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Effect of Organic Copper, Zinc and Manganese Supplementation on Immunity and Hatchability of Egg in Japanese Quail (*Coturnix coturnix japonica*)

A.K. Satapathy, S.K. Das, K. Sethy*, R.K Swain, S.K. Mishra K. Behera and S. Pati

Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry,
Odisha University of Agriculture and Technology, Bhubaneshwar-751 003, India

ABSTRACT

To assess the effect of organic copper (Cu), zinc (Zn) and manganese (Mn) supplementation on immunity and hatchability, 405 six week old Japanese quails (*Coturnix coturnix japonica*) were randomly distributed into nine dietary treatment groups and the experiment continued till the period up to 13th week. The dietary treatments were T_0:100 % inorganic minerals (Cu, Zn and Mn); T_1: 100 % organic minerals (Cu, Zn and Mn); T_2: 100 % inorganic Zn and Cu with 100 % organic Mn; T_3: 100 % inorganic Zn and Cu with 50 % organic Mn and 50% inorganic Mn; T_4: 100 % inorganic Zn and Mn with 100 % organic Cu; T_5: 100 % inorganic Zn and Mn with 50 % organic Cu and 50% inorganic Cu; T_6: 100 % inorganic Mn and Cu with 100 % organic Zn; T_7: 100 % inorganic Mn and Cu with 50 % organic Zn and 50% inorganic Zn; T_8: 50 % organic Zn, Cu and Mn with 50 % inorganic Zn, Cu and Mn. Basal diets were supplemented with Cu, Zn and Mn at the levels of 5, 25 and 60 ppm, respectively. Results revealed that supplementation of organic Cu, Zn and Mn had no effect on growth rate, blood chemistry and weight of lymphoid organs, but the hatchability of eggs and cutaneous basophil hypersensitivity response were significantly ($P<0.05$) higher in 100% organic supplemented groups as compared to other groups. So, it may be concluded that supplementation of 100% organic copper, zinc and manganese improved the immunity and hatchability of eggs in Japanese quail.

Key words: Growth, Hatchability, Immunity, Organic minerals

INTRODUCTION

Trace minerals, such as Cu, Zn and Mn are constituents of proteins involved in intermediary metabolism, hormone secretion pathways and immune defense systems (Dozier *et al.,* 2003). Traditionally, these trace minerals are supplemented in the inorganic form, such as sulfates, oxides and carbonates. However, an excess of supplemental inorganic minerals leads to waste and environmental contamination from excessive excretion (Leeson, 2003). Use of organically complexed trace minerals can help in preventing these losses, due to increased stability in the upper gastrointestinal tract of the birds (Aksu *et al.,* 2010). Organic complexed mineral is a type of mineral linked to protein/peptide/amino acids that has a higher bioavailability than those inorganic salts (Swiatkiewicz *et al.,* 2014). Therefore, organic complexed minerals are supposed to be more effective than the inorganic minerals in broilers (Abdallah *et al.,* 2009). Supplementation of organic minerals enhanced the enzyme secretion, immune response, tissue and bone development and integrity, eggshell formation, and protection against oxidative stress (Richards *et al.,* 2010). Organic Cu is related to iron metabolism and absorption, oxygen metabolism, collagen and elastin synthesis, bone formation, feathers development and colouring (Scheideler, 1991). Organic Zn and Mn improved utilization of feed via participating in the metabolism of carbohydrates, fats and proteins (Aksu *et al.,* 2010). Considering the importance of organic minerals in birds, the present study was envisaged to evaluate the combined effect organic Cu, Zn and Mn at different combinations on growth, blood chemistry, immunity and hatchability of egg in Japanese quail.

MATERIALS AND METHODS

Four hundred and five Japanese quail (*Coturnix coturnix japonica*) of 6 weeks of age were divided into nine treatment groups having three replicates of 15

*Corresponding author; Email: babuivri@gmail.com

birds each. The dietary treatments were T_0: 100 % inorganic minerals (Cu, Zn and Mn); T_1: 100 % organic minerals (Cu, Zn and Mn); T_2: 100 % inorganic Zn and Cu with 100 % organic Mn; T_3: 100 % inorganic Zn and Cu with 50 % organic Mn and 50% inorganic Mn; T_4: 100 % inorganic Zn and Mn with 100 % organic Cu; T_5 : 100 % inorganic Zn and Mn with 50 % organic Cu and 50% inorganic Cu; $T_{6:}$ 100 % inorganic Mn and Cu with 100 % organic Zn; T_7: 100 % inorganic Mn and Cu with 50 % organic Zn and 50% inorganic Zn; T_8: 50 % organic Zn, Cu and Mn with 50 % inorganic Zn, Cu and Mn. Cu, Zn and Mn were supplemented at the levels of 5, 25, and 60 ppm, respectively over the basal diet (BIS, 1992). Inorganic sources of Cu, Zn and Mn were in the form of sulphates and the organic forms were in the form of methionate complexes of respective minerals.

Before the onset of the experiment, the quail house was thoroughly cleaned, disinfected and dried. The quails were wing-banded, weighed group wise and transferred to experimental shed in the farm itself with standard management and health care practices. A basal diet was prepared to meet the nutrient requirement of birds (BIS, 1992). The experimental feed was analysed as per AOAC (1995). Calcium was measured according to the method of Talapatra *et al.* (1940). The ingredients and chemical composition of the experimental diets are presented in Table 1. The organic Cu, Zn and Mn were obtained from Surging Ahead (SA) Pharmaceuticals Pvt. Limited, Vadodara, Gujurat, India. The analytical report revealed that Cu, Zn and Mn content were (%) 18.20, 12.67 and 14.85 respectively in the organic minerals.

The quails were weighed treatment wise by top pan electronic balance in each week up to the end of 13[th] week. Blood samples were collected from the birds at 13[th] week of age and blood glucose, triglycerides, cholesterol, total protein, AST (asparate transaminase) and ALT (alanine transaminase) were estimated by using kit (Crest Biosystems, Mumbai, India). At the end of experiment, three birds from each replicate were slaughtered for collection of spleen and thymus.

At 13[th] week of age, three birds in each replicate were injected intra-dermally 100 micro gram of Phyto-haemaglutinin-P (PHA-p) in 0.1 ml of normal saline per bird in the foot pad to measure the cellular immune response by cutaneous basophillc hyper sensitivity (CBH) test (Edelman *et al.,* 1986). The

Table 1. Ingredients and chemical composition of basal diet

Dietary composition		Chemical composition	
Ingredients	**Parts per quintal**	**Parameters**	**Percentage on dry matter basis**
Yellow maize	50	Dry matter	90.20
Soya bean meal	35	On DM basis	
Deoiled rice bran	3	Crude protein	22.60
Vegetable oil	4	Ether extract	5.52
Dicalcium phosphate	2	Crude fibre	4.90
Oyster shell	5	Total ash	8.10
Mineral mixture[#]	1	Acid insoluble ash	2.90
		Nitrogen free extract*	58.88
		Calcium	2.50
		Metabolizable energy* (kcal/kg)	2918
		Copper (ppm)	3.83
		Zinc (ppm)	12.63
		Manganese (ppm)	5.68

[#]Mineral mixture (without zinc, copper and manganese) contained (g/100g): calcium carbonate 33.28, magnesium oxide 1.48, ferrous sulphate 1.40, potassium iodide0.001, potassium chloride 17.09 and sodium selinate 0.001. [*]Calculated value thickness of foot pad was measured using digital calliper before (0 h) and 24 h post inoculation and CBH response was calculated using the formula:

$$\text{CBH response} = \frac{\text{Post injection skin thickness}}{\text{Pre-injection thickness}} \times 100$$

At 13th weeks of the experiment, total of 1239 eggs were collected from different treatments. The eggs were candled on 15th day of incubation to remove the infertile eggs and dead in germ eggs, then the live embryos were transferred to the Hatcher. The chicks were taken out on 18th day. Hatchability was calculated on the basis total eggs set (TES).

Hatchability % (on TES) =

$$\frac{\text{Number of chicks hatched out}}{\text{Total number of eggs set}} \times 100$$

Data obtained were subjected to one-way analysis of variance using Software Package for Social Sciences (SPSS) version 17.0 (2008) and comparison among treatment means was made by Duncan's multiple range test (Duncan, 1955) with significance level of P<0.05.

RESULTS AND DISCUSSION

The weekly body weight (g) gain of quail birds of different dietary treatments did not differed significantly (P>0.05) among the groups (Table 2). The non-significant effect on body weight gain corroborated with the findings of Nollet *et al.* (2007). Various workers had reported non-significant effect of mineral supplementation on body weight of broilers either in inorganic or organic form (Rossi *et al.,* 2007; Zhao *et al.,* 2010). In contrast to this, Idowu *et al.* (2011) and Dozier *et al.* (2003) reported that the body weight gain was significantly higher in birds supplemented with organic minerals than control. The increased body weight in above experiments might be due to difference in age group of birds.

Blood biochemical parameters like glucose, triglycerides, cholesterol, total protein, AST and ALT did not differed significantly (P>0.05) among the treatment groups (Table.3). Similarly, non-significant change in serum glucose levels was reported by Parak and Strakova (2011) in breeding cocks supplemented with organic zinc. Similar to the present result, non-

Table 2. Body weight gain (g) of birds of different dietary treatments

Week	Treatments									P Value
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
6th	5.85±0.03	5.88±0.04	5.84±0.03	5.91±0.03	5.87±0.03	5.85±0.03	5.97±0.02	5.90±0.04	5.90±0.03	0.20
7th	6.46±0.03	6.43±0.04	6.50±0.07	6.49±6.49±	6.42±0.10	6.47±0.04	6.51±0.02	6.50±0.02	6.48±0.02	0.96
8th	6.91±0.03	6.98±0.06	6.93±0.04	6.97±0.05	7.02±0.05	7.01±0.06	6.96±0.04	6.99±0.02	6.98±0.03	0.74
9th	6.98±0.04	6.99±0.01	6.92±0.03	6.97±0.02	6.99±0.02	6.95±0.04	6.92±0.03	6.94±0.06	6.97±0.04	0.79
10th	6.88±0.02	6.89±0.02	6.87±0.05	6.85±0.07	6.83±0.05	6.88±0.07	6.89±0.08	6.88±0.02	6.88±0.01	0.99
11th	6.97±0.02	7.01±0.07	7.02±0.06	6.98±0.02	6.99±0.03	6.99±0.03	6.89±0.02	7.01±0.03	6.98±0.03	0.46
12th	6.98±0.03	7.04±0.03	7.01±0.09	7.04±0.02	6.96±0.03	6.99±0.03	7.01±0.03	6.99±0.02	6.93±0.02	0.55
13th	7.09±0.05	7.12±0.02	7.05±0.07	7.11±0.02	7.03±0.03	7.02±0.01	7.07±0.02	7.09±0.02	7.03±0.02	0.27

Table 3. Blood biochemical constituents of experimental quails on different dietary treatments

Attributes	Treatments									P Value
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Glucose (mg/dl)	312.59± 7.92	315.62± 8.56	320.50± 14.23	318.51± 9.33	323.17± 16.52	312.58± 13.52	310.16± 9.17	322.12± 8.72	305.13± 8.76	0.14
Cholesterol (mg/dl)	156.44± 1.98	152.67± 1.98	149.02± 2.47	154.95± 2.47	154.95± 4.45	154.03± 6.44	151.06± 3.46	153.47± 0.99	152.03± 9.41	0.18
Triglyceride (mg/dl)	120.27± 12.09	120.08± 2.55	120.17± 10.72	120.54± 4.44	120.08± 9.09	119.9± 0.40	119.45± 25.33	119.4± 6.36	119.4± 21.69	0.31
Protein (g/dl)	3.96± 0.03	4.06± 0.44	3.85± 0.24	3.68± 0.06	3.83± 0.13	4.04± 0.15	3.70± 0.18	3.91± 0.09	4.01± 0.04	0.10
AST(U/L)	59.18± 2.38	58.39± 2.22	57.59± 0.15	60.13± 0.15	61.75± 0.79	61.72± 1.74	60.14± 0.63	60.93± 3.81	60.45± 1.74	0.09
ALT(U/L)	50.64± 3.23	50.83± 0.85	50.58± 1.74	50.62± 0.45	50.66± 0.98	50.69± 4.33	50.68± 2.32	50.88± 3.66	50.55± 0.56	0.88

significant level of serum ALT in organic zinc fed groups was reported by Aksu *et al.* (2010) on feeding different levels of organic Zn, Cu and Mn in broiler ration. Idowu *et al.* (2011) reported that Zn sources had no significant (P>0.05) effect on serum glucose, protein and ALT concentrations which corroborated with the present findings.

Lymphoid organ parameters *viz.* thymus and spleen weight of quails as percentage of body weight at 13th week did not differ significantly (P>0.05) among various treatment groups (Table 4). The results were corroborated with the findings of Moghaddam and Jahanian (2009) and Feng *et al.* (2010). Supplementation of zinc did not improve the weight of the lymphoid organs as more nutrients being repatriated to develop body weight and production whereas immune system needed a small amount of nutrient in relation to that needed for growth and production (Badawy *et al.,*1987). However, Idowu *et al.* (2011) observed significantly higher percentage of spleen in zinc-proteanate group than the inorganic group.

Data pertaining to CBH response of quails at 13th week of age is presented in Table 5. Higher CBH response was observed in quails fed 100% organic mineral supplement than other treatments. Trace elements, especially Cu, Zn and selenium (Se) markedly influence humoral and cellular immunity (Allgower *et al.,* 1995). The quails provided diets supplemented with organic minerals might have increased thymulin activity, therefore enhancing immune response through increased maturation of T- lymphocyte (Hudson *et al.,* 2004). The better immune response might be due to the fact that antagonism between the minerals could have been avoided through using chelated forms of the mineral (Abdallah *et al.,* 2009).

Table 4. Weight of lymphoid organs (% BW) of experimental quails

Organs	Treatments									P Value
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
Spleen	0.25± 0.05	0.24± 0.03	0.24± 0.02	0.24± 0.02	0.24± 0.01	0.24± 0.05	0.26± 0.001	0.24± 0.03	0.24± 0.04	0.92
Thymus	0.14± 0.01	0.13± 0.02	0.13± 0.00	0.14± 0.01	0.13± 0.00	0.13± 0.00	0.13± 0.01	0.13± 0.00	0.14± 0.01	0.81

Table 5. Effect on immune response and hatchability of eggs in quails

Attributes	Treatments									P Value
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	
CMI	$273^{a}\pm$ 2.25	$445^{e}\pm$ 13.17	$309^{ab}\pm$ 4.19	$308^{ab}\pm$ 4.36	$328^{bc}\pm$ 3.80	$327^{bc}\pm$ 8.72	$382^{d}\pm$ 7.55	$324^{b}\pm$ 12.30	$370^{cd}\pm$ 20.16	<0.01
Hatchability (%)	$68.80^{a}\pm$ 1.36	87.2^{d} 1± 1.10	$80.00^{bc}\pm$ 1.09	$78.47^{bc}\pm$ 2.21	$80.30^{bc}\pm$ 0.75	$77.20^{b}\pm$ 0.42	$80.13^{bc}\pm$ 1.00	$80.76^{bc}\pm$ 2.31	$83.33^{cd}\pm$ 1.00	<0.01

[a,b,c,d]Means with different superscript in a row differed significantly (P<0.05)

Data on hatchability of eggs are presented on Table 5. The hatchability percent of T_1 group was significantly (P<0.05) higher as compared other treatment groups. The lower hatchability percent in all other treatments might be due to inadequate transmission of minerals from the hen to the hatching egg (Kienholz *et al.,* 1961). In contradiction to the present findings, Kidd *et al.* (1996) reported that feeding of zinc from inorganic and organic sources did not have any significant effect on the hatchability. This might be due to higher zinc concentration in the basal diet of the birds in the previous study.

CONCLUSION

It can be concluded that dietary supplementation of 100 percent organic copper, magnesia and zinc improved cell mediated immunity and egg hatchability of quails.

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