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Effects of Dietary Threonine and a Multi Strains Probiotic (Primalac) Supplementation on Growth Performance, Blood Metabolites and Carcass Characteristics in Japanese Quails

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Abstract
The objective of this study was to investigate the effects of three levels of threonine (100, 95 and 90% of NRC requirements) with or without of multi strains probiotic (Primalac) on performance, carcass characteristics and blood metabolites of Japanese quails. A total of 180 one-day-old male Japanese quails were randomly allocated to six treatments with three replicates and 10 birds per each. The experiment was done from 0 to 6 weeks of age. Growth performance traits including weight gain, feed intake and feed conversion ratio were recorded. At the end of the experiment, six birds per treatment were slaughtered and carcass characteristics were measured. Blood sampling was taken at 42 days of age. The results of this study indicated that supplementation of probiotic did not affect feed intake, weight gain and feed conversion ratio in quails. In contrast, feed conversion ratio was improved in birds fed with 100% of threonine requirements. All carcass traits, except for liver weight, were not influenced by threonine levels and dietary probiotic supplementation. The liver percentage was higher in birds fed diets without the probiotic. The results showed that serum glucose was affected by threonine levels. However, the other blood metabolites such as cholesterol, triglyceride, high-density lipoproteins and low-density lipoproteins were not influenced by the threonine levels. In this regard, none of the blood metabolites were influenced by probiotic supplement, except for cholesterol. In conclusions, the supplementation of probiotic did not affect growth performance in Japanese quails. However, use of threonine at 100% NRC requirements improved feed efficiency in Japanese quails.

Introduction

Nowadays livestock producers raise genetically animal yields and faster growing. It is very important to properly formulated a diet that meets the nutrient requirements of animals. Modern diet formulation is based on amino acid requirements of poultry, not crude protein.

Threonine is the third limiting amino acid, especially in a low crude protein diet. Using

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supplemental threonine allows nutritionists to feed low protein diets without compromising nutrition and performance. There are many reports on the threonine requirements of chicks (Rosa *et al*., 2001; Ahmadi and Golian, 2010; Rezaeipour *et al*., 2012; Rezaeipour and Gazani, 2014), but information on the effects of dietary L-threonine on quail performance are limited. Using inadequate levels of amino acids such as threonine can induce a lower performance in quails due to amino acid deficiency and consequently limitations in protein synthesis. It has been reported that threonine is an important component of mucus (40% of the protein in mucus glycoproteins) in the digestive tract (Carlstedt *et al*., 1993). Therefore, according to the above hypothesis, the balance of threonine in quail diets by adding L-threonine supplementation or use of soybean meal and meat meal as most important ingredients is necessary.

Antibiotics have long been used as a feed additive to increase the poultry growth performance and control of disease (Chen *et al*., 2009). Today, to maintain the microflora balance in poultry, non-antibiotic approach urgently requested. Use of products such as probiotics, prebiotics and organic acids as alternatives for antibiotics has increased in recent years. It is found that Probiotics act by competitive exclusion, lower gut pH, produce bacteriocins, lysozyme, and peroxides, and stimulate the immune system (Grashorn, 2010). The efficacy of probiotics may be potentiated by methods including the selection of more efficient strains and gene manipulation (Awad *et al.,* 2009). Several studies were conducted to investigate the effects of different probiotics in comparison with antibiotics on quail performance (Homma and Shinohara, 2004; Strompfova *et al*., 2005; Ayasan *et al*., 2006). But, there is a little information about the effects of probiotic supplement in combination with dietary threonine on quail performance.

Therefore, the aim of this study was to investigate the effects of different levels of dietary threonine in diets supplemented with a multi-strain probiotic on performance, carcass characteristics and blood metabolites of Japanese quail.

Materials and Methods

Birds and dietary treatments

The study was carried out in a poultry farm situated in Qaemshahr, Mazandaran province, Iran. All bird care and use procedures were approved by the Department of Animal and Poultry Science, Islamic Azad University of Qaemshahr Care and Use Committee. In this study litter pens with dimensions of 1 × 1.5 m and height of 0.8 m were used. The poultry farm was thermostatically controlled and feed and water were supplied *ad libitum*.

Three corn-soybean meal basal diets without any additives were formulated to meet or exceed the nutrient requirements, except for threonine amino acid, for Japanese quails recommended by the National Research Council (1994). The ingredients and calculated nutrients profile of the experimental diets are shown in Table 1. The experiment was carried out in a completely randomized design with 2 × 3 factorial arrangement, including two levels of a multi strains probiotic (with or without) and three levels of threonine (100, 95 and 90% of NRC requirements). The multi-strain probiotic was Primalac (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidum*) which was supplemented to basal diets with no additives. Each treatment was represented by three replicates and 10 male day-old Japanese quails were randomly assigned to each pen.

Traits measured

The birds were fed with the six experimental diets from 0 to six weeks of age. Feed intake and body weight gain of each pen was measured at the end of the experiment. Mortality was recorded daily and feed consumption data were corrected for body weight of mortality. Feed conversion ratio for each pen was calculated by dividing feed intake to body weight gain. At 42 days of age, six randomly selected birds from each treatment were weighed and sacrificed. The weight of intestinal tract (after digesta removal), breast, thigh, liver (without a gallbladder), gizzard and heart were recorded and their relative weight to body weight was measured.

Two birds per replicate were bled through the wing vein at 42 d of age to determine the blood biochemical metabolites. The blood sample (3 mL) was drawn and allowed to clot at room temperature (18 ^{o}C) for 2 hrs prior to serum collection. Serum was separated by centrifugation and stored at -20^{o}C for further analysis. Serum samples were thawed and serum glucose, cholesterol, triglyceride, high-density lipoproteins (HDL) and low-density

lipoproteins (LDL) were determined using an autoanalyzer (Autolab, BT 3500, Autoanalyzer medical system, Rome, Italy).

Table 1. The ingredients and chemical composition of experimental diets (%)

Ingredients	Threonine levels		
	100	95	90
Corn grain	55.57	55.57	55.57
Soybean meal	38.00	37.96	37.96
Wheat bran	2.00	2.09	2.13
Soybean oil	1.00	1.00	1.00
Dicalcium phosphate	0.86	0.86	0.86
Oyster shell	1.35	1.35	1.35
Salt	0.35	0.35	0.35
Mineral premix[1]	0.25	0.25	0.25
Vitamin premix[1]	0.25	0.25	0.25
DL-Methionine	0.07	0.07	0.07
L-Lysine	0.05	0.05	0.05
L-Threonine	0.15	0.10	0.06
Chemical composition (%)			
Metabolizable energy (kcal/kg)	2900	2900	2900
Crude protein	23.72	23.72	23.72
Calcium	0.81	0.81	0.81
Phosphorus, available	0.31	0.31	0.31
Threonine	1.03	0.98	0.93
Methionine + Cystine	0.81	0.81	0.81
Lysine	1.32	1.32	1.32

[1]Provides per kg of diet: 9000 I.U. vitamin A; 2000 I.U. vitamin D_3; 18 I.U. vitamin E; 2 mg menadione; 1.8 mg thiamine; 6.6 mg riboflavin; 30 mg niacin; 3 mg pyridoxine; 15 µg vitamin B_{12}; 100 mg D-pantothenic acid; 1 mg folic acid; 0.1 mg biotin; 500 mg choline chloride; 100 mg antioxidant; 100 mg manganese; 84.7 mg zinc; 50 mg iron; 10 mg copper; 1 mg iodine; 0.2 mg selenium.

Statistical analysis

Statistical analysis was carried out using general linear model (GLM Procedure) to evaluate the effects of treatments on performance, carcass characteristics and blood metabolites of Japanese quails in a completely randomized design with 2 × 3 factorial arrangements using SAS (2001) software. Statistical significance of differences among treatments was done using the Duncan's multiple range test.

Results

Results related to the effects of dietary treatments on Japanese quail performance are presented in Table 2. These findings indicated that effect of different levels of threonine on weight gain and feed intake was not statistically significant. However, quails fed diets with threonine at 100 and 90% of NRC requirements had better feed conversion ratio than those fed diets with 95% of threonine requirements ($P < 0.05$). Probiotic supplementation did not affect quails performance parameters. Results of carcass characteristics and internal organ weights of Japanese quails are shown in Table 3. These traits, except for liver, did not change significantly as a result of either dietary threonine levels or probiotic supplementation. The liver percentage was higher in birds fed diets with probiotic. The effects of dietary treatments on blood biochemical parameters are shown in Table 4. Results indicated that dietary threonine affected glucose concentration in blood of Japanese quails ($P < 0.05$). The birds fed diets with low levels of threonine had more serum glucose concentration. In this regards, serum cholesterol concentration of Japanese quails was influenced by probiotic supplementation ($P < 0.05$) and declined in quails fed diets without the probiotic. The other blood parameters were not influenced by threonine levels and probiotic supplementation.

Discussion

Feed conversion ratio was better in Japanese quails fed diets with 100% threonine requirements in the present study. These results contradict those obtained by Ton *et al*., (2013)

who observed that feed conversion ratio was not affected by threonine levels from 1 to 14 days of age in Japanese quails.

All carcass traits and internal organs were not influenced by L-threonine supplementation in the present study. These findings are in accordance with the results of Baylan *et al*., (2006) and Rezaeipour *et al*., (2012) who indicated that threonine supplementation to the diets did not affect growth performance and edible carcass parts in Japanese quails and broilers, respectively. In addition, Kerr *et al*., (1999) and Dozier *et al*., (2000) observed that carcass yield was not affected by threonine supplementation in broiler diets. It is reported that the formulation of diets to contain up to 544 g of L-threonine/ton did not alter growth performance of commercial broilers (Kidd *et al*., 2002). Besides, it is demonstrated that the threonine requirement of the classic strain of broiler chick is 0.69% for body weight gain and 0.68% for feed conversion ratio (Rosa *et al*., 2001).

Table 2. Effects of dietary threonine and probiotic on growth performance of Japanese quails

Threonine (%)	Probiotic	Feed intake (g/bird)	Weight gain (g/bird)	Feed intake (g/bird/d)	Weight gain (g/bird/d)	FCR (g/g)
100		695.9	222.0	16.75	5.29	3.13^{b}
95		673.0	202.1	16.02	4.81	3.33^{a}
90		660.2	206.5	15.72	4.92	3.20^{ab}
SEM		23.27	7.74	0.55	0.18	0.06
	-	665.6	205.53	15.84	4.89	3.25
	+	687.2	215.02	16.36	5.12	3.20
SEM		19.00	6.32	0.45	0.15	0.05
P-value						
Thr		0.56	0.19	0.56	0.19	0.08
Pro		0.44	0.31	0.43	0.30	0.50
Thr × Pro		0.93	0.95	0.93	0.96	0.08

Means within columns followed by different superscripts are significantly different ($P < 0.05$).

Table 3. Effects of dietary threonine and probiotic on carcass characteristics and internal organs of Japanese quails (% of live body weight)

Threonine (%)	Probiotic	Breast	Thigh	Heart	Liver	Intestine	Gizzard
100		24.8	15.3	0.98	2.12	2.10	1.59
95		25.4	16.2	1.06	1.82	1.90	1.69
90		24.5	15.3	1.00	1.86	2.04	1.68
SEM		0.73	0.53	0.06	0.18	0.17	0.12
	-	24.7	15.4	0.99	1.70^{b}	2.00	1.65
	+	25.1	15.8	1.03	2.16^{a}	2.02	1.66
SEM		0.60	0.43	0.05	0.15	0.14	0.10
P-value							
Thr		0.45	0.21	0.45	0.21	0.53	0.65
Pro		0.52	0.41	0.46	0.004	0.90	0.94
Thr × Pro		0.26	2.51	2.26	1.14	1.13	3.24

Means within columns followed by different superscripts are significantly different ($P < 0.05$).

Table 4. Effects of dietary threonine and probiotic on blood biochemical parameters of Japanese quails (mg/dL)

Threonine (%)	Probiotic	Glucose	Cholesterol	Triglyceride	HDL	LDL
100		271.50^{b}	207.16	176.83	129.60	34.84
95		304.51^{a}	205.33	176.17	123.00	33.01
90		299.60^{a}	199.00	180.00	118.66	31.00
SEM		10.52	3.41	18.47	4.58	2.20
	-	289.89	196.67^{b}	166.11	120.43	32.33
	+	293.88	211.01^{a}	189.22	127.10	33.56
SEM		8.60	2.78	15.09	3.74	1.81
P-value						
Thr		0.06	0.24	0.98	0.27	0.49
Pro		0.71	0.03	0.30	0.23	0.63
Thr × Pro		0.38	0.056	0.11	0.07	0.17

Means within columns followed by different superscripts are significantly different ($P < 0.05$).

Neither carcass traits nor internal organs were not influenced by L-threonine supplementation in the present study. These findings are in accordance with the results of Baylan *et al*., (2006) and Rezaeipour *et al*., (2012) who indicated that threonine supplementation to the diets did not affect growth performance and edible carcass parts in Japanese quails and broilers, respectively. In addition, Kerr *et al*., (1999) and Dozier *et al*., (2000) observed that carcass yield was not affected by threonine supplementation in broiler diets. It is reported that the formulation of diets to contain up to 544 g of L-threonine/ton did not alter growth performance of commercial broilers (Kidd *et al*., 2002). Besides, it is demonstrated that the threonine requirement of the classic strain of broiler chick is 0.69% for body weight gain and 0.68% for feed conversion ratio (Rosa *et al*., 2001). The serum glucose concentration of Japanese quails increased with the decrease of threonine levels in our experiment. The results of Edwards *et al*., (1997) did not support our findings. According to the findings of those authors, broiler chickens use threonine with high efficiency for protein accretion when dietary threonine is at or below the requirement whereas they use this amino acid as a glucogenic precursor in an excess amount. It is observed that lactate is the primary substrate for gluconeogenesis (glucose synthesis) than the threonine in the chickens (Myers and Klasing, 1999). A dearth of information exists in terms of blood parameters in Japanese quails response to threonine levels. Therefore, direct comparisons cannot be made.

Use of Primalac as a multi strains probiotic did not alter growth performance of Japanese quails in our study. In addition, except for liver percentage, the other carcass parameters were not influenced by probiotic supplementation. Several studies were conducted to investigate the effects of different probiotic supplements on broiler chickens performance (Lan *et al*., 2003; Kabir *et al*., 2004; Talebi *et al*., 2008; Král *et al*., 2012; Rezaeipour *et al*., 2012). However, our literature review showed that few studies have been conducted on quails. It was reported in the recent study that feed conversion ratio and overall growth performance of Japanese quails was improved by using a probiotic supplementation (Bagherzadeh Kasmani *et al*., 2012). These results are inconsistent with the findings of our experiment. In contrast, the results of Miles *et al*., (1981) and Sahin *et al*., (2008) are in accordance with our research. These authors observed that no significant differences existed in growth, feed efficiency or mortality in Bobwhite and Japanese quails fed the probiotic and combiotic supplements, respectively. The differences between investigations might be attributed to the used strains of microorganisms (Talebi *et al*., 2008). It is reported that probiotic supplementation changes the bacteria colonization in the poultry gastrointestinal tract and modified mucin formation, which in turn influences gut function resulting in improved nutrients absorption (Smirnov *et al*., 2005). In this regard, Rezaeipour *et al*., (2012) indicated that *Saccharomyces cerevisiae* as a probiotic supplement can improve broiler performance by increasing the villus height in the small intestine. In the present study, the effect of probiotic on the liver percentage of quails is in agreement with previous research (Awad *et al*., 2009). The main reason for this may be the greater weight gain and live weight of the probiotic group compared with control group.

Dietary inclusion of probiotic increased the serum cholesterol of Japanese quails in the present study. However, the other blood biochemical parameters were not affected by the probiotic supplementation. Sahin *et al*., (2008) reported that blood biochemical parameters of Japanese quails were not affected by diets supplemented with probiotic.

Conclusion

The results indicated that decreasing dietary threonine levels lower than NRC (1994) have no negative effect on carcass characteristics and internal organs, but lead to increased feed conversion ratio in Japanese quail. In addition, the supplementation of probiotic did not alter growth performance in Japanese quails.

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