

Bioavailability and Immunity Response in Broiler Breeders on Organically Complexed Zinc Supplementation

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ABSTRACT

Two hundred and sixty four broiler breeder hens of 32 weeks of age were distributed randomly in four dietary treatments. The dietary treatments were T₀: Broiler breeder ration containing 40 ppm zinc (basal 29.8 ppm + 10.2 ppm inorganic zinc), T₁: T₀ + organic zinc (zinc methionine) @ 20 ppm, T₂: T₀ + organic zinc @ 40 ppm and T₃: T₀ + organic zinc @ 60 ppm. The experiment was continued from 32 to 48 weeks of age. At 48 weeks, the weight of lymphoid organs, zinc levels in organs and immunity response were determined. The faecal zinc level was determined at monthly interval. The weight lymphoid organs of different treatment groups (both organic and inorganic zinc fed groups) of the broiler breeders did not differ significantly ($P > 0.05$). The cellular immune response of breeder birds to PHA-P was significantly ($P < 0.05$) higher in group T₃ than the rest of treated groups. The antibody titre to SRBC differed among the treated groups. The zinc content of serum of broiler breeders of all the groups did not differ significantly ($P > 0.05$) in all the periods of study. Zinc content in liver and tibia of broiler breeders in different dietary treatments of zinc differed significantly ($P < 0.05$) with higher levels were obtained on increasing zinc concentration in the diet. The zinc level in the spleen and kidney of the broiler breeders in different dietary treatments did not differ significantly ($P > 0.05$). The average zinc content in the faeces of broiler breeder during 35 to 43 week of age did not differ significantly ($P > 0.05$) among the treated groups. At 48 weeks of age, zinc content of the faeces of T₃ was found to be significantly ($P < 0.05$) higher than the rest of treated groups. Similarly, during the overall experimental period analysis, it was found that zinc levels in the faeces of T₂ and T₃ were significantly ($P < 0.05$) higher than T₁ and T₀.

Keywords: Organic Zinc; Immunity; Bioavailability; Broiler Breeders

1. Introduction

Zinc impacts immunity in poultry [1]. Zinc deficiency has been shown to decrease cellular immunity [2], thymus [3] and spleen development [4]. Zinc is important for proper disease resistance and its deficiency has resulted in bacteremia [5], parasitic infections [6] and alteration in high-density lipoprotein cholesterol [7]. Zn-methionine provides a source of zinc with greater biological availability than zinc from inorganic sources. Gill (1997) [8] reported that chelated (organic) minerals are more biologically available in animal digestive system than inorganic minerals and that perhaps resulted in less

mineral excretion and pollution of the environment. Today, large scale commercial livestock production system has given rise to many environmental concerns, since excess mineral concentrations in the manure can lead to mineral depositions that exceed crop nutrient requirement [9]. According to [10] and the opinion of Scientific Committee for Animal Nutrition on use of zinc in feeding stuffs, a clear indication of biological activity of zinc is: the content of this element in liver, methionine activity, accumulation of zinc in the bones and levels of zinc in blood serum are the method of estimation of availability of this element in live animals. Considering the higher bioavailability of organic zinc [11] the experiment was planned to study the effect of organic zinc

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supplementation in broiler breeder birds on bioavailability and immune response.

2. Materials and Methods

2.1. Selection and Management of Experimental Birds

Female broiler breeder birds of synthetic dam line, maintained under All India Coordinated Research Network Project on “Poultry Breeding” were selected for the study. A total of two hundred and sixty four broiler breeder hens of 32 weeks of age were distributed randomly in four dietary treatments with three replicate per treatment. The hens were selected on the basis of their body weight and egg production. The hens were maintained in individual layer cages. Experimental diets were offered @ 150 g/bird/day throughout the experimental period. The hens were provided 24 hours free access to clean drinking water. The experiment continued for a period of sixteen weeks *i.e.* 32 to 48 weeks of age of the hens. A basal diet was prepared to meet the nutrient requirement of broiler breeders [12]. The ingredient composition and proximate composition of the basal diet is given in **Table 1**. The basal diet was analyzed for proximate composition as per [13].

2.2. Dietary Treatments

The dietary treatments of the experiment were: T₀:

Broiler breeder ration containing 40 ppm zinc (basal 29.8 ppm + 10.2 ppm inorganic zinc supplementation in form of ZnSO₄), T₁: T₀ + Supplementation of organic zinc (zinc methionine) @ 20 ppm in the broiler breeder ration, T₂: T₀ + Supplementation of organic zinc @ 40 ppm in the broiler breeder ration and T₃: T₀ + Supplementation of organic zinc @ 60 ppm in the broiler breeder ration.

2.3. Collection and Processing of Experimental Samples

At the end of the experimental period (48 weeks of age), three birds were randomly chosen from each replicate and slaughtered for collection of liver, spleen and kidney. The birds were kept off feed overnight before bleeding and only water was provided. The live weight of the birds were recorded as pre slaughter weight. The broiler birds were bled by modified Kosher's method [14]. Liver, spleen, bursa of fabricus and kidney were weighed in a top pan electric balance. Both the tibia bones were removed from the slaughter birds. The tibia bones were pressure cooked in deionised water for 15 minutes, cleaned off all tissues and dried in an oven for 72 hours until constant weight. Then the tibia bones were extracted in petroleum ether for 72 hours to remove fat and dried for 24 hours in 105°C. The collected liver, kidney and spleen samples were oven dried at 100°C for 24 hours and finely grounded. The zinc content in the liver, kidney, bursa and spleen samples were determined by

Table 1. Ingredient and proximate composition of breeder basal diet.

Ingredients	Percentage	Proximate composition	Percentage on DM basis
Maize	60.00	Moisture	9.17
Soyabean meal	19.50	Crude protein	16.08
De oiled rice bran	12.00	Ether extract	4.20
Mineral mixture [#]	3.00	Crude fibre	4.82
Oyster shell meal	5.00	Total ash	10.61
Common salt	0.30	Acid insoluble ash	2.54
L-lysine	0.03	Nitrogen free extract [*]	64.39
DL-methionine	0.05	Calcium	2.97
Feed additives used (g/kg of diet)		Available phosphorus	0.42
Biocholine	0.50	Metabolisable energy [*] (Kcal/kg)	2751.25
Biobantox	0.50	Lysine [*]	0.84
Layvit	0.50	Methionine [*]	0.34
Livoline	0.25	Zinc	29.80 ppm
E-sel-powder	0.10		
K-zyme	0.50		

[#]Mineral mixture—without zinc, ^{*}Calculated value.

digesting of 0.5 g samples at 120°C using 5ml concentrate HNO₃ for 1 hour using KEL plus digestion system. The digested samples were cooled and further digested with 30% H₂O₂ at 200°C. The process continued until the content appeared clear and colourless. At 35, 39, 43 and 48 weeks of experiment, 5 ml of blood was drawn from the brachial vein of 12 birds per treatment (4 per replicate). The serum was obtained by centrifugation of coagulated blood at 700 × g for 5 min. The serum were frozen at -20°C until needed for analysis.

2.4. Measure of Immunity

2.4.1. Cellular Immunity

At 48 weeks of age, three birds from each replicate in each dietary treatment were injected intra-dermally in the right wattle with 100 micro gram of Phytohaemagglutinin-P (PHP-P) in 0.1 ml of normal saline to measure the cellular immune response by Cutaneous Basophilic Hyper Sensitivity (CBH) test [15]. The thickness of wattle was measured using digital calliper before inoculation and 24 h post inoculation and CBH response was calculated using the formula:

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

2.4.2. Humoral Immunity

The measure of humoral immunity was carried out as per the method described by [16]. Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyse specific antibody response as measure of humoral immunity. At 48 weeks of age, three birds from each replicate in each dietary treatment were immunized intravenously via a wing vein with 0.07 ml packed RBC mixed with 0.93 ml physiological saline (0.9% NaCl) for measure primary response. Seven days following the antigen challenge, blood samples were collected and serum samples were used to measure humoral immunity. Antibody production to SRBC was measured using micro titration hemmagglutination technique with micro titer plate U shape of 96 wells (8 rows × 12 column) according [17,18]. All SRBC antibody titers were expressed as log₂ of the reciprocal of the highest serum dilution causing agglutination of SRBC.

2.5. Collection of Faeces

The faeces of the experimental broiler breeder birds were

collected at 35, 39, 43 and 48 weeks of age. Three birds from each replicate of each group were taken for individual collection of faeces. A polythene sheet was attached under the cages of the birds and light was turn off for 1 hour. The faeces were homogeneously mixed replicate wise and representative samples of the faeces were collected in moisture cup and were oven dried at 105°C for 24 h. For determination of zinc content in faeces, 2 gm faecal samples were taken in a digestion tube and to it 12 ml of tri acid mixture (7 ml HNO₃, 3 ml H₂SO₄ and 2 ml Perchloric acid) were added and digested at 200°C.

2.6. Statistical Analysis

Data retrieved from the experiment was subjected to statistical analysis wherever required. The statistical analysis of the data was done according to [19].

3. Results and Discussion

3.1. Immunity

The effect of various levels of organic zinc on immune response of broiler breeders is presented in **Table 2**. The cellular immune response of breeder birds to PHA-P was significantly higher in higher zinc fed groups with T₃ recorded highest response. The influence on primary antibody titer to SRBC was significantly (P < 0.05) lower in T₀ group (inorganic zinc) than that of other three organic zinc fed groups. [20] reported that immune response to PHA-P injection was enhanced when dietary zinc supplementation was solely from ZnAA. They reported that PHA-P (mm) was 0.97 in ZnSO₄ group and 1.12 in ZnAA group and they observed significant difference between these two groups. [21] reported that zinc as zinc-methionine supplementation (100 mg/kg zinc to a basal diet containing 36.8 mg/kg zinc) had better effect on primary immune response to SRBC relative to control. Researcher has demonstrated supplementing broiler breeder hen diets with zinc-methionine rather than inorganic zinc sources increased cellular immune response of progeny to PHA-P [22]. [23] concluded that supplementation of 20 ppm zinc significantly improved immune response and impact was more prominent with ZnAA (organic zinc) compared to ZnSO₄. The broiler breeder hens provided diets supplemented with zinc from zinc amino acid which might have increased thymulin activity; therefore, enhancing immune response through increased maturation of T-lymphocyte and activation of B lym-

Table 2. Effect on immune response of broiler breeders in different dietary treatments of zinc.

Parameters	T ₀	T ₁	T ₂	T ₃	P value
CBH	165.89 ^c ± 10.98	192.66 ^c ± 6.023	243.96 ^b ± 7.76	291.48 ^a ± 12.49	<0.01
SRBC	4.33 ^b ± 0.40	5.88 ^a ± 0.30	6.22 ^a ± 0.52	6.44 ^a ± 0.68	0.02

Means with different superscripts in a row differ significantly (P < 0.05).

phocytes by T-helper cells [20]. Moreover, zinc has been shown to directly influence the immune system [24]. This element is required for normal immune function [25].

In contrast to this finding, [26] reported that zinc supplementation (80 mg/kg zinc from ZnSO_4) had lower effect on primary immune response to SRBC relative to control. Similarly, [1] reported that on feeding of zinc both from inorganic and organic sources did not have any significant effect on either cellular or humoral immunity. Also, [27] reported that supplementation of zinc methionine partially or completely in place of inorganic sources had no much influence on primary antibody titer to SRBC.

The weight of lymphoid organs (spleen and bursa) and liver of female broiler breeder of different dietary treatment has been presented in **Table 3**. The Weight lymphoid organs of different treatment groups (both organic and inorganic zinc fed groups) of the broiler breeders did not differ significantly ($P > 0.05$). [27] reported that dietary addition of organic zinc in place of inorganic source in broiler diet increased the relative weight of thymus as a proportion of live body weight but no significant effect on the weight of spleen and bursa of fabricius. [28] in their experiments in broiler reported that all the immune organs (bursa of fabricius, spleen and thymus) were significantly affected by the level of zinc-glycine in the diet at 21 day of age but at 42 day, no significant improvement in these organs were observed except for thymus. In contrast to this finding, [29] observed significant higher weight of spleen in zinc-proteanate group than the control group. Supplementation of zinc did not improve

the weight of the lymphoid organs as more nutrients being reparationed to develop body weight and production and immune system need a small amount of nutrient in relation to what is needed for growth and production [30]. The weight of the liver (percentage of body weight) increased from T_0 to T_3 with significant lower liver weight was recorded in T_0 group. This might be due to numerical higher body weight of birds in T_3 than that of T_0 group.

3.2. Bioavailability

Measuring the deposition or storage of minerals in selected tissues (tibia or plasma zinc, liver copper and tibia manganese) is most common output in trace mineral relative bioavailability experiments [31]. Tissue mineral concentrations are indicators of body storage and mineral status and have been used as biomarker in requirement and bioavailability study [32]. According to some authors [33] and the opinion of Scientific Committee for Animal Nutrition on use of zinc of feeding stuffs, a clear indication of biological activity of zinc are: the content of this element in liver, methionine activity, accumulation of zinc in the bones and levels of zinc in blood serum are the method of estimation of availability of this element in live animals.

The zinc content in the serum of broiler breeder in different dietary treatment during the experimental period is presented in **Table 4**. The zinc content of serum of broiler breeders of all the groups did not differed significantly in all the periods of study. No increasing or decreasing trend was observed even on feeding higher le-

Table 3. Weight lymphoid organs and liver (percentage of live weight) of broiler breeders in different dietary treatments of zinc.

Immune organ	T_0	T_1	T_2	T_3	P value
Spleen	1.73 ± 0.15	1.82 ± 0.12	1.98 ± 0.13	2.15 ± 0.17	0.21
Liver	$2.42^c \pm 0.14$	$2.82^{cb} \pm 0.18$	$2.97^b \pm 0.22$	$3.60^a \pm 0.19$	<0.01
Bursa	0.14 ± 0.04	0.12 ± 0.02	0.14 ± 0.03	0.17 ± 0.04	0.72

Means with different superscripts in a row differ significantly ($P < 0.05$).

Table 4. Zinc content (ppm) of serum of broiler breeders in different dietary treatments of zinc.

Weeks	T_0	T_1	T_2	T_3	P value
35 week	11.06 ± 0.45	10.58 ± 0.31	11.29 ± 0.25	11.27 ± 0.30	0.42
39 weeks	11.15 ± 0.36	11.26 ± 0.51	11.81 ± 0.52	11.66 ± 0.40	0.70
43 weeks	12.04 ± 0.47	11.26 ± 0.35	11.76 ± 0.54	12.24 ± 0.50	0.50
48 week	11.92 ± 0.38	12.10 ± 0.34	11.81 ± 0.45	12.58 ± 0.44	0.56
Average	11.54 ± 0.21	11.30 ± 0.20	11.67 ± 0.22	11.94 ± 0.21	0.21

Means with different superscripts in a row differ significantly ($P < 0.05$).

vels of zinc in organic form or replacement of inorganic zinc with organic zinc in the diet of broiler breeders. [34] in their experiment on broilers reported that zinc content of serum of both organic and inorganic fed groups did not differ significantly which is in agreement with our finding. In contrast to this, [29] reported significantly higher level of serum zinc in zinc-proteanate group than control. Similarly, [35] reported that broiler chicken received diet containing zinc-methionine had increased level serum zinc compared to the content of this element in birds received zinc sulphate. The non-significant level of serum zinc in different periods of study might be due to mineral homeostasis which is precisely maintained in the body and is predominantly achieved by balancing tissue storage and excretion [36].

Zinc content in liver, tibia, kidney and spleen of broiler breeders in different dietary treatments is presented in **Table 5**. Zinc content in liver and tibia of broiler breeders in different dietary treatments of zinc differed significantly ($P < 0.05$) with higher levels obtained on increasing zinc concentration in the diet. The increased level of zinc in tibia as observed in the present study is in agreement with the findings of [29,34,35, 37,38]. In contrast to this finding, [39] reported dietary treatment by replacing 50% of the inorganic Zn, Cu, Mn in the control ration with chelated minerals at 14 and 52 day, there was no significant difference between the control and treatment group for tibia zinc content. This might be due to lower duration of feeding. [26] reported that feeding broiler chicken with feed containing either zinc-methionine or ZnO had no significant effect on the presence of microelements in the bone. Moreover, based on tibia zinc content, [11] reported that zinc from zinc methionine, is more bio-available than zinc from ZnSO_4 or ZnO. Similarly, [9] reported the impact of different dietary concentrations of minerals on their deposition rate in tibia. Their results showed that with feeding different diets containing same concentration of zinc, manganese and copper, birds were able to deposit organic form of minerals more efficient than their inorganic form.

[37] reported that the increased dietary addition of zinc from both organic and inorganic sources increased the

liver zinc content. [40] reported that on supplementation of 100 ppm zn-methionine chelate over the NRC level in control group exhibited higher zinc content in the liver (104.53 ppm) of zinc-methionine supplemented group than control (99.50 ppm) in spite of any significant difference between the two groups. In support to our finding, [26] reported that feeding broiler chicken with feed containing either zinc-methionine or ZnO had no significant effect on the presence of microelements in the liver and pancreas at the same level of feeding but they observed significant difference in zinc levels of those organs at different levels of feeding.

The zinc level in the spleen and kidney of the broiler breeders in different dietary treatments did not differ significantly. No such reported work was available on this study. Probably, the weight of immune organ have significance in birds on feeding different source and level of zinc rather than the level of zinc in those organs.

3.3. Faecal Excretion

The zinc content in the faeces of broiler breeder in different dietary treatment during the experimental period has been presented in **Table 6**. The zinc content in the faeces (excretion) of broiler breeders in different dietary treatments of zinc did not differ significantly among all the treatments during the periods of study viz. 32 - 35 weeks, 36 - 39 weeks and 40 - 43 weeks of age. Even the zinc level was higher in T_3 and T_2 but the zinc excretion was not significant which implied better bioavailability of organic zinc. [41] reported that one of the benefits of feeding chelated minerals to broilers was to improve mineral bioavailability, allowing reduction in dietary mineral supplementation and thus reduced excretion. Organically complexed trace minerals provide alternative pathways for absorption, thus leading to a reduction in the excretion of minerals [42]. But during 44 - 48 weeks of age, the zinc excretion in faeces was significantly higher in T_3 than that of T_2 , T_1 and T_0 groups. The overall zinc excretion in faeces of T_3 did not differ significantly with T_2 which implied higher retention of zinc in T_3 as it contained 20 ppm which is more organic zinc

Table 5. Zinc content (ppm) in organs and chicks of broiler breeders in different dietary treatments of zinc.

Organs	T_0	T_1	T_2	T_3	P value
Liver	75.76 ^c ± 2.28	83.37 ^{bc} ± 3.29	90.92 ^b ± 3.27	106.12 ^a ± 3.065	<0.01
Kidney	91.65 ± 1.184	89.85 ± 0.82	90.51 ± 0.89	92.73 ± 0.74	0.15
Spleen	85.62 ± 1.43	82.33 ± 1.68	84.89 ± 2.51	85.69 ± 1.72	0.56
Tibia	203.0 ^c ± 9.54	320.81 ^b ± 16.71	375.22 ^a ± 12.98	381.84 ^a ± 13.99	<0.01
chick	55.25 ^d ± 1.51	59.38 ^c ± 1.37	65.6 ^b ± 1.43	71.81 ^a ± 0.97	<0.01

Means with different superscripts in a row differ significantly ($P < 0.05$).

Table 6. Zinc content (ppm) in the faeces (excretion) of broiler breeders in different dietary treatments of zinc.

Weeks	T ₀	T ₁	T ₂	T ₃	P value
35 Week	119.24 ± 2.98	118.55 ± 2.43	122.88 ± 3.079	127.81 ± 2.24	0.08
39 weeks	124.74 ± 2.57	126.33 ± 2.76	133.58 ± 2.002	129.36 ± 2.99	0.10
43 weeks	119.81 ± 2.42	124.88 ± 2.47	126.85 ± 2.41	125.80 ± 1.45	0.14
48 week	120.01 ^c ± 2.16	122.25 ^c ± 1.91	128.47 ^b ± 1.37	137.79 ^a ± 1.32	<0.01
Average	120.95 ^b ± 1.27	123.008 ^b ± 1.26	127.95 ^a ± 1.27	130.19 ^a ± 1.27	<0.01

Means with different superscripts in a row differ significantly (P < 0.05).

than that of T₂. This implied that in the 20 ppm organic zinc group where the diet contained 60 ppm of total zinc as compared to 40 ppm in control group, the bioavailability was significantly improved as the faecal loss in both groups were comparable. [43] fed broilers with basal diet contained 30 mg/ kg zinc in control and added inorganic zinc at the levels of 20, 40, 80 mg/kg. Similarly organic zinc was added to other groups at the levels of 20, 40, 80 mg/kg. They reported that neither in ZnSO₄ or organic alone supplemented groups, there was a reduction in zinc excretion. But they observed significantly lower zinc excretion in both organic and inorganic supplemented group. They also reported that the cumulative zinc excretion of organic zinc fed groups were significantly lower than inorganic zinc fed groups. [44] reported that the total amount of inorganic minerals in a broiler premix could be totally replaced by 20% organic minerals without affecting growth performance and at the same time reducing environmental pollution. [45] reported that organic minerals that are chelated to small peptides have much greater bioavailability through increased selective transport of peptide at gut level. Enhanced bioavailability of mineral source can potentially reduce the amount of a mineral that is added to a diet to meet nutritional requirement, leading to reduced amount of mineral excreted by birds [46].

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