Growth Performance, Carcass Yield, and Immune Competence of Broiler Chickens as Influenced by Dietary Supplemental Zinc Sources and Levels

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Abstract This study was conducted to determine the effect of sources and levels of zinc (Zn) on growth performance, carcass characteristics, and immunocompetence of broilers. A 6-week (0–6 weeks of age) feeding trial was conducted as per 3×4 factorial design involving three Zn sources (zinc propionate—ZnP, zinc oxide—ZnO and zinc sulfate—ZnS) each with four levels of Zn (40, 60, 80, and 100 mg/kg) during starting (0–3 weeks) and finishing (4–6 weeks) phase in a standard broiler diet. Overall body weight gain, feed intake, feed conversion ratio, and percent Zn retention in broilers from 0 to 6 weeks were not differed significantly due to the interaction of different sources and levels of Zn. Growth traits were non-significant due to the main effect of Zn level. Percent Zn retention was decreased due to increase in Zn levels (L effect, $P < 0.001$; Q effect-non-significant) among different groups. The humoral (HA titre to-SRBC) and cellular (foot pad index (FPI) to phytohaemagglutinin-P) immune response did not differ significantly due to main effect of Zn sources and interaction effect of Zn sources and levels. However, HA titre to SRBC was maximum ($P < 0.05$) at 80 mg Zn/kg diet and FPI to PHA-P was maximum ($P < 0.05$) at 60 mg Zn/kg diet. The L effect of Zn level was non-significant for HA titre and FPI. The Q effect of Zn level was significant at $P < 0.05$ and $P < 0.01$ for HA titre and FPI, respectively.

Keywords Growth · Carcass quality · Immune competence · Broiler · Zinc

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Introduction

Optimizing and updating nutrient requirements of poultry is a continuous process because of improved performance, variations in nutrient availability, interaction of different nutrients at sites of absorption and metabolism, and to achieve specialized designer products. Zinc (Zn) is an essential trace mineral for normal growth/development of animal, and is involved in a variety of metabolic processes [19]. In poultry Zn plays a vital role in skin nucleic acid, keratin, collagen synthesis, and production and maintenance of epithelial cells. The Zn requirement of the chicks was first defined to be 30 mg/Kg [17]. Subsequently, the NRC [15] has recommended that the broiler diet should contain 40 ppm Zn, which appeared to be based on the results that considered growth performance as the only criterion [4, 20]. However, higher levels of Zn are supplemented in diets for enhancing immune response in chicken but the fortification of diets with excess minerals

may cause antagonism affecting mineral bioavailability and masking the advantages of supplementation [7].

In view of the above, the effect of supplemental Zn sources and levels in relation to the growth performance, carcass yield, and immune competence in broiler chickens was studied during 6 weeks experimental period.

Materials and Methods

Experimental Design

A total of 252 broiler chicks were randomly allotted to cabins or tiers of the electrically heated battery brooders with the provision of wire-mesh floor, feeder, and waterer, located in the well-ventilated room, with uniform management. The chicks had free access to feed and water throughout 6 weeks (wks) feeding period.

Twelve dietary treatments with three Zn sources (Zn propionate–ZnP, Zn oxide–ZnO, and Zn sulfate–ZnS) and four levels (40, 60, 80, and 100 mg/kg) of each source were evaluated with triplicate lot of seven broiler chicks, accommodating 21 broiler chicks per treatment. As shown in Table 1 corn soybean basal diet was formulated and the final Zn content of each diet was set with the addition of Zn content of basal diet plus supplemented Zn content. Thus, different ZnP, ZnO, and ZnS were added to achieve final Zn contents of 40, 60, 80, and 100 ppm Zn in both starter and finisher diet. Birds were fed starter diet for the initial 21 days followed by finisher diet for next 21 days.

Determination of Proximate Principal of Feed Ingredients and Diets

The representative samples of feed ingredients and test diets used in the study were analyzed for moisture, crude protein (CP), total phosphorus (TP); [2], calcium [22], and Zn content [2] using atomic absorption spectrometer (AAS, *Varian SpectrAA 220 Model*). The ingredients and nutrient composition of basal diet used during starting (0–3 weeks) and finishing phase (4–6 weeks) have been presented in Table 1.

Growth Traits, Mortality, and Zn Retention

The overall body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) were recorded during 0–6 weeks. Daily monitoring and recording on individual basis was carried out to study the mortality among experimental birds. Two birds per replicate, thus six birds from each treatment were selected randomly and a 3-day balance study was conducted from 38 to 40 days of age during which the net feed consumed by each bird in the respective dietary group was recorded and the dropping voided over

Table 1 Ingredient and nutrient composition of basal diet used during starting phase (0–3 weeks) and finishing phase (4–6 weeks)

Ingredients	Composition (%)	
	Starting phase (0–3 weeks)	Finishing phase (4–6 weeks)
Yellow maize	57.5	64.6
Deoiled rice bran	4.9	4.8
Soybean meal	34.2	27.2
Limestone powder	0.5	0.5
Dicalcium phosphate	2	2
DL-Methionine	0.11	0.1
Lysine	0.09	0.1
Common salt	0.4	0.4
Trace mineral premix ^a	0.15	0.15
Vitamin premix ^b	0.15	0.15
Nutrient composition—analyzed (% DM)		
Crude protein	22.5	19.49
Calcium	1.05	1.18
Total phosphorus	0.71	0.73
Zinc (mg/kg) ^c	30	28
Calculated (%)		
ME (KCal/kg)	2850	2927
Available phosphorus	0.5	0.5
Lysine	1.2	1.2
Methionine	0.5	0.5

^a Trace mineral mixture provided (mg/kg diet): Cu—7.25 mg, Fe—80 mg, I—1.2 mg, Mn—65 mg, Zn—variable

^b Vitamin mixture provided (mg/kg diet): Choline Chloride—500, Niacin—12, Pyridoxine Hydrochloride—1.6, Vitamin A—8250 IU, Vitamin B₁—0.8, Vitamin B₂—5, Vitamin B₁₂—8, Vitamin D₃—1200 IU, Vitamin E—10 and Vitamin K—1

^c Further supplemented with four desired Zn levels to achieve 40, 60, 80, and 100 mg Zn/kg diet from each of three Zn sources

the same period were collected to estimate % Zn retention. The total excreta voided from six replicates per treatment were collected to estimate the concentration of Zn.

Evaluation of Carcass Yield Factors

At the end of experiment (42 days), two birds per replicate, thus six birds from each treatment were selected randomly by maintaining average body weight, slaughtered, and de-feathered by machine. These six birds represented as six replicates in each treatment and evaluated for different carcass yield parameters.

Immune Response

The immune response of broiler chicks fed different supplemental Zn levels and sources were analyzed by the

humoral immune response and cell-mediated immune response on completing 3 and 4 weeks of age, respectively. For this 72 chicks (6 chicks per treatment) were selected.

Humoral Immune Response

Modified method of Allan and Gough [1] was followed for assaying the immune response to sheep red blood cells (1 % SRBC) at 21 days of feeding trial and blood was collected 72 h post SRBC injection. The antibody titre was determined by haemagglutination test (HA) in which microliter plates with U bottom were used. 50 µl of PBS was distributed in each well of the plate. Same amount of serum was added in the first well. Two-fold serial dilutions were made up to 11th well, 12th well being the control. Then, 50 µl of 1 % SRBC in PBS was added to each well and mixed. The plates were covered and kept at 37 °C for 1 h for incubation. The plates were read under bright light. The reciprocal of the highest dilution showing clear agglutination was the end titre.

Cell-Mediated Immune Response

In vivo cell-mediated immune response to PHA-P was evaluated at 28 days of age by the method [9]. PHA-P 0.1 ml (1 mg/ml in sterile phosphate buffer saline, PBS) was injected intradermally in inter-digital space between 3rd and 4th toe of the right foot of the individual chick. The left foot served as controls and received the same amount of sterile PBS. The individual skin index for the right and left foot was calculated as the difference between the skin thicknesses measured by micrometer before and after 24 h of injection and expressed as millimeters. FPI was determined as the difference in skin index of right and left foot for individual bird.

Statistical Analysis

The data pertaining to various parameters were analyzed statically as general linear model with multivariate analysis. The significant mean differences were tested as per Duncan's multiple range test (DMRT). Polynomial linear (L) and quadratic (Q) contrasts were used to test the effect of levels of Zn on immune parameters.

Results and Discussion

Growth Traits, % Zn Retention, and Mortality

In the present study the overall BWG, FI, FCR, and percent Zn retention in broilers from 0 to 6 weeks were not differed significantly due to the interaction of different sources and

levels of Zn. Due to the main effect of Zn source FI and FCR were differed significantly (Table 2). The FI were more ($P < 0.05$) in ZnO group than other two groups and FCR were better ($P < 0.05$) in ZnP group. Again all the growth traits were non-significant due to the main effect of Zn level except for % Zn retention which was decreased as Zn level were increased (L effect, $P < 0.001$; Q effect-nonsignificant) in different groups. Non-significant interaction between Zn sources and Zn levels as seen in the present study is in close agreement with earlier observation reported by Mohanna and Nys [14] who did not observe any significant interaction between Zn sources and levels on BWG of broiler chicks and concluded that a dietary Zn concentration above 45 mg/kg did not give additional response in terms of BWG. Collins and Moran [7, 8] also reported that when dietary Zn concentrations are provided in excess of NRC recommendation of 40 mg/kg supplemental Zn, growth rate remained unaffected. The present results received support from the earlier report of Collins and Moran [7, 8], who observed that the FCR was not improved at dietary levels of Zn exceeding the NRC recommendations of 40 mg/kg diet. Present results on Zn retention received support from the earlier work reported by Stahl et al. [21], who reported that chicks given either moderate or excess Zn, excreted more than twice as much Zn from a test diet as those fed on the control diet. Furthermore, the high dietary Zn explained the low percentage of Zn retention as body Zn concentration remained stable when dietary Zn was higher than 60 mg/kg. Similarly, Burrell et al. [5] reported that increased supplemental Zn concentration significantly increased the Zn excretion and reduced Zn utilization in experimental chicks. In the present study the ZnP had better Zn retention numeric value than inorganic sources ZnO and ZnS. These results can be corroborated with earlier work reported by Wedekind et al. [23], who observed that in production animals, organic Zn sources such as Zn methionine or Zn propionate are significantly more bioavailable than inorganic Zn sources such as Zn oxide or Zn sulfate. Contrary to present finding, Burrell et al. [5] reported that the Zn sources either organic or inorganic did not alter Zn utilization. The overall mortality of the chicks in present study was 4.76 % and there was no difference in the mortality due to the treatments.

Carcass Yield Factors

The various carcass yield factors (live weight, shrinkage loss, blood loss, feather loss, dressed weight, and eviscerated weight) and organ weight (giblet, heart, liver, and gizzard) did not differ significantly due to interaction between Zn sources and Zn levels. Similarly, carcass yield and organ weight did not differ statistically due to different

Table 2 Growth and immune parameters of broiler fed different sources and levels of zinc

Parameters of broiler fed different sources and levels of zinc	Treatments		Parameters						
	Interaction effect (<i>n</i> = 21) (Zn sources × Zn levels)		BWG (g)	FI (g)	FCR	Zn retention (%)	HA Titre (log2)	FPI (mm)	
Values containing common superscripts within a column are non-significant (<i>P</i> < 0.05) BWG body weight gain, FI feed intake, FCR feed conversion ratio, FPI foot pad index, Zn zinc, ZnP zinc propionate, ZnO zinc oxide, ZnS zinc sulfate	ZnP	40	1130	2587	2.29	45.43	5.00	0.43	
		60	1132	2592	2.30	33.67	7.50	0.85	
		80	1141	2621	2.30	29.97	7.67	0.75	
		100	1079	2497	2.32	26.19	6.67	0.62	
	ZnO	40	1127	2630	2.33	41.14	6.50	0.48	
		60	1076	2554	2.38	35.44	8.33	0.81	
		80	1075	2664	2.48	30.19	9.33	0.76	
		100	1078	2613	2.43	20.43	7.83	0.55	
	ZnS	40	1110	2551	2.30	39.27	5.83	0.52	
		60	1098	2548	2.32	38.25	8.00	0.77	
		80	1096	2535	2.32	27.68	8.16	0.80	
		100	1115	2585	2.31	23.58	7.00	0.63	
	Pooled SEM			6.45	11.02	0.01	2.27	0.35	0.03
	Main effects								
	Zinc sources (<i>n</i> = 84)	ZnP	1120	2574 ^{ab}	2.30 ^b	33.81	6.71	0.66	
		ZnO	1089	2615 ^a	2.41 ^a	31.80	8.00	0.65	
ZnS		1105	2555 ^b	2.31 ^b	32.20	7.25	0.68		
Zinc levels (<i>n</i> = 63)	40	1122	2589	2.31	41.95 ^a	5.78 ^b	0.48 ^c		
	60	1102	2565	2.33	35.79 ^b	7.94 ^a	0.81 ^a		
	80	1103	2607	2.37	29.28 ^c	8.39 ^a	0.77 ^{ab}		
	100	1092	2565	2.35	23.40 ^d	7.17 ^{ab}	0.60 ^{bc}		

Values containing common superscripts within a column are non-significant ($P < 0.05$)

BWG body weight gain, FI feed intake, FCR feed conversion ratio, FPI foot pad index, Zn zinc, ZnP zinc propionate, ZnO zinc oxide, ZnS zinc sulfate

dietary sources and levels of Zn, whereas the average shrinkage loss due to fasting differed significantly ($P < 0.01$) due to different dietary Zn sources. Significantly, lower shrinkage loss value was observed in ZnS than ZnP and ZnO. Present results received support from the earlier report of Collins and Moran [8] who also reported that the broiler fed with inorganic Zn at levels exceeding NRC recommendations did not alter carcass weight after processing at 49 days. These authors also reported that supplementing broiler feed with substantial levels of inorganic Zn generally did not improve processing yield of different broiler strains. Furthermore, the carcass yield was not influenced by supplemental Zn above 40 mg/kg diet. Rossi et al. [18] also stated that carcass

yields were not influenced by providing increasing levels of dietary organic Zn to broilers.

Immune Response

Humoral and cellular immune response did not differ significantly due to Zn sources and interaction between Zn sources and Zn levels (Table 2). However, Humoral and cellular immune response differed significantly ($P < 0.05$) due to different dietary Zn levels. HA titre was significantly ($P < 0.05$) higher at 80 mg Zn/kg diet and FPI was significantly ($P < 0.05$) higher at 60 mg Zn/kg diet than other levels, whereas the diet having 100 mg Zn/kg both immune parameters were found intermediary. The contrast

Table 3 Effects of zinc levels ($n = 63$) on the immune parameters of broiler chicks

Parameters	Zinc levels				Contrast		
	40	60	80	100	SEM	L	Q
HA titre (log2)	5.78 ^b	7.94 ^a	8.39 ^a	7.17 ^{ab}	0.35	NS	*
Foot pad index (mm)	0.48 ^c	0.81 ^a	0.77 ^{ab}	0.60 ^{bc}	0.03	NS	**

Values containing common superscripts within a row are non-significant ($P < 0.05$)

NS non-significant, L linear, Q quadratic

* $P < 0.05$, ** $P < 0.01$

effect of Zn levels on the immune parameters is presented in Table 3. As the Zn level was increased, the HA titre increased up to 80 mg/kg Zn level but again decreased at 100 mg/kg Zn level. The L effect was non-significant and the Q effect was significant at $P < 0.05$ for HA titre. The increase in Zn level led to increase the FPI from 40 to 60 mg/kg Zn level but a continuous drop in FPI was observed after 60 mg/kg Zn level in a quadratic manner (Q, $P < 0.01$).

Present results on immune parameters are in agreement with earlier report [6], where optimum supplemental level of Zn was suggested to be 80–120 mg/kg diet for broiler chickens to maximize immune response. Supplementing the Zn in diets fed to broiler breeder and turkey hens increased the cellular immune response of progeny as measured by cutaneous basophil hypersensitive tests [12, 13]. Similarly, earlier researchers [3, 10] reported that the immune response of broilers can be affected by the levels of ZnAA in the diet. Khajareem et al. [11] reported that high level of Zn supplementation (75 versus 175 mg/Kg) resulted in higher antibody titre to viral diseases in broiler breeder hens.

Contrary to present finding, some researchers reported that Zn concentration did not affect antibody titres of broilers in response to SRBC injections [14]. In agreement with these data Pimentel et al. [16] indicated that dietary Zn concentration (8–58 mg/Kg) or source (Zn methionine or ZnO) did not influence antibody titres to human gamma globulin or delayed-type hypersensitivity to phytohaemagglutinin in broilers.

Conclusions

The optimum supplemental level of Zn should be 60–80 mg/kg diet for broiler chickens to get better immune response. However, the zinc source did not significantly influence zinc utilization. Most of the growth and carcass yield factors were found to be non-significant due to different Zn sources and their levels.

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