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Effect of Dietary Organic Zinc and Prebiotic on Productive Performance and Immune Response of Growing Quails

Research Article

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ABSTRACT

The aim of the present study was to investigate the possibility of improving productive performance and immunity of growing Japanese quail reared during summer in Egypt by supplementing the diets with organic zinc (Bioplex zinc) and prebiotic (mannan oligosaccharides). Diets were a basal diet or the basal diet supplemented with 20 mg Bioplex zinc (3.0 mg Zn) (Zn1), 40 mg Bioplex zinc (6.0 mg Zn) (Zn2), 1.0 g prebiotic (P), P + Zn1 or P + Zn2. Ambient temperature and relative humidity ranged between 34 to 36 ˚C and between 45 to 51%, respectively. No significant difference was observed in body weight, body weight gain, feed intake, feed conversion and mortality of the treated quails when compared to the control group. The effect of treatments on the carcass traits and relative weights of the lymphoid organs and thyroid gland was not significant. The primary and secondary immune responses were the highest in the treated quails. The results showed that plasma total protein of the (P) and (P+Zn2) treated quails increased significantly when compared to the control group. The level of plasma total lipids and cholesterol decreased significantly ($P<0.05$) in quails fed the supplemented diets. No significant change was observed in the plasma AST and ALT enzyme activities and triiodothyronine concentration. It is concluded that, supplementing diets of growing Japanese quails reared during summer in Egypt with 20 or 40 mg Bioplex zinc/kg each alone or in combination with 1.0 g/kg prebiotic (mannan oligosaccharides) had no significant effect on productive performance but improved their immune response.

KEY WORDS growing quail, immune response, organic zinc, performance, prebiotic.

INTRODUCTION

When the ambient temperature is above the thermo neutral zone, which is between 18-22 ˚C for Japanese quail, growth rate, feed intake and feed efficiency decrease, but the excretion of minerals increases (Ensminger *et al.* 1990). Moreover, temperatures > 30 ˚C represent the heat-stress conditions for birds (Ensminger *et al.* 1990). In addition, when elevated ambient temperatures coupled with high humidity, the combination can be more harmful (Defra, 2005). Broilers exposed to high ambient temperature (32 ˚C) showed a decrease in feed intake (Geraert *et al.* 1996). Mashaly *et al.* (2004) reported that high ambient temperatures had deleterious effects on productive performance of laying hens. Heat stress adversely affects production and inhibits immune function of chickens (Puvadolpirod and Thaxton, 2000). Stress causes abnormal accumulation of zinc in the liver, therefore, there is a decrease in plasma zinc concentration; hence, it may exacerbate a marginal zinc deficiency or an increase in zinc requirement (Beisel, 1982). It is reported that zinc deficiency results in reduced appetite, depressed growth rate and abnormalities of the skin and its appendages (McDowell, 2003). Zinc is an integral part of many enzymes involved in carbohydrate and protein metabolism and plays a key role in the immune system (Rahman *et al.* 2002; Ibs and Rink, 2003). Organic zinc sources, such as zinc proteinate or amino acid chelate have been used increasingly in recent years due to their higher bioavailability (Cao *et al.* 2000). Mannan oligosaccharides (MOS) might have a positive effect on immunity due to its influence on immune system, intestinal absorption of some nutrients, such as Zn; or both (Shashidhara and Devegowda, 2003). In addition, MOS are a group of prebiotic that works by providing alternate binding sites for pathogenic bacteria. 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, thus the digestive tract remained healthy, works more efficiently and finally, more nutrients are available for absorption. Pelicano *et al.* (2004) mentioned that adding MOS to broiler diets increased live weight and improved feed conversion. Therefore, this study was conducted to evaluate the effects of organic zinc (Bioplex zinc) and prebiotic (mannan oligosaccharides) on the productive performance, carcass traits, immune response and some plasma metabolites of growing Japanese quail reared during summer in Egypt.

MATERIALS AND METHODS

Five hundred and ninety-day old Japanese quail (*Coturnix coturnix japonica*) chicks were fed *ad libitum* on a basal diet (Table 1) during the first week of age. On day 7, the chicks were weighed and a total number of 450 chicks with similar body weight (45.74±0.37) were selected and randomly allocated to 18 pens with 25 chicks in each pen. The chicks in the 18 pens were randomly subjected to one of the following treatments (3 pens/treatment); a basal diet (control and diet 1; Table 1); the basal diet supplemented with 20 mg Bioplex zinc (provided 3.0 mg zinc)/kg of diet (Zn1), 40 mg Bioplex zinc (provided 6.0 mg zinc)/kg of diet (Zn2), 1.0 g a prebiotic (mannan oligosaccharide)/kg of diet (P), 1.0 g prebiotic plus 20 mg Bioplex zinc/kg of diet (P+Zn1) and 1.0 g prebiotic plus 40 mg Bioplex zinc/kg of diet (P+Zn2). Bioplex® zinc is a chelated zinc proteinate contains 15% Zn, was provided by Alltech Inc., Nicholasville, KY, USA. Bio-Mos® is a mannan oligosaccharide derived from the cell walls of the yeast *Saccharomyces cerevisiae* 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). The quails were fed experimental diets for five weeks (from seven days to 42 days of age). The diets and fresh water was provided *ad libitum*. The birds were exposed to 23 hours photoperiod daily. The natural ambient temperature and relative humidity percent for bird's room was recorded daily. The weekly average ambient temperature during the five weeks of the experimental period (from 6 July to 10 August) was 34 ˚C, 35 ˚C, 34.5 ˚C, 34.3 ˚C and 36 ˚C, respectively, with an overall average of 34.8 ˚C. The weekly average relative humidity during the experimental period was 46, 47, 51, 51 and 45%, respectively with an overall average of 48%. Live body weight and feed intake were recorded weekly. Mortality rate was also recorded during the experimental period. At the 3rd week of age, six quail chicks (three males and three females) of each treatment were injected intramuscularly (IM) with 0.05 mL packed sheep red blood cells (SRBCs) mixed with 0.95 mL physiological saline (0.9% NaCl). Seven days post SRBCs antigen challenge; blood samples were collected, centrifuged (4000 rpm/min) and sera was decanted and stored frozen at -20 ˚C until used for determination of primary immune response. At the 5th week of age, second injection was administered to the same chicks in a similar manner and then the blood samples were collected after seven days for the measurement of secondary immune response. The antibody production (Abs) was measured using 96 wells microtitre plate according to the method described by Van Der Zijpp *et al.* (1983).

Table 1 Composition and calculated analyses of the basal diet

Ingredients	g/kg
Yellow corn	532
Soybean meal (48%)	335
Corn gluten meal (62%)	45
Sunflower oil	9
Wheat bran	45
Di-Ca-P	14.4
Limestone	10
Premix*	3
NaCl (salt)	2.5
L-lysine-HCL	1.9
DL-methionine	1.2
Mold guard	1.0
Calculated analyses g/kg	
Crude protein	241
Ether extract	31.6
Calcium	8.1
Available P	4.2
Lysine	13
Methionine	5
Methionine + cystine	8.9
Zinc mg/kg	84.0
ME kcal/kg	2905

* Vitamin premix per kg of diet: vitamin A: 4000000 IU/kg; vitamin D: 833333 IU/kg; vitamin E: 3.33 g/kg; vitamin K_3: 0.67 g/kg; vitamin B_1: 0.33 g/kg; vitamin B_2: 1.67 g/kg; vitamin B_6: 0.50 g/kg; vitamin B_{12}: 0.003 g/kg; Niacin: 10 g/kg; Folic: 0.33 g/kg; Biotin: 0.017 g/kg; Pantothenic acid: 3.33 g/kg; Cu: 3.33 g/kg; I: 0.33 g/kg; Se: 0.03 g/kg; Fe: 10 g/kg; Mn: 20 g/kg; Zn: 16.67 g/kg and CO: 0.033

At the 4th week of age, six birds (three males and 3 females) from each treatment were randomly selected, weighed and slaughtered. Weights of spleen, bursa, thymus and thyroid gland were recorded and their relative weights to body weights were calculated. At the end of the experiment (6^{th} week of age), six birds (3 males and 3 females) from each group were randomly taken, weighed and slaughtered. Feather was manually removed, and birds were re-weighed and eviscerated. Carcass weight and weights of gizzard, liver, heart, alimentary canal and abdominal fat were recorded. Blood samples in heparinized tubes, were taken from birds during slaughter time (three samples/treatment). These samples were centrifuged (4000 rpm) for 10 minutes and plasma samples were decanted in Eppendorf tubes and stored at -20 ˚C for further analysis. Plasma total protein (Henry, 1964) and albumin (Dumas and Biggs, 1972) were colormetrically determined. Plasma globulin concentration was calculated by subtraction of plasma albumin from plasma total protein and then albumin (A) to globulin (G) ratio was calculated. Plasma total lipids (Knight *et al.* 1972), cholesterol (Richmond, 1973) and activities of alanine transaminase (ALT) and aspartate aminotransferase (AST) (Reitman and Frankel, 1957) were also colorimetrically determined. Plasma triiodothyronine (T_3) was determined by radioimmunoassay Gamma- Coat 125I RIA Kits, Clinical Assay, Cambridge, Medical Diagnostics, Boston, MA, as reported by Akiba *et al.* (1982).

The data was subjected to the analysis of variance by one-way ANOVA using the general linear models (GLM) procedure of the satistical analysis system (SAS, 1994), according to the following model:

$$Y_{ij} = \mu + T_i + £_{ij}$$

Where:
Y_{ij}: observation.
μ: population mean.
T_i: diet effect (i=1 to 6).
$£_{ij}$: random error.

Duncan's multiple range test (Duncan, 1955) was performed when the differences were significant. Mean values were considered significantly different at P<0.05. The data were expressed as mean values ± standard error.

RESULTS AND DISCUSSION

Productive performance and carcass traits

Initial body weight of quail chicks was similar in all treatments (Table 2). No significant difference was observed in body weight (BW), weight gain (WG), feed intake, feed conversion (FC) and mortality of the treated quails when compared to the control group (Table 2). Natural ambient temperature (34 to 36 ˚C) and relative humidity (45 to 51%) were high in the present study. Temperatures > 30 ˚C represent heat-stress conditions for birds (Ensminger *et al.* 1990). Moreover, when high ambient temperatures coupled with high humidity, the combination can be more harmful (Defra, 2005). Growth rate, feed intake and feed efficiency decrease when the ambient temperature is above the thermo neutral (TN) zone (18-22 ˚C, for Japanese quail) (Ensminger *et al.* 1990). In accordance with the present results, Yalçınkaya *et al.* (2012) showed that the supplementation of organic zinc and / or MOS in broiler diets had no effect on the BW, FC and carcass yield. Mohanna and Nys (1999) reported that WG and FC in broilers were not influenced by Zn-Met dietary supplementation. Rossi *et al.* (2007) found that body weight, body weight gain, feed intake, feed conversion and mortality were not influenced by addition of organic Zn to broiler diets. Similar results were also obtained in broiler fed MOS-supplemented diet (Bozkurt *et al.* 2008). Additionally, Yang *et al.* (2007) reported that MOS supplementation had no effect on FC of broiler chickens. Supplementation of broiler diet with MOS did not improve BW and FC (Waldroup *et al.* 2003). Khalaji *et al.* (2011) showed that MOS had no significant effect on growth performance and FC of broiler chicks. There was no significant difference in carcass traits of quail chicks among treatments (Table 3). Yalçinkaya *et al.* (2012) found that carcass yield of broiler was not influenced by dietary addition of organic Zn and / or MOS. Hudson *et al.* (2004) and Rossi *et al.* (2007) also reported that carcass yield of broilers was not influenced by dietary supplementation with organic zinc. Moreover, Waldroup *et al.* (2003) and Yalcinkaya *et al.* (2008) found no significant improvement in carcass yield of broilers fed Bio-Mos-supplemented diet. Mannan oligosaccharides did not affect carcass weight and carcass dressing percentage of quails (Sarica *et al.* 2009; Bonos *et al.* 2010) and heart to live weight percentage of quails (Bonos *et al.* 2010).

Humoral immune response

Higher (P<0.05) primary immune response (antibodies (Abs) production after 7 days post the 1^{st} challenge with sheep red blood cells (SRBCs)) was observed with all the tested supplements (Table 4). Moreover, the identical trend was observed after seven days post the 2^{nd} challenge with the same antigen (secondary immune response; at 6 week of age), but with greater (P<0.05) response reaching almost double as much as those of the control. Mashaly *et al.* (2004) demonstrated that antibody production significantly inhibited in heat stressed hens. The present results are in consistent with those reported by Burns (1983) who found that diets supplemented with zinc tend to improve the

Table 2 Productive performance of growing quails fed diets supplemented with organic zinc (Zn) and / or prebiotic (P)

Traits	Control	Zn1	Zn2	P	P + Zn1	P + Zn2	SEM*	P-value
Initial body weight (g)	46.43	45.31	46.48	45.21	45.35	45.63	0.162	0.250
Final body weight (g)	219.94	236.44	233.58	249.47	232.79	240.60	3.017	0.088
Weight gain (g)	173.51	191.13	187.10	204.26	187.44	194.97	3.132	0.085
Feed intake (g)	814.09	841.23	867.88	878.58	864.07	887.07	8.393	0.097
Feed conversion	7.56	5.59	5.79	5.51	5.88	5.66	0.262	0.185
Mortality rate	1.96	0.00	1.11	0.00	1.23	0.00	1.348	0.675

*SEM: standard error of means.

Table 3 Carcass traits of growing quails fed diets supplemented with organic zinc (Zn) and / or prebiotic (P)

Traits	Control	Zn1	Zn2	P	P + Zn1	P + Zn2	SEM*	P-value
Body weight (g)	209.4	254.0	233.0	251.1	240.2	241.02	29.35	0.444
Carcass (g/kg)	674.6	639.9	658.3	661.7	636.9	675.5	46.1	0.874
Liver (g/kg)	21.03	22.97	27.25	19.03	21.38	20.49	06.6	0.817
Gizzard (g/kg)	19.20	13.00	13.41	13.92	14.73	12.39	02.4	0.175
Heart (g/kg)	7.12	8.40	7.32	9.05	6.74	7.84	1.3	0.223
Alimentary canal (g/kg)	37.16	39.12	43.11	44.97	38.13	35.23	11.1	0.917

*SEM: standard error of means.

Table 4 Primary and secondary immune response of growing quails fed diets supplemented with organic zinc (Zn) and / or prebiotic (P)

Traits	Control	Zn1	Zn2	P	P + Zn1	P + Zn2	SEM*	P-value
Primary	3.58[c]	5.517[ab]	5.650[ab]	5.32[b]	5.933[ab]	6.100[a]	0.214	<0.001
Secondary	4.75[b]	8.200[a]	8.383[a]	8.12[a]	8.417[a]	8.717[a]	0.338	<0.001

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

ability of the birds to produce antibodies. Kidd *et al.* (1994) found that dietary addition of Zn-methionine in the chick diet significantly increased antibody titer when chicks were challenged with sheep red blood cell (SRBC). Abou El-Wafa *et al.* (2003) found that antibody titer against SRBC was significantly increased in broiler chicks fed diets supplemented with Zn-methionine. In agreement with the present results, mannan oligosaccharides can enhance immune response by promoting the growth of lactic acid bacteria.

They produce immune stimulating substances that react with the immune system at different levels, including the production of cytokines, mononuclear cells and macrophage phagocytosis as well as the induction of synthesis of large amounts of immunoglobulin, especially IgA (Sarica *et al.* 2009; Silva *et al.* 2009). Ferket (2004) observed that mannan oligosaccharides markedly increased concentrations of IgA and IgG. Shashidhara and Devegowda (2003) found that antibody responses against the infectious bursal disease virus were higher in the broiler breeder fed diets supplemented with mannan oligosaccharides. Adding prebiotic to broiler diets resulted in a significant increment in the antibody titer against SRBCs (Riad *et al.* 2010).

No significant differences among treatments were observed in the relative weights of the lymphoid organs and thyroid gland (Table 5). Stress responses include an involution of immunoglobulin producing organs (spleen, thymus and bursa of fabricius). In accordance with the present results, El-Kaiaty *et al.* (2001) found that Zn-methionine had no effect on bursa, thymus and spleen relative weights at six weeks of age for Fayoumy breed. Osman and Ragab (2007) found no significant differences among diets supplemented with Zn-methionine in spleen of broiler chick.

Plasma constituents

Significant increase in plasma total protein and globulin concentration was observed with P and P + Zn2 diets (Table 6). The low level of Zn resulted in a decrease in plasma albumin level. Albumin to globulin ratio decreased (P=0.001) with Zn1, P + Zn1 and P + Zn2 supplemented diets (Table 6). Similarly, plasma total protein was increased with Zn-Gly dietary supplementation in broiler Feng *et al.* (2010) and Zn proteinat in laying hens Bulbul and Kuçukersan (2004). Moreover, Vytautas *et al.* (2006) reported that feeding broiler chickens on a prebiotic increased serum total protein and globulin.

Plasma total lipids and cholesterol were decreased (P=0.020 and 0.001, respectively) with the tested supplements (Table 6). Uyanik *et al.* (2001) indicated that zinc supplementation decreased serum cholesterol concentration of broilers. Mannans prevent cholesterol from absorption in gastro intestinal tract (Tizard *et al.* 1989) and can promote the growth and activity of lactic acid bacteria (Gibson and Roberfroid, 1995), which reduces the cholesterol level by producing enzymes disintegrating bile salts and making them unconjugated, as well as by reducing the pH in the intestinal lumen (Klaver and Van der Meer, 1993). Serum total cholesterol concentration was significantly lower in broilers fed a mannan oligosaccharides diet when compared to the control broilers (Yalçinkaya *et al.* 2008). Furthermore, Taherpour *et al.* (2009) reported that adding

Table 5 Relative weights of lymphoid organs and thyroid gland of growing quails fed diets supplemented with organic zinc (Zn) and / or prebiotic (P)

Traits	Control	Zn1	Zn2	P	P + Zn1	P + Zn2	SEM*	P-value
Body weight (g)	167.2	191.1	176.0	193.2	185.3	179.1	3.221	0.162
Thymus (g/kg)	3.65	3.30	3.01	3.60	3.58	3.99	0.05	0.269
Spleen (g/kg)	0.79	0.41	0.62	0.54	0.62	0.74	0.20	0.847
Bursa (g/kg)	1.07	1.34	1.40	1.63	1.30	1.40	0.08	0.501
Thyroid (g/kg)	0.07	0.09	0.07	0.07	0.08	0.08	0.061	0. 847

* SEM: standard error of means.

Table 6 Plasma constituents of growing quails fed diets supplemented with organic zinc (Zn) and / or prebiotic (P)

Traits	Control	Zn1	Zn2	P	P + Zn1	P + Zn2	SEM*	P-value
Total protein (g/dL)	3.81^{b}	3.78^{b}	3.71^{b}	4.90^{a}	3.77^{b}	5.11^{a}	0.164	0.002
Albumin (g/dL)	1.97^{b}	1.34^{c}	1.55^{bc}	2.67^{a}	1.52^{bc}	1.99^{b}	0.125	0.003
Globulin (g/dL)	1.84^{b}	2.45^{c}	2.16^{bc}	2.24^{bc}	2.25^{bc}	3.12^{a}	0.1045	0.001
A/G ratio	1.08^{a}	0.55^{b}	0.71^{b}	1.20^{a}	0.68^{b}	0.65^{b}	0.066	0.001
Total lipids (mg/dL)	1029^{a}	857^{bc}	949^{ab}	850^{bc}	793^{c}	859^{bc}	23.54	0.020
Cholesterol (mg/dL)	198^{a}	174^{b}	162^{bc}	146^{c}	160^{bc}	151^{c}	4.744	0.001
AST (IU/L)	40.30^{ab}	45.07^{ab}	49.30^{a}	28.67^{c}	40.87^{ab}	39.03^{b}	1.852	0.008
ALT (IU/L)	8.23	8.40	9.17	8.37	7.87	7.97	0.287	0.874
T3 (ng/dL)	2.753	3.037	2.650	3.107	2.970	2.677	0.065	0.160

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

prebiotics to broiler chicken's diet decreased total cholesterol. There were no effects of the test supplements on plasma ALT and AST enzyme activity and triiodothyronine (T_3) level, except a significant (P=0.008) increase in plasma AST enzyme activity with P supplemented diet. Yalçinkaya *et al.* (2012) found that supplementing broiler diets with organic zinc and / or MOS had no effect on serum ALT. Osman and Ragab (2007) reported non significant differences in plasma AST of broiler chicks fed diets supplemented with Zn-methionine compared with the control. Kaya *et al.* (2001) reported that zinc supplementation to diets had no effect on thyroid hormone levels of laying hens. Spears *et al.* (2003) also observed that plasma T_3 level was not affected by organic zinc supplementation in the diets of broiler chicks. Al-Kassi and Witwit (2010) found 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

It is concluded that, supplementing diets of growing Japanese quails reared during summer in Egypt with 20 or 40 mg Bioplex zinc/kg each alone or in combination with 1.0 g/kg prebiotic (mannan oligosaccharides) had no significant effect on productive performance but improved their immune response.

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