The effect of dietary protein levels and synbiotic on performance parameters, blood characteristics and carcass yields of Japanese quail (Coturnix coturnix Japonica)

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Abstract

The aim of this study was to evaluate the effect of synbiotic on performance parameters, blood characteristics and carcass yields of Japanese quails fed diets containing different levels of protein. In a completely randomized design with 3 x 3 factorial arrangements, 720-day-old healthy Japanese quails were randomly allocated into 9 groups with 4 replicates of 20 chicks. Treatments consisted of combination of 3 levels of crude protein (CP): A) sufficient protein diet (24%, high CP) from 0 to 42 days of age; B) low protein diet (22.08%, low CP) from 0 to 42 days of age; C) sufficient protein diet from 0 to 21 days-low protein diet from 21 to 42 (medium CP) days of age and three levels of synbiotic, without, recommended and 150% of recommended levels, respectively. The results showed that there were no significant differences in feed conversion ratio, feed intake and body weight among treatments due to the interaction of CP and synbiotic levels. However, body weight and daily weight gain and feed conversion ratio improved (P<0.05) for bird fed medium and high CP compared with birds fed low CP in diet. The level of serum triglyceride decreased significantly (P<0.05) by adding different levels of synbiotic in diet. However, the effects of synbiotic supplementation on other blood parameters (cholesterol, total protein serum, uric acid, HDL and LDL) were not statistically significant among the groups (P>0.05). The effect of CP and synbiotic levels on the carcass yields of quail were not statistically significant (P>0.05).

Introduction

Poultry industry is one of the most dynamic agribusiness trades worldwide. The importance of feed supplementation in poultry production has increased in the last years with the aim of improving the economic situation of poultry projects (Zeweil et al., 2006). Nowadays, the efficiency of poultry to convert the feed into meat, plays a key role in economics of broiler industry. Therefore, it is highly essential to improve feed efficiency of poultry to produce meat economically and also food safety is more seriously considered than before. On the other hand, economy of food production is also a factor that can not be ignored. A huge amount of antibiotics have been used to control diseases and improve performances in livestock. The use of antibiotics to promote growth and control diseases in farm animals has been the usual practice for many decades among farmers (Plail, 2006; Zeweil et al., 2006; Akinleye et al., 2008). But by long-term use, side effects of antibiotics occur, like residues in meat. Many countries are either regulating the use of antibiotics in feed or setting up programs to reduce the overall use of antibiotic. Therefore, the use of probiotic and prebiotic in poultry diet has become popular as an alternative to antibiotic for animal production and health worldwide in recent years (Ayasan et al., 2006; Zeweil et al., 2006; Sahin et al., 2008; Vali, 2009; Erdogan et al., 2010; Skvortsova, 2010; Sahin et al., 2011). A way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics, which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract. Those effects are due to activating the metabolism of one or a limited number of health-promoting bacteria or by selectively stimulating their growth, which improved the welfare of the host, or both (Gibson and Roberfroid, 1995).

One of the most expensive and deficient compounds of feeds for farm animals is the protein source. On the other hand, the protein quantity and quality are the main factor limiting the animal performance. For this reason the attempt of nutritionists is to find cost effective and environment friendly solutions for utilization of protein rich feeds (Djouvinov and Mihailov, 2005). In addition to the stimulation of development of desirable bacteria, the probiotic and prebiotic effect of lactobacillus is also manifested in the prevention of development of coli-bacteria and in its inhibition of enterotoxins in the digestive system (Fuller, 2001), which reduces the breakdown of proteins to nitrogen. In this way, the utilization of proteins (amino acids) is improved, particularly from food that does not contain them in optimum quantities (Mikulec et al., 1999). As seen in the previous studies, there has not been information in the literature on the effect of synbiotic supplementation on performance parameters, blood characteristics and carcass yields of Japanese quail. Therefore, the present study was conducted to investigate the effects of synbiotic supplementation and different protein levels on growth and blood parameters and carcass yields of Japanese quail.

Materials and methods

Seven hundred and twenty-day-old healthy Japanese quail were randomly allocated into 9 groups with 4 replicates of 20 birds. The birds were reared in cages of identical size (100x100 cm floor area and 80 cm height) for 42 days of experimental period. All groups were subjected to similar management practices (brooding, lighting, feeding and watering) throughout the experiment except the diets offered. Quails were benefited from day-light (average 16 h/day) and water, and experimental diets were continuously available and room temperature was gradually decreased from 37°C at first day to 23°C after 21 days of age. No vaccination was performed.

Dietary treatments were used with 3 levels of crude protein (CP): A) sufficient protein
diet (24%, high CP) from 0 to 42 days of age; B) low protein diet (22.08%, low CP) from 0 to 42 days; and C) sufficient protein diet from 0 to 21 days of age - low protein diet during 21 to 42 days of age and 3 levels of synbiotic: i) basal diet-without synbiotic; ii) basal diet supplemented with synbiotic (1 g/kg of Bioimin® IMBO (Bioimin GmbH, Herzogenburg, Austria) for the 0-21 days and 0.5 g/kg for the 21-42 days periods); iii) basal diets supplemented with synbiotic (1.5 g/kg of Bioimin® IMBO for the 0-21 days and 0.75 g/kg for the 21-42 days periods). Nutrients compositions of the diets except for protein for quails were based on the National Research Council (NRC, 1994) recommendations (Table 1). Representative samples of feed ingredients (corn, soybean meal and fish meal) were analyzed for CP (AOAC, 1990).

Bioimin® IMBO (Bioimin GmbH, Herzogenburg, Austria) is a combination of the probiotic strain Enterococcus faecium (DSM 3330), a prebiotic (derived from chicory), and immune modulating substances (derived from sea algae).

Weight of all collected Japanese quails determined at 1 and 42 days of age were treated as initial weight and final weight, respectively. The daily weight gain (DWG) was calculated as (final weight - initial weight)/42. Feed conversion ratio was calculated weekly as the amount of feed consumed per unit of body weight gain.

At 42 days of age, blood samples were collected from the brachial vein of 2 quails from each replicate to determine serum cholesterol, triglyceride, total protein, uric acid, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). The blood was collected in a test tube to obtain serum. The collected blood samples were centrifuged at 3000rpm for 10 min and the serum was decanted into aseptically treated vials and stored at -20°C until further analysis. The serum concentrations of total triglyceride, cholesterol, total protein, uric acid, HDL cholesterol in serum samples were analyzed by an automatic biochemical analyzer (Cima, R.A. S.A., St. Joan Despi, Spain), following the instructions of the corresponding reagent kits (Teif Amzon Pars Co., Tehran, Iran); very-low-density lipoprotein (VLDL) cholesterol was calculated from triglycerides divided by the factor 5 (Ashayerizadeh et al., 2009). The LDL cholesterol was calculated by using the formula LDL cholesterol = Total cholesterol – HDL cholesterol – VLDL cholesterol. For slaughter process, feed was removed from each pen 12 h prior to processing. Eight quails per group (2 birds per replicate, one male and one female) were randomly selected and slaughtered for determining the carcass yields. The relative weights of carcass, thigh and breast muscles to live body weights were calculated. The obtained data regarding growth performance, blood parameters and carcass characteristics were subjected to analysis of variance using one-way ANOVA procedure of SAS (1998). The significant means were ranked using Duncan’s Multiple Range Test (Duncan, 1955).

### Results and discussion

The initial body weight of chicks did not differ (P>0.05) among the dietary treatments (Table 2). At the end of the experiment (day 42), the body weight did not differ (P>0.05) due to interaction of CP and synbiotic and by synbiotic levels, while there were significant differences among treatments of body weight and daily body weight gain for CP levels (P<0.05). The body weight and daily weight gain (from days 1 to 42) were increased (P<0.05) for birds received medium and higher CP compared with birds received low CP.

Djouvinov and Mihailov (2005) reported that the changes in body weight of growing quails during a 42-day experimental period did not demonstrate any significant effect by different levels of protein. Symbiotic levels were not demonstrating any significant effect. Similar results were observed in other studies (Cakir et al., 2006; Akinleye et al., 2008). However, beneficial effects of these supplements in poultry nutrition have been widely documented (Awan et al., 2008, 2009; Chimote et al., 2009). Total feed intake and daily feed intake of the Japanese quail in this study were not significantly influenced (P>0.05) by the interaction and main effects of CP and symbiotic levels. These results agree with the work by Kaur et al. (2006), who reported that feed intake did not differ significantly between the groups receiving di levels of CP. Sahin et al. (2008) showed that the feed intake was not statistically different in Japanese quail due to combination of probiotic and prebiotic.

The interaction of CP levels and symbiotic levels did not influence (P>0.05) on the feed conversion ratio during 0 to 42 days of age. Feed conversion ratio was lower for the quails received diets with high and medium CP level than low protein diet. An improvement in feed conversion ratio in growing quails during 0-3 weeks of age with increasing dietary CP level was also reported earlier (Kaur et al., 2006; 2008). During overall phase, however, the feed

### Table 1. Ingredients and nutrient composition of experimental diets.

|                     | Sufficient protein | Low protein diet |
|---------------------|--------------------|------------------|
| Ingredients, %      |                    |                  |
| Corn 7.89%CP        | 50.51              | 56.96            |
| Soybean meal 43.68%CP | 42.04              | 36.47            |
| Fish meal 55.32%CP  | 3.00               | 3.00             |
| Soy oil             | 2.97               | 1.05             |
| Dicalcium phosphate | 0.32               | 0.38             |
| Limestone           | 1.16               | 1.17             |
| Salt                | 0.30               | 0.29             |
| Hcl-Lysine          | -                  | 0.05             |
| DL-Methionine       | 0.10               | 0.13             |
| Vitamin premix°     | 0.25               | 0.25             |
| Mineral premix°     | 0.25               | 0.25             |
| Total               | 100.0              | 100.0            |

**Analysis results**

|                     | Metabolisable energy, kcal/kg | Crude protein, % | Calcium, % | Available phosphorus, % | Sodium, % | Lysine, % | Methionine, % | Methionine + cystine, % | Threonine, % |
|---------------------|------------------------------|------------------|------------|------------------------|-----------|-----------|----------------|------------------------|-------------|
|                     | 2900                         | 24.00            | 0.80       | 0.30                   | 0.15      | 1.39      | 0.50           | 0.88                   | 1.07        |

**Vitamin premix content per kg**: vitamin A, 3,450,000 U; vitamin D, 3,500,000 U; vitamin E, 7,200 U; vitamin K, 800 mg; vitamin B₆, 720 mg; vitamin B₁₂, 3000 mg; vitamin B₁₂, 4000 mg; vitamin B₁₂, 12,000 mg; vitamin B₆, 500 mg; vitamin B₁₂, 600 mg; vitamin B₆, 2000 mg; choline chloride, 200,000 mg; antioxidant, 400 mg. **Mineral premix content per kg**: Mn, 61,140 mg; Fe, 100,000 mg; Zn, 117,600 mg; Cu, 16,000 mg; I, 640 mg; Se, 8000 mg; choline chloride, 133,340 mg.
Table 3. Effect of protein and synbiotic levels on blood parameters of Japanese quails.

| Initial body weight, g | CP × Synbiotic | Final body weight, g | CP × Synbiotic | Daily body weight gain, g | CP × Synbiotic | Total feed intake, g | CP × Synbiotic | Daily feed intake, g | CP × Synbiotic | FCR, feed/gain |
|------------------------|----------------|----------------------|----------------|---------------------------|----------------|----------------------|----------------|----------------------|----------------|-------------------|
| CP levels              |                | Synbiotic levels     |                |                            |                |                      |                |                      |                |                   |
| Low                    | 8.57±0.08      | 243.2±5.2            | 5.9±0.13       | 865.2±15.8                | 20.65±0.38     | 3.69±0.02            |                |                      |                |                   |
| Medium                 | 8.47±0.09      | 250.0±3.7            | 5.89±0.06      | 887.1±10.0                | 21.12±0.24     | 3.55±0.02            |                |                      |                |                   |
| High                   | 8.61±0.08      | 258.8±3.31           | 5.98±0.06      | 889.2±7.8                 | 21.17±0.19     | 3.54±0.05            |                |                      |                |                   |
| Low R                  | 8.43±0.07      | 254.4±3.3            | 5.86±0.05      | 887.2±4.2                 | 21.13±0.10     | 3.60±0.03            |                |                      |                |                   |
| Medium R               | 8.46±0.10      | 259.3±2.8            | 5.97±0.06      | 886.9±19.0                | 21.12±0.45     | 3.54±0.06            |                |                      |                |                   |
| High R                 | 8.60±0.05      | 260.7±5.1            | 6.00±0.12      | 891.4±11.5                | 21.23±0.23     | 3.53±0.04            |                |                      |                |                   |
| Low 50%R               | 8.5±0.09       | 259.4±9.0            | 5.65±0.10      | 862.7±16.8                | 20.54±0.40     | 3.63±0.03            |                |                      |                |                   |
| Medium 150%R           | 8.6±0.08       | 262.2±3.0            | 6.04±0.09      | 893.0±6.2                 | 21.27±0.15     | 3.52±0.04            |                |                      |                |                   |
| High 150%R             | 8.48±0.09      | 259.8±2.9            | 5.99±0.04      | 883.8±3.4                 | 21.14±0.08     | 3.53±0.02            |                |                      |                |                   |

Main effects

CP levels

Low 8.50±0.05 247.8±2.5 P≤0.05 872.3±7.7 20.77±0.19 3.64±0.02

Medium 8.51±0.05 251.8±2.4 P≤0.04 889.0±6.8 21.17±0.16 3.54±0.03

High 8.56±0.05 262.0±6.6 P≤0.00 889.5±4.3 21.18±0.10 3.53±0.02

Synbiotic levels

0 8.5±0.05 253.0±2.8 5.82±0.06 880.8±6.8 20.97±0.16 3.61±0.03

R 8.49±0.06 258.2±3.0 5.94±0.05 888.5±6.8 21.16±0.15 3.55±0.03

150%R 8.52±0.04 258.2±2.9 5.89±0.07 881.2±6.8 20.98±0.16 3.57±0.02

Probability

CP × Synbiotic ns ns ns ns ns

CP level ns P≤0.05 ns ns ns

Synbiotic level ns ns ns ns ns

FCR, feed conversion rate; CP, crude protein; R, recommended level; *P means with different superscripts are significantly different (P<0.05); values are reported as means ± SE, ns, not significant (P>0.05).

Table 2. Effect of protein and synbiotic levels on performance parameters of Japanese quails.

| Parameter                  | Initial body weight, g | Final body weight, g | Daily body weight gain, g | Total feed intake, g | Daily feed intake, g | FCR, feed/gain |
|----------------------------|------------------------|----------------------|---------------------------|----------------------|----------------------|----------------|
| CP × Synbiotic             |                        |                      |                           |                      |                      |                |
| Low                       | 8.57±0.08              | 243.2±5.2            | 5.9±0.13                  | 865.2±15.8           | 20.65±0.38           | 3.69±0.02      |
| Medium                    | 8.47±0.09              | 250.0±3.7            | 5.89±0.06                 | 887.1±10.0           | 21.12±0.24           | 3.55±0.02      |
| High                      | 8.61±0.08              | 258.8±3.31           | 5.98±0.06                 | 889.2±7.8            | 21.17±0.19           | 3.54±0.05      |
| Low R                     | 8.43±0.07              | 254.4±3.3            | 5.86±0.05                 | 887.2±4.2            | 21.13±0.10           | 3.60±0.03      |
| Medium R                  | 8.46±0.10              | 259.3±2.8            | 5.97±0.06                 | 886.9±19.0           | 21.12±0.45           | 3.54±0.06      |
| High R                    | 8.60±0.05              | 260.7±5.1            | 6.00±0.12                 | 891.4±11.5           | 21.23±0.23           | 3.53±0.04      |
| Low 50%R                  | 8.5±0.09               | 259.4±9.0            | 5.65±0.10                 | 862.7±16.8           | 20.54±0.40           | 3.63±0.03      |
| Medium 150%R              | 8.6±0.08               | 262.2±3.0            | 6.04±0.09                 | 893.0±6.2            | 21.27±0.15           | 3.52±0.04      |
| High 150%R                | 8.48±0.09              | 259.8±2.9            | 5.99±0.04                 | 883.8±3.4            | 21.14±0.08           | 3.53±0.02      |

Main effects

CP levels

High 8.47±0.09 250.0±3.7 5.89±0.06 887.1±10.0 21.12±0.24 3.55±0.02

Synbiotic levels

0 8.5±0.09 259.4±9.0 5.65±0.10 862.7±16.8 20.54±0.40 3.63±0.03

R 8.49±0.06 258.2±3.0 5.94±0.05 888.5±6.8 21.16±0.15 3.55±0.03

150%R 8.52±0.04 258.2±2.9 5.89±0.07 881.2±6.8 20.98±0.16 3.57±0.02

Probability

CP × Synbiotic ns ns ns ns ns

CP level ns P≤0.05 ns ns P≤0.05

Synbiotic level ns ns ns ns ns

FCR, feed conversion rate; CP, crude protein; R, recommended level; *P means with different superscripts are significantly different (P<0.05); values are reported as means ± SE, ns, not significant (P>0.05).
ommended and 150% recommended levels) compared to quails fed by diet without synbiotic. Similar results were obtained by other researchers (Paryad and Mahmudi, 2008; Rabie et al., 2010).

These results also agree the work by Ashayarizadeh et al. (2009), who reported that a reduction in the serum triglyceride level with use of probiotic and prebiotic can be due to an increase in the population of lactic acid bacteria in the gastrointestinal tract. Santose et al. (1995) have reported that supplementation of Bacillus subtilis to the ration of broiler chickens, in addition to reducing the carcass fat, reduces the serum triglyceride with or without synbiotic supplementation (Mohan et al., 1996; Ghiyasi et al., 2008).

Lactobacillus, which has a high bile salt hydrolytic activity, is responsible for deconjugation of bile salts (Taherpour et al., 2009). Deconjugated bile acid are less soluble at low pH and less absorbed in the intestine and is more likely to be excreted in feces (Klaver and Van der Meer, 1993). In addition, probiotic microorganism inhibits hydroxymethyl-glutaryl-coenzyme A, an enzyme involved in the cholesterol synthesis (Fukushima and Nakano, 1995).

The most important mechanism by which prebiotic eliminates cholesterol would likely be through reducing lipid absorption in intestine by binding bile acids, which results in increased cholesterol elimination and hepatic synthesis of new bile acid (Zhang et al., 2003, Taherpour et al., 2009). Other blood parameters include total protein, uric acid, HDL and LDL were not affected by any of the levels of synbiotic supplementation and dietary protein. These findings are in agreement with those of Sahin et al. (2008) who found that combination of probiotic and prebiotic did not effect on the cholesterol and total protein in Japanese quail. Moreover, also these observations support data published by several authors (Akinleye et al., 2008; Cakir et al., 2008; Ghiyasi et al., 2008; Taherpour et al., 2009; Alkhalf et al., 2010).

Carcass and cut yields (breast and thigh) were not significantly influenced (P>0.05) by synbiotic inclusion or levels of protein in diet (Table 4). The results of this study are in agreement with previous researches (Maiorka et al., 2001; Pelicia, et al., 2004; Akinleye et al., 2008; Sahin et al., 2008; Chumpawadee et al., 2009), that reported that synbiotic, probiotic and prebiotic had no significantly (P>0.05) positive effect on carcass yields of quails and broilers. As well, no differences were seen in carcass and cut yields, due to the use of protein levels, similarly to the findings reported by Correa et al. (2005) and Ghiyasi et al. (2008).

### Conclusions

It can be concluded that the CP requirement for optimum performance in Japanese quail of up 21 days of age was 24% and, after this age, there are evidences of reduction to 22%. The inclusion of synbiotic, under conditions of good hygiene, did not show a clear positive effect on performance and blood parameters but only a small positive effect on blood parameters.

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