºC during the third week of age. Consequently, during the pre-starter phase, temperature values were close to the quails' thermal comfort range, whereas during the starter and grower I phases, birds experienced heat stress, which may have reduced their feed intake and productivity.

In Experiment 1, evaluating the pre-starter phase (1 to 7 days old), increasing digestible Thr:Lys ratios resulted in a linear ($p$<0.01) increase in feed intake (FI), Thr intake (TI) and weight gain (WG), as estimated by the equations: FI = 25.366 + 13.416X, $R^2$ = 0.88; TI = -10.025 + 57.567X, $R^2$ = 0.99; and WG = 16.943 + 12.013X, $R^2$ = 0.94 (Table 4). Using the LRP model, an optimal ratio Thr:Lys of 0.85% was estimated for maximum FI (36.83 g), according to the equation: FI = 36.8314 - 14.052 (0.848 - X), $R^2$ = 0.99. The digestible Thr:Lys ratios recommended by Silva & Costa (2009) for meat-type quails during the starter phase (1 to 21 days old) and by Rostagno *et al.* (2017) for Japanese quails during the starter phase (1 to 14 days old) are 76% and 67%, respectively. Amino acids requirement estimates to achieve optimal performance are influenced by age, growth phase, lean meat accretion rate, dietary protein levels, and the statistical methods applied (Rangel-Lugo *et al.*, 1994; Smith *et al.*, 1998; Kidd, 2000, Rosa *et al.*, 2001; Dozier *et al.*, 2015). Furthermore, Silva *et al.* (2012) compared the genotypes of European and Japanese quails and reported that, although the protein and energy requirements of European quails are similar to those of Japanese quails, European quails require higher amino acids levels, possibly due to their higher growth rate and a heavier body weight.

In Experiment 2, evaluating the starter phase (8 to 14 days old), no effects of digestible Thr:Lys ratios were detected ($p$>0.05) on weight gain and feed conversion ratio. However, linear and quadratic effects ($p$<0.01) were observed on feed intake, given by the equations: FI = 84.400 + 9.252X, $R^2$= 0.38; and FI= 190.01 – 271.69X + 185.24$X^2$, $R^2$=0.90, respectively. The quadratic model estimated 0.73% digestible Thr:Lys ratio at the lowest feed intake (90.38%), after which feed intake gradually increased. Threonine intake (TI = -8.249 + 124.754X; $R^2$ = 0.98) linearly increased as dietary digestible Thr:Lys ratio increased ($p$<0.01) (Table 5). Adequate threonine intake is important particularly during more advanced growth stages in quails with high potential for lean meat accretion, because threonine requirements for maintenance is proportionally high relative to the other amino acids due to its high content in endogenous intestinal secretions (Fernandez *et al.*, 1994; Kidd 2001; Sigolo *et al.*, 2017). Ton *et al.* (2013) evaluated the digestible

**Table 4 –** Growth performance of meat-type quails fed increasing digestible Thr:Lys ratios from 1 to 7 days of age (pre-starter phase).

| Variable | Digestible Thr:Lys ratios % | | | | | CV (%) | P-value |
|---|---|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 | | |
| Feed intake (g) * | 34.29 | 35.07 | 34.94 | 36.67 | 36.83 | 4.11 | 0.0003 |
| Threonine intake (mg) * | 28.12 | 30.86 | 33.19 | 37.04 | 40.17 | 4.93 | 0.0001 |
| Weight gain (g) * | 24.96 | 25.58 | 25.68 | 26.80 | 27.35 | 4.89 | 0.0001 |
| Feed conversion (g/g) | 1.349 | 1.396 | 1.362 | 1.369 | 1.346 | 3.27 | 0.5587 |
| Livability (%) | 94.17 | 96.19 | 94.17 | 96.67 | 95.83 | 6.27 | 0.5478 |

CV= Coefficient of variation; *Effect linear ($p$< 0.01).

**Table 5 –** Growth performance of meat-type quails fed digestible Thr:Lys ratios from 8 to 14 days of age (starter phase).

| Variable | Digestible Thr:Lys ratios % | | | | | CV (%) | P-value |
|---|---|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 | | |
| Feed intake (g)** | 91.18 | 90.91 | 90.65 | 90.93 | 93.64 | 1.21 | 0.001 |
| Threonine intake (mg)* | 74.77 | 80.00 | 86.12 | 91.84 | 100.20 | 3.69 | 0.001 |
| Weight gain (g) | 49.64 | 49.84 | 48.09 | 48.52 | 50.88 | 4.46 | 0.094 |
| Feed conversion (g/g) | 1.838 | 1.829 | 1.885 | 1.877 | 1.832 | 3.95 | 0.118 |
| Livability (%)** | 100.00 | 100.00 | 100.00 | 100.00 | 95.00 | 1.44 | 0.001 |

CV= Coefficient of variation; *Linear effect ($p$< 0.01); **Quadratic effect ($p$< 0.01).

threonine requirements for 1- to 14-d-old meat-type quails, and reported a quadratic effect on feed intake and weight gain, estimating a requirement of 12.60 g digestible threonine/kg of diet, which is equivalent to a 67% Thr:Lys ratio. Dozier *et al.* (2015) reported an average minimum digestible Thr:Lys ratio of 0.67% for 1- to 14-d-old male broilers for weight gain and feed conversion ratio. In the present study, increasing dietary digestible Thr:Lys ratios reduced quail livability, as estimated by the linear (LIV = 114.65 – 20.556X, $R^2$=0.50) and quadratic (LIV= -52.789 + 423.257X – 291.588$X^2$, $R^2$= 0.85) equations (Table 6).

The effects of dietary digestible Thr:Lys ratios on the performance of quails during grower phase I (15 to 21 days old; Experiment 3) are shown in Table 6. Threonine intake (TI = -3.819 + 172.516X; $R^2$ = 0.99) linearly increased as dietary digestible Thr:Lys ratios increased ($p$<0.01) as a result of Thr supplementation in the diets. However, body weight linearly decreased, as determined by the equation: WG = 79.651 – 22.673X ($R^2$= 0.76. Harper et al (1970) reported that amino acids in excess did not cause signs of toxicity, differ substantially from those considered to be toxic in their ability to depress feed intake, and threonine is one of the least toxic amino acids. Thus, the evaluated increasing Thr:Lys ratios did not influence feed intake due to the limited effect of an excess of threonine on feed intake depression. However, quail weight gain was negatively influenced possibly due to excessive dietary nitrogen, which demands rapid removal, catabolism and excretion of the amount ingested in excess of the requirements, resulting in excess of uric acid in the excreta. Therefore, energy will be utilized to eliminate that nitrogen excess rather than being used for protein synthesis.

**Table 6 –** Growth performance of meat-type quails fed digestible Thr:Lys ratios from 15 to 21 days of age (grower I phase).

| Variable | Digestible The:Lys ratios % | | | | | CV (%) | P-value |
|---|---|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 | | |
| Feed intake (g) | 133.99 | 135.49 | 133.95 | 134.28 | 135.62 | 1.37 | 0.3305 |
| Threonine intake (mg)* | 109.87 | 119.23 | 127.25 | 135.62 | 145.11 | 1.32 | 0.0001 |
| Weight gain (g)* | 64.75 | 63.67 | 61.26 | 62.75 | 59.53 | 3.95 | 0.0003 |
| Feed conversion (g/g)* | 2.072 | 2.124 | 2.196 | 2.141 | 2.280 | 3.49 | 0.0001 |
| Livability (%) | 99.17 | 100.00 | 99.05 | 96.67 | 99.17 | 2.48 | 0.2563 |

CV= Coefficient of variation; *Linear effect ($p$< 0.01).

Increasing dietary Thr:Lys ratios also linearly increased feed conversion (FCR = 1.500 + 0.871X, $R^2$=0.75). The best Thr:Lys ratio to achieve the highest weight gain (64.75 g) and the best feed conversion ratio (2.072) was estimated as 0.66%. Although Rostagno *et al.* (2017) recommended a digestible Thr:Lys ratio of 0.734% for 15- to 35-d-old Japanese quails, the results of the present study indicate that Thr:Lys ratios higher than 0.66% have a negative effect on performance parameters. These results are in agreement with the findings of Edmonds & Baker (1987), Carew *et al.* (1998), and Sigolo *et al.* (2017), who reported that excessive Thr supply reduces broiler feed intake and growth rate. Wang *et al.* (2007) observed that excessive dietary threonine reduced the synthesis of intestinal mucus proteins, mucin and muscle protein in weaned piglets, and impaired their performance. According to those authors, a possible explanation would be a reduction in the absorption of neutral amino acids (including branched-chain amino acids) because they share common transport system with threonine, consequently limiting protein synthesis. Rossoni *et al.* (2008) also reported a negative effect of excessive dietary threonine on pig feed intake and fat deposition. Ospina-Roja *et al.* (2014) attributed the worse performance observed in 22- to 42-d-old broilers fed digestible Thr:Lys ratios higher than 57% to impaired protein synthesis caused by dietary amino-acid imbalance. According to those authors, the deamination of threonine excess relative the other amino acid into uric acid requires energy, which consequently impair animal performance.

The evaluated digestible Thr:Lys ratios did not influence ($p$>0.05) duodenal or jejunal villus height. However, ileal villus height linearly increased as Thr:Lys increased (VHi = 77.07 + 284.00X; $R^2$=0.89), as shown in Table 7. It is important to highlight that the quails evaluated in this study were reared under controlled conditions (environment and disease control), which may explain this effect. Ospina-Roja *et al.* (2013) reported that healthy broilers fed diets that supplied their threonine requirements and containing amino acid levels in excess of their requirements did not present any small intestine morphological changes. Moreover, proteins involved in physiological functions have high threonine content, which requirements increase under health challenges due to the strong stimulation of the body's defense systems, increasing

**Table 7 –** Effects of different dietary digestible Thr:Lys ratios on the intestinal morphological parameters of meat-type quails during the pre-starter phase (1 to 7 days of age).

| Variable | Digestible Thre:Lys ratios % | | | | | CV (%) | P-value |
|---|---|---|---|---|---|---|---|
| | 0.66 | 0.71 | 0.76 | 0.81 | 0.86 | | |
| VHd (µm) | 879.20 | 928.47 | 912.31 | 850.58 | 823.39 | 12.20 | 0.3368 |
| VHj (µm) | 440.39 | 384.99 | 390.12 | 394.67 | 385.26 | 11.67 | 0.2000 |
| VHi (µm)* | 258.69 | 289.52 | 294.48 | 298.14 | 324.55 | 17.22 | 0.0168 |

CV= Coefficient of variation; *Linear effect ($p$< 0.05). VHd: duodenal villus height; VHj: jejunal villus height; VHi: ileal villus height.

mucus secretion and antibody synthesis (Ajinomoto, 2003). Reis *et al.* (2016), evaluating dietary digestible threonine levels (0.98, 1.04, 1.10 and 1.16%) for starter: meat-type quails (1 to 21 days old), estimated a requirement of 1.1% for optimal villus height in the duodenum, jejunum and ileum.

The observed increase in villus height indicates that efficiency of nutrient (amino acids) digestion and absorption. According to Rutz (2002), large membrane carriers are located in the ileum, suggesting that this is the main site of threonine absorption. Similarly, Macari & Maiorka (2000) reported that the development of the intestine mucosa is characterized by an increase in villus height and density, corresponding to an increase in the number of epithelial cells and consequent better nutrient digestion and absorption. On the other hand, Ospina-Roja *et al.* (2013), evaluating the effects of glycine and threonine supplementation on the performance and intestinal mucosa development of growing broilers (21-35 days old), reported that increasing dietary glycine+serine and threonine levels enhanced mucin secretion, but had no effect on villus height. Significant amounts of threonine are required by growing poultry, particularly for maintenance, due to their high body protein turnover rate (Sá *et al.*, 2007). According to Myerie *et al.* (2001) and Berres *et al.* (2007), approximately 60% of dietary threonine is retained in the intestine for mucin synthesis. However, because mucin is resistant to digestion, it is fermented by intestinal microorganisms or excreted in feces, and therefore, is not utilized for body protein synthesis.

## CONCLUSION

The estimated digestible Thr:Lys ratios recommended for meat-type quails are 0.85, 0.73 and 0.66%, for the pre-starter, starter and grower I phases, respectively.

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