



ABSTRACT

To evaluate the effects of supplementing diets of growing Japanese quails (n=450; 7 days old) with chromium (Cr) yeast and / or mannan oligosaccharides (MOS) on productive performance, carcass traits and the immune system are reported. Diets were a basal diet (control) or the basal diet supplemented with 600 mg Cr-yeast/kg (Cr 1), 1200 mg Cr-yeast/kg (Cr 2), 1.0 g MOS/kg, Cr 1 + MOS or Cr 2 + MOS. Quails fed Cr 1, MOS or Cr 1 + MOS diets had similar final live body weight and weight gain, with both groups being higher than the control. Feed conversion tended to be improved with dietary supplements. Carcass traits did not differ among treatments. Primary and secondary humoral immune responses of quails fed the supplemented diets were higher than the control. Plasma total proteins and triiodothyronine were increased, while plasma total lipids and cholesterol were decreased by the supplements. In conclusion, dietary supplementation with Cr yeast (600 mg/kg) and / or mannan oligosaccharides (1.0 g) improved productive performance and immunity and reduced total cholesterol of growing Japanese quails.

KEY WORDS chromium, immunity, mannan, performance, quail.

INTRODUCTION

Japanese quails (*Coturnix coturnix japonica*) have become an important class of livestock in the world. The advantages of Japanese quail, which have been widely used for biological and genetic studies, because it has a small body size, because it is easily handled, and because a large number of birds can be kept in a limited space (Ayasan, 2013). Chromium (Cr) is essential for activating certain enzymes and for stabilizing proteins and nucleic acids (Linder, 1991). Cr is also a cofactor of insulin, promoting insulin activity (McCarty, 1993) and enhancing amino acid uptake into muscle cells for protein synthesis (Ohba *et al.* 1986). In recent years, there has been considerable research interest in the utilization of organic Cr in animal feed. Published research related to Cr supplementation of poultry diets is

very limited; however, most of the studies prior to 1991 evaluated inorganic Cr effects on poultry (Suksombat and Kanchanatawee, 2005). A number of organic Cr preparations are commercially available for poultry and animals (Yildiz et al. 2004). Dietary Cr supplementation has been reported to positively affect growth rate and feed efficiency of growing poultry (Lien et al. 1999; Sahin et al. 2001). Mannan oligosaccharides (MOS) are natural substances derived from the outer cell wall of yeast (Saccharomyces cerevisiae) (Bonos et al. 2010), providing alternate binding sites for pathogenic bacteria (Lomax and Calder, 2009). Bozkurt et al. (2008) reported that due to the ability of MOS to limit the growth of potential pathogens in the digestive tract of animals, the digestive tract remains more healthy, works more efficiently and absorbs more nutrients. Pelicano et al. (2004) reported that adding MOS to broiler

diets increased live weight and improved feed conversion. Mannan oligosaccharides might have a positive effect on immunity due to their influence on the immune system (Shashidhara and Devegowda, 2003). The objectives of this study were to evaluate the effects of dietary Cr yeast and/or mannan oligosaccharides on the performance and immunity of growing Japanese quails.

MATERIALS AND METHODS

Japanese quails (Coturnix coturnix japonica) (n=450; 7 days old) were randomly and equally divided into six groups, equal in mean body weight (45.49 g±0.147), each of 75 chicks (3 replicates of 25 chicks). Chick groups were assigned randomly to six experimental diets: a basal diet (control Table 1); the basal diet supplemented with either 600 mg of Cr yeast/kg diet; 1200 mg Cr yeast/kg diet; 1.0 g of mannan oligosaccharides/kg diet (MOS; known commercially as Bio-MOS); 600 mg Cr yeast plus 1.0 g MOS/kg diet; or 1200 mg Cr yeast plus 1.0 g MOS/kg diet. Chromium yeast containing 0.1% chromium was provided by Alltech Inc., Nicholasville, KY, USA. The basal diet was formulated to cover the recommended nutritional requirements of growing Japanese quail (NRC, 1994). Quails were fed the experimental diets for 5 weeks. Diets and fresh water were provided ad libitum. Birds were exposed to a 23 hour photoperiod daily starting from the day of hatching until the end of the experiment. The experimental conditions complied with animal welfare and proper care of animals.

Live body weight and feed consumption were recorded weekly. At the 3rd week of age, six quail chicks (3 males and 3 females) of each treatment were injected intramuscularly (i.m.) with 0.05 mL packed SRBCs mixed with 0.95 mL physiological saline (0.9% NaCl). Seven days post SRBCs antigen challenge, blood samples in nonheparinized tubes, were collected and centrifuged (4000 rpm) and serum were decanted and stored frozen at -20 °C until used for determination of primary immune response.

At the 5th week of age, a second injection was given to the same chicks in a similar manner and then blood samples were collected after seven days for the measurement of a secondary immune response. The antibody production (Abs) was measured using a U-shape microtitre plate with 96 wells, according to the method described by Van Der Zijpp *et al.* (1983).

At the 4th week of age, six birds (3 males and 3 females) from each treatment were randomly taken, weighed, and slaughtered. Spleen, bursa, thymus and thyroid glands were manually. Spleen, bursa, thymus and thyroid glands were manually removed. The thyroid gland was taken from the right and left sides. Weights of lymphoid organs and thy-

roid gland were recorded and their weights relative to body weights were calculated.

At the end of the experiment (6th week of age), six birds (3 males and 3 females) from each group were randomly taken, weighed and slaughtered.

Table 1 Composition and calculated analyses of the basal diet

Ingredients	g/kg
Yellow corn	532
Soybean meal (48 g/100)	335
Corn gluten meal (62 g/100)	45
Sunflower oil	9
Wheat bran	45
Di-Ca-P	14.4
Limestone	10
Premix ¹	3
NaCl (salt)	2.5
L-lysine-HCL	1.9
DL-methionine	1.2
Mold Guard	1
Calculated analyses ²	
Crude protein g/kg	241
Crude fiber g/kg	30.3
Ether extract g/kg	31.6
Calcium g/kg	8.1
Available P g/kg	4.2
Lysine g/kg	13.0
Methionine g/kg	5.0
Methionine + cystine g/kg	8.9
Metabolizable energy MJ/kg	12.154

¹ Each 1 kg contain: vitamin K: 0.67 g; vitamin B₁: 0.33 g; vitamin B₂: 1.67 g; vitamin B₆: 0.50 g; vitamin B₁₂: 0.003 g; Retinyl acetate: 1.2 g; Cholecalciferol: 0.0208 g; A-tocopherol acetate: 3.33 g; Niacin: 10 g; Folic: 0.33 g; Biotin: 0.017 g; Pantothenic acid: 3.33 g; Copper: 3.33 g; Iodine: 0.33 g; Selenium: 0.03 g; Iron: 10 g; Manganese: 20 g; Zinc: 16.67 g and Cobalt: 0.03 g. ² Calculated using NRC (1994) analytical values.

Feathers were manually removed and birds were re-

weighed and eviscerated. Carcass weight and weights of gizzard, liver and heart were recorded. Blood samples in heparinzed tubes, were collected during birds slaughter by severing the jugular vein and collecting blood for 120 sec. These samples [three samples (from male and female) / treatment] were centrifuged (4000 rpm) for 10 minutes and plasma was then decanted in Eppendorf tubes and stored at -20 °C until biochemical analysis. Plasma total protein (Henry, 1964) and albumin (Dumas and Biggs, 1972) were colorimetrically determined. Plasma globulin concentration was calculated by sub.

Plasma globulin concentration was calculated by substraction of plasma albumin from plasma total protein, and then the albumin (A) to globulin (G) ratio was calculated. Plasma totals lipids (Knight *et al.* 1972) and cholesterol (Richmond, 1973) were also colormetrically determined. Plasma triiodothyronine (T₃) was determined by an RIA technique using Gamma-Coat (125I) RIA Kits (Cambridge Medical Diagnostics, Boston, MA) as reported by Akiba *et al.* (1982). Statistical analyses were performed using the SAS program package (v 6.0) (SAS, 1994). All data were analyzed by one-way ANOVA (GLM procedure) to test for the effects of the dietary treatments. Differences among treatment means were detected using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Initial body weight (BW) of quail chicks was similar for all treatments (Table 2). Final BW and weight gain (WG) of quails fed Cr 1, MOS or Cr 1 + MOS supplemented diets were similar, being higher (P < 0.05) than the control. Feed intake of quails fed the Cr 1 + MOS supplemented diet was higher (P<0.05) than the control. Feed conversion of quails was improved (P<0.05) by dietary Cr 1, Cr 2, MOS or Cr 1 + MOS supplementation. The high rate of feed conversion in this study may be because the quails were reared in the summer season (average of temperature is 34.8 °C). Chromium promotes insulin activity (McCarty, 1993). Moreover, insulin regulates metabolism of carbohydrate, fat and protein, stimulating amino acid uptake and protein synthesis as well as glucose utilization (Colgan, 1993). If insulin levels are low, glucose cannot be effectively utilized by body cells and it is converted into fat and stored in fat cells. Furthermore, if adequate amino acids cannot enter the cells, muscles cannot grow (Hossain et al. 1998). Moreover, due to the ability of MOS to limit the growth of potential pathogens in the digestive tract of animals, the digestive tract remains healthy, functions more efficiently and more nutrients are available for absorption (Bozkurt et al. 2008). These effects of Cr and MOS explain the increases in final BW, WG and feed intake with the better-feed conversion noticed in the supplemented quails of the present study. In accordance with the present results, Cupo and Donaldson (1987) reported that Cr supplementation increased the rate of glucose utilization of chicks by 16%.

Hossain *et al.* (1998) also observed that both body weight and body weight gain of broilers were significantly increased by including 300 ppb Cr from Cr yeast in the diet. Al-Mashhadani *et al.* (2010) reported that live body weight, weight gain, feed intake and feed efficiency in broilers were improved by dietary chromium supplementation. Ghosh *et al.* (2007) reported a lower feed conversion ratio for quails fed MOS. Bonos *et al.* (2010) observed higher BW and feed consumption for quails consuming MOS.

Carcass traits did not differ significantly among treatments (Table 3). No significant effects were observed for any of the supplemented diets for the relative weights of thymus, spleen, bursa of Fabricius or thyroid gland compared to the control group (Table 4). In accordance with the present results, Uyanik *et al.* (2005) found that carcass yield percentage of Japanese quails was not affected by dietary Cr yeast supplementation. Mannan oligosaccharides did not affect carcass yield of broilers (Yalcinkaya *et al.* 2008; Yalçınkaya *et al.* 2012). Also, dietary MOS had no effect on carcass weight, carcass dressing percentage (Ghosh *et al.* 2008; Sarica *et al.* 2009) and heart percentage (Bonos *et al.* 2010) of growing Japanese quails.

Primary and secondary humoral immune responses of quails fed the supplemented diets were higher (P<0.05) than the control (Table 5).

Mannan oligosaccharides can enhance immune response by promoting the growth of lactic acid bacteria, and they simultaneously produce antibacterial substances and stimulate the production of immunoglobulin, especially IgA (Sarica *et al.* 2009; Silva *et al.* 2009).

Adding MOS to broiler diets enhanced immunity and markedly increased concentrations of IgA antibodies (Kogan and Kocher, 2007; Rehman *et al.* 2009) and resulted in a significant increase in the antibody titer against SRBCs (Riad *et al.* 2010).

Table 2 Productive performance (from 1 week to 6 weeks of age) of growing quails fed diets supplemented with chromium yeast (Cr) and / or mannan oligosaccharides (MOS)

nan ongosacchandes (MOS)							
Item	Control	Cr 1	Cr 2	MOS	Cr 1 + MOS	Cr 2 + MOS	SEM
Initial body weight, g	46.43	45.16	45.48	45.21	45.22	45.42	0.147
Final body weight, g	219.9 ^b	250.4 ^a	235.2 ^{ab}	249.5 ^a	248.9^{a}	236.9 ^{ab}	3.233
Total weight gain, g	173.5 ^b	205.2ª	189.7 ^{ab}	204.3 ^a	203.7 ^a	191.5 ^{ab}	3.342
Total feed intake, g	814.1 ^b	885.3 ^{ab}	823.7 ^b	878.6^{ab}	936.8ª	871.8^{ab}	13.00
Feed conversion ratio	7.560^{a}	5.51 ^b	5.343 ^b	5.510 ^b	5.658 ^b	6.122 ^{ab}	0.269
(g feed/g weight gain)							

(g feed/g weight gain)

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 3 Carcass traits (at the 6 th week of age) of q	uails fed diets supplemented with chromium	n yeast (Cr) and / or mannan oligosaccharic	des (MOS)

Item	Control	Cr 1	Cr 2	MOS	Cr 2 + MOS	Cr 1+ MOS	SEM
Live body weigh, g	209.4	249.0	220.3	251.1	230.7	220.5	29.35
Carcass, g/100 g	67.46	65.89	63.41	66.17	65.21	67.84	4.487
Liver, g/100 g	2.103	2.013	2.178	1.903	1.854	2.013	0.483
Gizzard, g/100 g	1.920	1.333	1.439	1.392	1.439	1.236	0.210
Heart, g/100 g	0.712	0.847	0.912	0.905	0.751	0.757	0.117

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

 Table 4
 Relative weights of lymphoid organs and thyroid glands (at the 4th week of age) of quails fed diets supplemented with chromium yeast (Cr) and / or mannan oligosaccharides (MOS)

Item	Control	Cr 1	Cr 2	MOS	Cr 1 + MOS	Cr 2 + MOS	SEM
Live body weight, g	167.2	185.7	183.4	193.2	189.7	177.5	3.220
Thymus, %	0.365	0.375	0.400	0.360	0.348	0.339	0.014
Spleen, %	0.079	0.060	0.062	0.054	0.060	0.142	0.008
Bursa, %	0.107	0.146	0.148	0.163	0.143	0.100	0.017
Thyroid, %	0.007	0.007	0.008	0.007	0.010	0.009	0.004

The means within the same row with at least one common letter, do not have significant difference (P>0.05). SEM: standard error of the means.

Table 5 Primary and secondary humoral immune responses of quails fed diets supplemented with chromium yeast (Cr) and / or mannan oligosaccharides (MOS)

Item	Control	Cr 1	Cr 2	MOS	Cr 1 + MOS	Cr 2 + MOS	SEM
Primary (at the 4 th week of age)	3.583 ^d	4.083 ^{cd}	4.500 ^{bc}	5.317 ^a	5.117 ^{ab}	5.467 ^a	0.187
Secondary (at the 6 th week of age)	4.750 ^c	5.950 ^b	5.917 ^b	8.117ª	7.483 ^a	7.733ª	0.310

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Chromium supplementation of quail chick diets increased total antibody, IgG and IgM titers and immunoglobulin against SRBCs (El-Hommosan, 2008). Increases (P<0.05) in plasma triiodothyronine (T₃) and total proteins were observed for quails fed the supplemented diets (Table 6). Quails fed Cr 1 + MOS supplemented diets had higher (P<0.05) plasma albumin and globulin compared to the control (Table 6).

Albumin to globulin ratio was not affected by the tested supplements. Plasma total lipids and cholesterol were decreased (P<0.05) by the tested supplements. Dietary Cr supplementation increased serum total protein and albumin and decreased serum cholesterol of Japanese quails (Sahin *et al.* 2002) and increased serum T_3 of broilers (Sahin *et al.* 2003). Mannans prevent cholesterol absorption in the gastrointestinal tract (Tizard *et al.* 1989).

 Table 6
 Plasma constituents (at the 6^{th} week of age) of quails fed diets supplemented with chromium yeast (Cr) and / or mannan oligosaccharides

 (MOS)

Item	Control	Cr 1	Cr 2	MOS	Cr 1 + MOS	Cr 2 + MOS	SEM
Total protein, g/dL	3.813 ^c	4.920 ^{ab}	4.123 ^{bc}	4.903 ^{ab}	5.570 ^a	4.833 ^{ab}	0.167
Albumin, g/dL	1.973 ^c	2.540^{abc}	2.047 ^{bc}	2.667^{ab}	2.917 ^a	2.437 ^{abc}	0.106
Globulin, g/dL	1.840 ^c	2.380 ^{ab}	2.077 ^{bc}	2.237 ^{abc}	2.653 ^a	2.400^{ab}	0.079
A / G ratio	1.077	1.083	0.977	1.203	1.107	1.017	0.039
Total lipids, mg/dL	1029 ^a	866.7 ^b	719.3°	850.0 ^b	699.0 ^c	611.0 ^c	35.24
Cholesterol, mg/dL	198.3ª	142.0 ^b	138.0 ^b	146.3 ^b	150.0 ^b	142.0 ^b	6.087
T ₃ , ng/dL	2.753 ^d	3.890 ^a	3.750 ^{ab}	3.107 ^c	3.497 ^{bc}	3.673 ^{ab}	0.107

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Moreover, mannan oligosaccharides promote lactic acid bacteria activity, which can be effective in reducing the cholesterol level by producing enzymes that cause disintegration of bile salts making them unconjugated, as well as by reducing the pH in the intestinal lumen (Sarica *et al.* 2009; Silva *et al.* 2009). Yalçinkaya *et al.* (2008) found that dietary MOS lowered blood cholesterol of broilers. Feeding broiler chickens a prebiotic increased serum total proteins and globulins (Vytautas *et al.* 2006). Al-Kassi and Witwit (2010) reported that adding a mixture of herbal plants and dandelion as a source of prebiotics to broiler diets had no significant effect on T_3 level.

CONCLUSION

In conclusion, dietary supplementation with Cr yeast (600 mg/kg) and / or mannan oligosaccharides (1.0 g/kg) impro-

ved the productive performance, increased the levels of immunity and reduced total blood cholesterol of growing Japanese quails.

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