Protein, Methionine+Cystine and Lysine Levels for Japanese Quails During the Production Phase

ABSTRACT

An experiment was conducted at Faculdade de Medicina Veterinária e Zootecnia/Unesp - Botucatu for 168 days to evaluate the effects of protein, Met + Cys and lysine diet levels on egg production and egg quality of laying Japanese quails. Quails with 42 days of age were reared in a completely randomized design. There were 1,944 quails distributed in four replicates of 27 birds per pen, according to a factorial 3x3x2 with three crude protein levels (16, 18 and 20% CP), three Met + Cys levels (0.700; 0.875 and 1.050%) and two lysine levels (1.100 and 1.375%). Birds fed diets with 18 and 20% CP had higher feed intake and egg production than those fed diets with 16% CP. There was significant interaction (p<0.05) between protein and Met + Cys levels on egg weight. There was no effects (p>0.05) of the protein level on feed conversion per dozen eggs; however, improved feed conversion per egg mass was seen for birds fed diets with 20% CP compared to those fed diets with 16% and 18% CP. Protein and lipid percentage in the yolk increased when dietary protein level increased from 16 to 18%. Increasing Met + Cys from 0.700% to 0.875% reduced yolk protein percentage. Higher lipid percentage in the yolk was seen in eggs from quails fed diets with 1.050% Met + Cys, whereas 1.375% lysine in the diet of resulted in decreased egg production and egg mass, besides poorer feed conversion per dozen eggs and per egg mass.

INTRODUCTION

Diet formulation for Japanese quails is usually based on nutrient requirement tables from other countries, which are not ideal for the tropical climatic conditions of Brazil. No new reports on the nutrient requirements for quails have been described since 1984 (NRC, 1994), evidencing the need of new information.

On the other hand, the increasing prices of corn and soybean meal and the greater commercial availability of the two most important limiting amino acids for birds (methionine and lysine) have drawn interest to diet formulation based on reduced protein levels with adequate amino acid supplementation.

Protein requirements have been reported to change according to age, so that quails aged 4, 8 and 12 months required for maximal performance 14.6, 16.5 and 18% of protein in the diet, respectively (Schwartz & Allen, 1981). Such results evidence the decrease in the ability of protein utilization in older birds; however, levels of 20% CP and 3,000 kcal ME/kg are suggested by NRC (1984). Besides, levels recommended for Japanese quails during the laying period, no matter the age, were 20%CP and 2,800 kcal ME/kg (AEC, 1987) and 20% CP and 2,900 kcal ME/kg (NRC, 1994).

Murakami (1991) evaluated nutrient requirements for laying quails...
after 35 days of age and reported as adequate levels 2,700 kcal ME/kg and 18% CP in the diet during the laying period. Improved egg weight and feed conversion per egg mass were seen when the protein level of the diet was increased, whereas feed intake, laying rate, feed conversion per dozen eggs, shell percentage and shell thickness were not significantly affected.

The effects of two energy levels (2,850 and 2,950 kcal/ME/kg) and five protein levels (16; 18; 20; 22 and 24%) in the diet were evaluated for quails during the production phase (Pinto, 1998). Protein levels had a linear effect on the fourth period and a quadratic effect on the laying rate in two from the four periods that were studied, and maximum egg production was seen with 21.5 and 22.5% CP. Egg weight, shell weight, and feed conversion per dozen eggs and per egg mass were improved with increasing protein in the diet. There was a quadratic effect of the protein level on the produced egg mass, and maximum production was observed with 23.1% CP in the diet.

On the other hand, egg production, feed intake and feed conversion were not different among treatments containing three different crude protein levels (19, 20 and 22%) and three metabolizable energy levels (2,400, 2,600 and 2,800 kcal ME/kg), although egg weight was greater with 2,600 kcal and 20% CP (Vidal et al., 2000).

Protein and amino acid requirements vary considerably according to the growth rate and egg production. Shim & Cheng (1989) reported better egg production with 0.62% Met+Cys compared to 0.52; 0.57; 0.67 and 0.72% Met+Cys. Similarly, during production, quails after 5 weeks of age require 0.375% of Met and 0.30% available Cys in a diet with 20% CP (Shim & Shim, 1989). Intake levels of 79.5 mg available Met were required for maximum egg production. Shim & Lee (1984) reported similar estimated requirements for maximal egg production and egg weight (1.07% and 1.08% lysine, respectively), and it was considered that the control diet with 19% CP and 1.0% Lys fulfilled the nutritional requirements for this amino acid.

Considering the contradictory results that have been reported and the few data available in Brazil about the protein and amino acid level requirements in Japanese quails, this study was carried out to evaluate the effects of protein, Met+Cys and lysine levels on the performance and egg quality of laying quails in the production period.

**MATERIAL AND METHODS**

The experiment was carried out at the Fazendas de Ensino, Pesquisa e Produção from Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade Estadual Paulista (UNESP), in Botucatu, SP, Brazil.

Two thousand and two hundred quails were reared from one to 35 days of age under similar management and feeding conditions. At 35 days of age, 1,944 birds were transferred to a production poultry house and housed in conventional quail cages throughout the experimental period (168 days).

Treatments were comprised of three protein levels (16, 18 and 20%), thee Met+Cys levels (0.700; 0.875 and 1.050%) and two lysine levels (1.100 and 1.375%) in the diet.

Ingredients used to produce the diet were analyzed in Laboratório de Análises Bromatológicas from FMVZ. Protein, calcium and phosphorus levels were determined, and metabolizable energy and amino acid levels were estimated according to Andriguetto et al. (1998). Chemical compositions and estimated
percentages of the experimental diets are shown in Table 1.

Egg production, feed intake, mean egg weight, egg mass, feed conversion per egg dozen and per egg mass, eggshell percentage, yolk percentage and albumen percentage were evaluated. Dry matter (DM) and percentages of protein, ether extract and ashes were determined in the yolk and albumen (AOAC, 1990).

Performance was analyzed according to a completely randomized design in a factorial arrangement 3 x 3 x 2 (three protein levels, three methionine + cystine levels, two lysine levels) with 4 repetitions and 27 birds per parcel. Egg quality was assessed in five eggs collected per repetition during three days at the end of each 28-day period.

Results were submitted to analysis of variance and Tukey’s test (p<0.05) was used to compare significantly different means.

RESULTS AND DISCUSSION

Performance results of the Japanese quails submitted to the experimental treatments are shown in Table 2. Protein (p<0.01) and lysine (p<0.01) levels significantly affected laying rate. There were no significant effects of Met+Cys levels and there were no significant three-way interaction among the factors.

Birds fed 16% protein in the diet had decreased egg production compared to the other birds. Although birds fed 20% CP showed a tendency to higher egg production than birds fed 18% CP, no significant differences were seen. These findings are different from results reported previously by Vohra & Roudybush (1971), Crivelli-Espinosa et al. (1980) and Sakurai (1981), who suggested that protein levels higher than 24% CP would maximize egg production. On the other hand, the results are similar to the value of 20% CP suggested by NRC (1994) and AEC (1987).

Egg production was not affected by Met+Cys levels in the diet, although production tended to be higher with increasing Met+Cys. Conversely, some previous studies have reported significantly increased egg production with higher Met+Cys levels (Resende, 1993; Murakami et al., 1994; Dabbert et al., 1996; Bello, 1997).

The increase in protein level from 16% to 18% resulted in higher feed intake, but feed intake was not significantly different between birds fed 18% or 20% CP. Increased feed intake was also reported with increasing protein levels in commercial layers (Yakout et al., 2000), and in studies that assessed protein levels for Japanese quails (Sakurai, 1981; Arscott & Pierson-Goeger, 1981). On the other hand, Cherici (1987) and Murakami (1991) reported no significant effects of protein diet levels on intake. These discordant results among studies may have resulted from the different levels of other nutrients in the diets, such as energy or calcium levels.

Feed intake was not affected by the increase in lysine and Met+Cys levels. Resende (1993) and Murakami et al. (1994) concluded that Met levels and sulfur amino acid levels in the diet had no effect on

### Table 1 - Percentage and calculated composition of experimental diets.

| Ingredients                        | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      | 11      | 12      | 13      | 14      | 15      | 16      | 17      | 18      |
|------------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
|                                    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    |
| ME (kcal/kg)                       | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    | 2800    |
| Antioxidant (BHT)                  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  | 0.0200  |
| Total                              | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     | 100     |

* Levels per kg of diet: Manganese, 80.0 mg; Iron, 50.0 mg; Copper, 10.0 mg; Zinc, 50.0 mg; Cobalt, 1.0 mg; Iodine, 1.0 mg; Selenium, 0.075 mg. **Levels per of diet: Vit. A, 120,000IU; Vit D3, 3,600 IU; Vit B1, 2.5 mg; Vit B2, 8 mg; Vit B6, 5 mg; Folic acid, 12 mg; Biotin, 0.2 mg; Vit K3, 3.0 mg; Folic acid, 1.5 mg; Nicotinic acid, 40 mg; Vit B12, 20 mcg.
the feed intake of quails in the laying period. Oliveira (1998) evaluated lysine levels varying from 0.65 to 1.45% in the diet of quails during production and reported no significant effects of lysine on feed intake.

There was a significant interaction between protein and Met+Cys levels in the diet (Table 3) on egg weight, which indicates that the main factors are dependent. Birds fed 18% CP and 0.875% Met+Cys had greater egg weight than birds fed 0.700% Met+Cys, whereas the birds fed 1.050% showed intermediate values. Within 16 and 20% CP, egg weight was not affected by Met+Cys levels. At 0.700% Met+Cys, eggs were heavier when birds were fed 20% CP. Within the Met+Cys level of 0.875%, eggs were heavier at 18 and 20% CP. Finally, the increase in protein levels of diets containing 1.050% Met+Cys increased egg weight.

### Table 2 - Effects of protein, Met+Cys and lysine levels on the performance of Japanese quails

<table>
<thead>
<tr>
<th>Nutrient Levels</th>
<th>Egg Production (%)</th>
<th>Feed intake (g)</th>
<th>Egg weight (g)</th>
<th>Egg Mass (g)</th>
<th>Feed conversion kg/dozen</th>
<th>Feed conversion kg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>73.09</td>
<td>74.94</td>
<td>76.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>24.45</td>
<td>25.08</td>
<td>25.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>11.32</td>
<td>11.52</td>
<td>11.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met + Cys (%)</td>
<td>0.700</td>
<td>0.875</td>
<td>1.050</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.700</td>
<td>74.53</td>
<td>74.61</td>
<td>75.54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.875</td>
<td>24.98</td>
<td>24.91</td>
<td>24.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.050</td>
<td>11.44</td>
<td>11.59</td>
<td>11.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.100</td>
<td>1.375</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.100</td>
<td>75.92</td>
<td>73.74</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1.375</td>
<td>25.00</td>
<td>24.90</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>11.32</td>
<td>11.44</td>
<td>11.52</td>
<td>11.69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by different letters in the column are different by Tukey’s test (p<0.05). 1 - Significant interaction Protein:Met+Cys.

Garcia EA, Mendes AA, Pizzolante CC, Saldanha ESPB, Moreira J, Mori C, Pavan AC

Protein, Methionine+Cystine and Lysine Levels for Japanese Quails During the Production Phase

Crivelli-Espinosa et al. (1980) and Pinto (1998) reported that Japanese quails fed 24% CP in the diet produced heavier eggs than birds fed lower protein levels. On the other hand, Costa et al. (1985), Cherici (1987) and Shrivastav et al. (1993) reported that egg weight increased when levels CP were increased up to 25% CP in the diet. The present findings corroborate results from Pires Júnior (1981) and Murakami (1991), who reported that egg weight may be improved with 20% CP levels. Although it has been previously reported that Met+Cys levels higher than 0.72% in the diet affect egg weight negatively (Shim & Lee, 1988), in the present study there were no negative effects of increasing amino acid levels in egg weight at 16, 18 or 20% CP in the diet.

Higher protein levels in the diet improved egg mass. Nevertheless, an opposite behavior was seen with the increase in diet lysine levels. The effects of protein levels on egg mass may reflect the greater laying rate and egg weight that were also seen with increased protein levels, since egg mass is directly related to these parameters. Crivelli-Espinosa et al. (1980) reported maximum egg mass production with 19.4% CP in the diet.

There was no significant effects of Met+Cys levels on egg mass. On the other hand, Bello (1997) reported an increase in egg production and egg weight when sulfur amino acid levels up to 0.692 and 0.839% were used in the diet. It was reported that lysine levels affected egg mass significantly, and best results were seen with 1.1% Lys.

There was no significant effect of protein and Met+Cys, or of the interaction between these factors, on feed conversion (kg feed: kg dozen eggs), although the worst results were seen with 1.375% Lys. Arscott & Pierson-Goeger (1981), Schwartz & Allen (1981) and Pinto (1998) reported significant protein effects on feed conversion. Feed conversion was improved by the increase in protein levels. Considering the sulfur amino acid levels, the present results partially corroborate the
results reported by Bello (1997). Feed conversion was affected by treatments containing Met+Cys levels from 0.549 to 0.839%, so that feed conversion was poorer only at the higher lysine levels (1.375%) (Bello, 1997). Therefore, recommended levels of lysine from 0.97% to 1.0% (Shim & Lee, 1984) and from 1.0 to 1.1% (Shim & Lee, 1985) seem to be adequate for laying quails.

Feed conversion (kg diet : kg eggs) was improved with 20% protein and poorer results were seen with 16%. Feed conversion with 18% CP was not statistically different from the other treatments.

Murakami (1991) observed improved feed conversion per egg mass with diet protein levels between 19 and 20%, whereas Pinto (1998) reported improvement in feed conversion per egg mass with up to 22% CP in the diet. Feed conversion was also improved as a function of Met+Cys (Resende, 1993; Bello, 1997). It was suggested that increased Met+Cys levels improved egg weight, and consequently, feed efficiency.

Although Shrivastav et al. (1990) suggested that 0.79% Lys in the diet is adequate for egg production, Shim & Lee (1984), Shim & Lee (1985) and NRC (1994) suggest levels of 0.97; from 1.0 to 1.1; and 1.0%, respectively. The present findings and previous findings reported in the literature suggest that lysine levels between 1.0 to 1.1% are adequate for the optimization of feed conversion.

The effects of treatments on egg quality are shown in Table 4. There were no significant treatment effects on eggshell percentage. Murakami (1991) and Pinto (1998) also found that protein levels had no effect on this parameter. On the other hand, Bello (1997) reported increased egg weight and decreased eggshell percentage with increasing Met+Cys levels in the diet. In commercial laying hens, Novak & Scheideler (1998) and Yakout et al. (2000) suggested that eggshell percentage may be reduced by higher lysine levels and by lower Met+Cys levels.

Improved yolk percentage was obtained with Met+Cys levels of 0.875% and 1.050%, whereas protein levels had no effect on yolk percentage. Akbar et al. (1983) reported that higher protein levels in the diet increased yolk percentage and reduced albumen percentage in eggs from commercial laying hens. Nevertheless, Scheideler & Elliot (1998) did not report influence of Met+Cys levels on the yolk percentage in laying hens, when daily intake was between 520 and 800 mg/bird/day, whereas Novak et al. (1999) and Yakout et al. (1999) reported decreased yolk percentage with the increase in lysine intake in laying hens.

No significant effects of protein, Met+Cys and lysine levels were seen on the percentage of albumen, and a significant interaction (p<0.05) between Met+Cys and lysine was seen. The simple main effects from the factors (Table 5) showed higher albumen percentages with 1.10% lysine and 1.050% Met+Cys.

Akbar et al. (1983) reported that an increase in protein levels resulted in increased yolk percentage and decreased albumen percentage. On the other hand, the percentage of albumen in eggs from commercial laying hens was not affected by Met+Cys levels between 0.52 and 0.8% (Scheideler & Elliot, 1998) or by lysine levels from 800 to 900 mg/day and Met+Cys levels of 490 and 730 mg/day (Yakout et al., 1999). Furthermore, Scheideler et al. (1996) reported that albumen percentage was not significantly affected by lysine levels between 500 and 1,000 mg/bird/day.

The percentage of protein in the yolk was significantly affected by protein levels (p<0.01) and Met+Cys levels (p<0.05). Birds fed 18% or 20% CP had higher protein levels in the yolk compared to the

### Table 4 - Effects of protein, Met+Cys and lysine levels on egg quality.

<table>
<thead>
<tr>
<th>Nutrient levels</th>
<th>Eggshell (%)</th>
<th>Yolk (%)</th>
<th>Albumen (%)</th>
<th>Yolk protein (%)</th>
<th>Albumen protein (%)</th>
<th>Yolk ether extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (%)</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>0.700</td>
<td>0.875</td>
<td>1.050</td>
</tr>
<tr>
<td></td>
<td>8.57</td>
<td>8.62</td>
<td>8.68</td>
<td>8.65</td>
<td>8.61</td>
<td>8.62</td>
</tr>
<tr>
<td></td>
<td>31.34</td>
<td>31.11</td>
<td>31.21</td>
<td>31.03</td>
<td>31.54</td>
<td>31.09</td>
</tr>
<tr>
<td></td>
<td>60.09</td>
<td>60.16</td>
<td>60.10</td>
<td>60.21</td>
<td>59.86</td>
<td>60.29</td>
</tr>
<tr>
<td>Met+Cys (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.303</td>
<td>0.306</td>
<td>0.304</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>79.43</td>
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<tr>
<td>Lysine (%)</td>
<td></td>
<td></td>
<td></td>
<td>30.03 A</td>
<td>30.60 A</td>
<td>30.72 B</td>
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<td></td>
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<td></td>
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<td>79.43</td>
<td>79.68</td>
<td>79.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>61.72 c</td>
<td>61.41 b</td>
<td>58.99 A</td>
</tr>
</tbody>
</table>

Means followed by different letters in the column are different by Tukey’s test (p<0.05). 1 - Significant interaction Methionine + Cystine:Lysine. 2 - Significant interaction Protein:Methionine + Cystine. 3 - Significant interaction Methionine + Cystine:Lysine.
birds fed 16% CP in the diet. Such results corroborate previous findings from Andersson (1979) and Akbar (1983), who reported that yolk protein contents increased with higher protein levels in the diet.

Significant effects of diet lysine levels on the protein percentage of albumen in eggs from laying hens have been reported by Vogt & Krieg (1982) and Prochaska et al. (1996) in one of two studies that were conducted. Such effect was not observed in the present study.

Protein levels (p<0.01) and Met+Cys levels (p<0.05) had significant effects on the ether extract percentage in the yolk. The increase in the protein levels resulted in a proportional decrease in the yolk ether extract. Nevertheless, eggs from birds fed the diet with 1.050% Met+Cys had yolk protein levels similar to the other two groups.

Shafer et al. (1996) reported positive effects of the increase in total sulfur amino acid levels on yolk protein. As for lysine levels, Prochaska et al. (1996) reported no significant effects of 638 to 1,165 mg/bird/day on the protein contents of the yolk in eggs from 23-week-old laying hens.

Significant interactions were seen between protein and Met+Cys levels (p<0.01) and between protein and lysine levels (p<0.05) on the albumen protein levels (Table 6).

The simple main effects of the interaction between protein and Met+Cys shows that, for birds fed 18% CP in the diet, those fed with 0.700% Met+Cys had higher albumen protein than those fed with 1.050% Met+Cys. Considering the birds fed the 20% CP diet, higher protein in the albumen was present in eggs from birds fed 1.050% Met+Cys than from birds fed 0.700% Met+Cys. On the other hand, within the group of 0.700% Met+Cys, birds fed 18% CP had higher albumen protein than birds fed the other diets. Finally, for the birds fed 1.050% Met+Cys, those fed 20% of protein had higher percentage of albumen protein than those fed 18%, whereas birds fed 16% protein were not different from the other groups.

Higher protein percentage in the yolk was seen with 0.700% Met+Cys compared to 0.875% Met+Cys. Nevertheless, eggs from birds fed the diet with 1.050% Met+Cys had yolk protein levels similar to the other two groups.

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Significant effects of diet lysine levels on the protein percentage of albumen in eggs from laying hens have been reported by Vogt & Krieg (1982) and Prochaska et al. (1996) in one of two studies that were conducted. Such effect was not observed in the present study.

Protein levels (p<0.01) and Met+Cys levels (p<0.05) had significant effects on the ether extract percentage in the yolk. The increase in the protein levels resulted in a proportional decrease in the yolk ether extract. Nevertheless, the increase in Met+Cys level from 0.700% to 1.050% increased yolk ether extract. Similarly, Gardner & Young (1972) reported that the increase in protein levels from 12 to 18% CP decreased ether extract percentage in the yolk and increased yolk protein percentage in the eggs from laying hens in the early laying period.

**CONCLUSIONS**

Egg production, feed intake, egg mass, protein and ether extract in the yolk were increased by diets with 18% CP and daily intake of 4.48 g CP when compared to diets containing 16% CP, although there were no effects on feed conversion per dozen eggs.

Production performance or egg quality were not improved by Met+Cys levels higher than 0.700% and Met+Cys daily intake higher than 174 mg/bird/day.

Diet lysine levels higher than 1.10% and lysine daily intake higher than 275 mg/bird/day were inadequate since there was decreased egg production, egg mass and feed conversion per egg mass.

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