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Effect of graded concentration of organic zinc (zinc glycinate) on skin quality, hematological and serum biochemical constituents in broiler chicken

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ABSTRACT

Day-old chicks (120) were randomly distributed to 4 dietary groups with 6 replicate in each and raised for 42 days under uniform managemental conditions to determine the effect of supplementing organic zinc (zinc glycinate, Zn-gly) at lower levels on skin quality, haematological and serum biochemical constituents. Dietary treatments consisted of adding 30, 20 and 10 ppm Zn from Zinc glycinate and 40 ppm Zn (NRC 1994) from $ZnSO_4$ (inorganic) (control) to a corn-soybean meal basal diet prepared as per NRC (1994) nutritional requirements, except Zn. Feed and water was provided *ad-libitum*. Haematological and serum biochemical constituents were estimated in blood collected at 35 d. At the end of experiment, representative samples of skin were collected from slaughtered birds for histological examination. Haematological constituents (WBC, RBC, Hb concentration, PCV, MCV and lymphocyte concentration), serum total protein, albumin and glucose concentrations did not vary with concentration (10–40 ppm) and source ($ZnSO_4$ or Zn-gly) of Zn supplementation in diet. The cholesterol and albumin to globulin ratio was lowest ($P<0.05$), while globulin, Zn concentrations and alkaline phosphatase (ALP) activity in serum was highest ($P<0.05$) at 30 ppm Zn supplementation from Zn- gly compared to other groups and these attributes were comparable between 20 ppm Zn as Zn-gly and 40 ppm Zn as $ZnSO_4$. An increased epithelial cell layer and collagen content was observed with 30 ppm Zn supplementation as Zn-gly compared to other dietary treatments. While epithelium cell layers and collagen content was moderate with addition of 40 ppm Zn as $ZnSO_4$ or 20 ppm Zn as Zn-gly. The skin of birds supplemented with 10 ppm Zn from Zn-gly had few epithelial layers and low amount of collagen. Higher ALP activity indicating higher bioavailability, better skin quality and globulin concentration in serum was observed in birds fed 30 ppm Zn from Zn-gly compared to 40 ppm Zn supplemented from inorganic source. Reducing the dietary Zn supplementation by 50% (20 ppm) from organic Zn had comparable skin quality, serum ALP activity and globulin concentration as 100% (40ppm) supplementation from inorganic source.

Key words: Hematology, Serum biochemical constituents, Skin quality, Zinc glycinate

Zinc (Zn) is one of the important trace mineral involved in carbohydrate, protein and fat metabolism and thus affecting all phases of life. Zn is a cofactor for more than 300 metalloenzymes which play important role in growth, reproduction and immune responses. Zn is necessary for production and maintenance of epithelial cells (Costa 2005) which is the first barrier to infection (Gallo and Hooper 2012) and very few microorganisms has ability to penetrate the intact skin (Baroni *et al.* 2012). McDowell (2003) stated that Zn deficiency results in abnormalities of the skin and its appendages due to its role in collagen (fibrous protein) synthesis, which functions as an extracellular structural element in connective tissue. Zinc affects carbohydrate and lipid metabolism by stimulating the glucose transport (May and Contoreggi 1982), incorporation of glucose into lipids (Coulston and Dandona 1980), and glucose oxidation by glycolysis and hexose monophosphate shunt pathways (Shisheva *et al.* 1992). It is also speculated that Zn also has positive correlation with serum cholesterol concentration (Al-Daraji and Amen 2011).

In poultry, Zn is mostly supplemented in diets from inorganic source to meet its requirements. Mostly, the Zn supplemented from inorganic source is poorly absorbed with low bioavailability (Ao *et al.* 2011) and thus supplemented at higher levels than the recommended to avoid deficiency. Higher supplemental levels might interfere with the absorption and metabolism of other minerals like copper (Ao *et al.* 2009). To avoid such problems, concept of organic minerals was developed in which mineral is in chemically inert form, more stable and less prone to interactions (Ao *et al.* 2009) and have greater bioavailability (Zhao *et al.* 2010). An enhanced bioavailability of mineral source could reduce the amount of mineral excreted by birds thereby, could lower the requirement (Jahanian *et al.* 2008 and Ao *et al.* 2011). Zinc glycinate (Zn-gly) is one such organic

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source of Zn having amino acid glycine. Glycine has a slightly higher stability constant for Zn than methionine (Kratzer and Vohra 1986) and this could result in the zinc glycinate complex being more available for absorption. In view of above, present study was carried out to investigate effect of supplementing lower levels of Zn as zinc glycinate on skin quality, haematological and serum biochemical constituents compared to $ZnSO_4$ supplementation at recommended levels in commercial broiler chicken.

MATERIALS AND METHODS

Birds, management and diet: Day-old commercial broiler chicks (200; 46.28±0.33g) were randomly allotted to 24 replicates with 5 chicks in each. On day 1, chicks were wing banded and housed in wire-floored stainless battery brooders. The brooder temperature was maintained at 35±0.5 °C until 7 days of age and gradually decreased to 27 °C by 21 days of age, after which, chicks were maintained at room temperature (20–27 °C). Birds were immunized for Marek's disease at hatchery, New Castle disease (ND) at 7th day and 21st day of age with Lasota vaccine and infectious bursal disease at 14th day of age with Georgia strain vaccine. The experiment was approved by Institutional animal ethics committee.

A corn-soybean meal basal diet (BD) was prepared for starter and finisher broiler chicks to meet the nutritional requirements, as recommended by NRC (1994) except Zn (Table 1). According to zinc concentration in the inorganic or organic source, four experimental diets were prepared. The mineral premix in the BD was prepared without Zn. The inorganic source of zinc was $ZnSO_4$ (22% Zn) and the organic source of Zn was zinc glycinate (21.94% Zn). The inorganic Zn ($ZnSO_4$) was added in BD to supply 40 ppm Zn (control diet), while organic Zn (*Zn-gly*) was added to BD so as to supply 30, 20 and 10 ppm Zn (experimental diets). The 24 replicates in turn were randomly allotted to these 4 dietary treatments with 6 replicates in each treatment. The birds were fed the respective diets *ad-lib* from 1 to 42 days.

Skin histology, hematology and biochemical constituents: Blood was collected from 2 randomly selected birds per replicate at 35 d of age aseptically from brachial vein in two sets, first set in a clean sterilized heparin coated vacutainers for hematology. Immediately after blood collection the samples were analysed for haematological constituents i.e. white blood cell count (WBC, 1000/ µl), red blood cell count (RBC, millions/µl), hemoglobin (Hb, g%), packed cell volume (PCV,%), mean corpuscular volume (MCV, Fl) and lymphocytes using automatic whole blood analyzer (Huma count, Med source Ozone biomedical Pvt. Ltd, India). Second set of blood samples were collected in clean sterilized glass tubes for serum collection and the serum was stored in eppendorf tubes at – 20°C for estimation of biochemical constituents i.e., total protein (Reinhold 1953), albumin (Gustafsson 1976), glucose (Cooper and Mc Daniel 1970), cholestrol (Wybenga *et al.* 1970) and alkaline phosphatase (Kind and King 1954).

Table 1. Ingredient (%) and nutrient composition (% dry matter) of basal diet fed to broilers

Ingredient	Starter	Finisher
Maize	52.9	58.05
Soybean meal	40.3	32.9
Oil (veg)	3	5.2
Salt	0.35	0.35
DL-Methionine	0.22	0.17
Threonine	0	0.05
Di-Calcium phosphate	1.97	2
Shell grit	0.73	0.75
Vitamin and trace mineral premix[1,2]	0.03	0.03
Choline chloride, 50%	0.1	0.1
Toxin binder	0.2	0.2
Antibiotic	0.05	0.05
Coccidiostat	0.05	0.05
Tylan	0.05	0.05
Liver tonic	0.05	0.05
Analyzed Zn	27.9	28.6
Nutrient composition *		
Metabolizable energy (kcal/kg)	2968	3166
Crude protein	22.6	19.72
Crude fibre	3.69	3.72
Calcium	0.84	0.81
Available phosphorus	0.47	0.46
Lysine	1.31	1.16
Methionine	0.57	0.53

[1] Vitamin premix provided per kg diet: Vitamin A, 20000IU; D_3, 3000IU; E, 10mg; K, 2mg; riboflavin, 25mg; B_1, 1mg; B_6, 2mg; B_{12}, 40mcg; Niacin, 15 mg. [2] Trace mineral premix provided per kg diet: Cu, 8 mg; Mn, 60 mg; Fe,80 mg.* Calculated values.

At the end of experiment (42 d), randomly selected two birds were slaughtered and approximately 1 cm^2 defeathered skin sample was collected from the back next to the thigh and one set was immediately preserved in 10% formalin and another set was stored in deep freeze (-20 °C) for estimation of Zn. The 1st set of samples were embedded in paraffin, sectioned, mounted, and stained with hematoxylin and eosin for observing the skin quality under microscope (Rossi *et al*. 2007).

For Zn estimation in skin and serum, the samples were first thawed to room temperature and approximately 1 g (skin) or 1 ml (serum) of sample was wet digested by diacid method in which samples were kept overnight with 10 ml of concentrated HNO_3. Next day again10 ml HNO_3 and 2–3 ml perchloric acid was added and digested on hot plate at 180–200°C till the dense white colour fumes appeared. The digested samples were then transferred to a 50 ml volumetric flask by several washing with double distilled water through Whatman filter paper No.42 and final volume was made to 50 ml. These processed samples were analysed for Zn with atomic absorption spectrophotometer (Arenza *et al.* 1977).

Statistical analysis: The data was subjected to one way analysis of variance as per the procedures of Snedecor and Cochran (1994). The differences between the means were tested by significance using Duncan's multiple range test

(Duncan 1955).

RESULTS AND DISCUSSION

Hematological and biochemical constituents: Organic trace minerals are stable in digestive tract and mineral would be protected from forming complexes with other dietary components that inhibit interactions and thus allow for great absorption (Ledoux *et al.* 1991). Ao *et al.* (2009) also stated that supplementation of Zn in organic form reduces the interaction with other nutrients and minerals, thus, improves the bioavailability of Zn. The blood WBC, RBC, Hb concentration, PCV, MCV and lymphocyte concentration in broiler chicks fed lower levels (30, 20 and 10 ppm) of Zn from organic source was comparable with those on 40 ppm Zn from inorganic source (Table 2). Thymulin being a Zn dependent hormone, essential for maturation of T lymphocytes (Dardenne 2002), and its deficiency causes apoptosis of lymphoid cells and leads to reduction in total leucocytic and lymphocyte count (Someya *et al.* 2007). In spite of lower Zn supplementation (up to 10 ppm) as Zn-gly, higher Zn bioavailability from Zn-gly probably could have regulated the adverse effect of lower Zn on various haematological constituents.

Zinc is an integral component of enzyme alkaline phosphatase (ALP), whose activity could be used as an indicator of animal Zn status in addition to serum Zn concentration and lower serum ALP activity was observed during Zn deficiency, that increased with increase in Zn status (Miller and Cragle 1965). Zn supplementation resulted in significant ($P<0.05$) improvement in ALP activity in broiler breeders (Al-Daraji and Amen 2011) and pigs (Revy *et al.* 2002). In the present study the highest ($P<0.01$) ALP activity was observed in chicks supplemented with 30 ppm Zn from Zn-gly compared to those on 40 ppm Zn as $ZnSO_4$. The activity decreased ($P<0.05$) with reduction in Zn supplementation (20 and 10 ppm) as Zn-gly (Table 3) but was comparable to 40 ppm Zn supplementation from $ZnSO_4$. Similar to ALP activity, significantly ($P<0.05$) higher serum Zn concentration (11.09 ppm) was observed in birds supplemented with 30 ppm Zn as Zn-gly and the mineral concentration was comparable ($P>0.05$) among birds supplemented with 40 ppm (8.75 ppm) Zn as $ZnSO_4$ and 20 (9.63 ppm) or 10 ppm (8.39 ppm) Zn as Zn-gly (Fig. 1). The ALP activity and serum Zn concentration clearly indicated greater Zn availability from Zn-gly than $ZnSO_4$. Idowu *et al.* (2011) observed an increase in serum ALP activity and serum Zn concentration in laying hens with supplementation of Zn (140 mg/kg) from organic source (Zn proteinate, Zn-prot) compared to inorganic sources (ZnO, $ZnSO_4$, $ZnCl_2$, $ZnCO_3$). In concurrence to our results, Wang *et al.* (2010) observed no significant($P>0.05$) difference in ALP enzyme activity and serum Zn concentration in weaning pigs with Zn supplementation of 100 mg/kg as Zn-gly compared to 3000 mg Zn/kg from ZnO in weaning pigs.

Zinc is a component of a number of enzymes, which play a vital role in protein and energy metabolism (Bhowmik and Chiranjib 2010). The serum total protein,

Table 2. Effect of organic zinc supplementation on haematological constituents in broiler chicken

Zn(mg/kg diet)	WBC ($\times10^3$/µl)	RBC ($\times10^6$/µl)	Hb(g%)	PCV (%)	MCV (Fl)	Lymphocyte (%)
Inorganic-40	17.91	1.99	7.61	19.93	103.08	55.66
Organic-30	21.58	2.06	7.92	21.39	107.50	57.39
Organic-20	19.38	1.92	7.55	18.58	104.08	55.55
Organic-10	19.02	2.13	7.56	21.64	103.00	54.87
SEM	0.709	0.060	0.091	0.585	0.982	0.472
N	12	12	12	12	12	12
P value	0.325	0.650	0.447	0.223	0.332	0.278

SEM, Standard error mean; WBC, white blood cells; RBC, Red blood cells; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume.

Table 3. Effect of organic zinc supplementation on serum biochemical constituents in broilers

Zn(mg/kg diet)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	ALP (KA units/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)
Inorganic-40	3.99	2.02	1.97^{ab}	1.05^{a}	13.97^{bc}	155.6^{a}	225.6
Organic-30	4.31	2.01	2.30^{a}	0.87^{b}	15.77^{a}	137.8^{b}	218.6
Organic-20	3.89	1.99	1.89^{ab}	1.07^{a}	14.67^{b}	151.5^{a}	229.5
Organic-10	3.64	1.93	1.72^{b}	1.13^{a}	13.66^{c}	161.9^{a}	227.5
SEM	0.128	0.060	0.075	0.022	0.179	2.172	2.912
N	12	12	12	12	12	12	12
P value	0.333	0.953	0.040	0.001	0.001	0.001	0.588

^{abc} Means with different superscripts in a row differ significantly: $P<0.01$, $P<0.05$; SEM, standard error mean; A/G, albumin/ globulin; ALP, alkaline phosphatase.

albumin and glucose levels in chicks in the present study were not influenced by source and concentration of Zn supplementation. Burns (1983) reported that diets supplemented with ample Zn tend to improve the ability of producing antibody. However, in our study, highest ($P<0.05$) serum globulin concentration was ($P<0.05$) noticed in birds receiving 30 ppm Zn as Zn-gly (Table 3) which could be due to higher bioavailability of organic Zn compared to inorganic source (Ao *et al.* 2009) as indicated by higher ALP activity and serum Zn concentration. In concurrence to our results, Feng *et al.* (2010) observed no effect on serum total protein and albumin concentration in broilers with dietary addition of Zn as zinc-gly chelate @ 90 to 120 mg/kg compared to 120 mg Zn/kg diet as $ZnSO_4$, while noticed higher immunoglobulin production with 90 mg/kg Zn supplementation as Zn-gly compared to 120 mg Zn/kg diet as $ZnSO_4$. Idowu *et al.* (2011) observed increased ($P<0.05$) total protein concentration in laying hens with 140 mg/kg Zn supplementation from Zn-prot compared to various inorganic sources (ZnO, $ZnSO_4$, $ZnCl_2$, $ZnCO_3$).

The anti-atherogenic effect of Zn in hypercholesterolemic rabbits was reported by many workers (Rashtchizadeh *et al.* 2008). Mice with Zn-deficient diets exhibited increased cholesterol and triglycerides levels in blood plasma (Reiterer *et al.* 2005). Bolkent *et al.* (2006) proved the protective effect of Zn supplementation on lipid metabolism indices (total lipids, cholesterol, HDL cholesterol) in laboratory rats with induced type 1 diabetes.

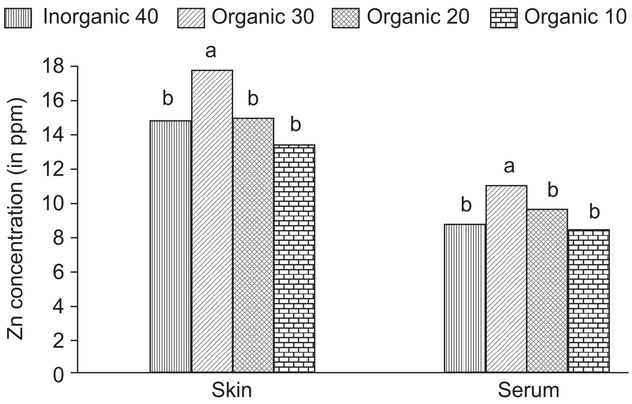

Fig. 1. Effect of organic Zn supplementation on Zn concentration in skin and serum means with different superscripts for skin and serum differ significantly; $P<0.05$.

Zinc supplementation could reduce the blood plasma cholesterol concentration and the reduction was significantly higher with organic Zn supplementation compared to inorganic (Parak and Strakova 2011) corroborating with present findings, where in serum cholesterol concentration reduced ($P<0.05$) with supplementation of 30 ppm Zn from Zn-gly (Table 3) compared to other dietary treatments. Similarly, Sahin *et al.* (2005) observed decrease in serum cholesterol concentrations in heat stressed Japanese quails by addition of 30 or 60 mg Zn/kg basal diet either from organic (zinc picolinate) or inorganic ($ZnSO_4$) source and comparatively higher improvement observed with organic Zn.

Skin histology: The skin is rich in Zn (McDowell 2003) and involved in nucleic acid and collagen synthesis (Close 1999). More number of epithelial cell layers in the outer parts of body, indicates higher protection, preventing multiple invasion by pathogen (Janhson *et al.* 2001). In present study highest ($P<0.05$) Zn deposition (17.97 ppm) in skin was noticed with 30 ppm Zn supplementation in diet as Zn-gly compared to 20 (15.10 ppm) or 10 (13.50 ppm) ppm levels of Zn supplementation from the same source or 40 (15.05 ppm) ppm Zn supplementation from the $ZnSO_4$ (Fig 1). Also, the histological examination revealed increase in epithelial cell layer and collagen content in skin with 30 ppm Zn supplementation as Zn-gly compared to 40 ppm Zn supplemented as $ZnSO_4$ (Fig 2) indicating better skin quality with organic Zn. The collagen, major fibrous element of skin holds the skin more tightly together in discrete units and further gives flexibility and resistance to skin. Higher Zn deposition with 30 ppm Zn supplementation as Zn-gly compared to 40 ppm Zn as $ZnSO_4$ might be the reason for better skin quality. In addition the birds fed diets supplemented with 40 ppm Zn as $ZnSO_4$ or 20 ppm Zn as Zn-gly (Fig. 2) had moderate number of epithelium cell layers and collagen content. The skin of birds supplemented with 10 ppm Zn in form of Zn-gly had few epithelial layers and low amount of collagen (Fig. 2). Salim *et al.* (2011) observed increase in thigh skin epidermis and dermis thickness in group of birds supplemented with organic Zn @ 20, 40, 80 mg Zn/kg diet than group of birds fed without Zn supplementation. These authors also observed increase in collagen content of skin by organic Zn supplementation and the increase was significantly higher in birds supplemented with 80 mg/kg

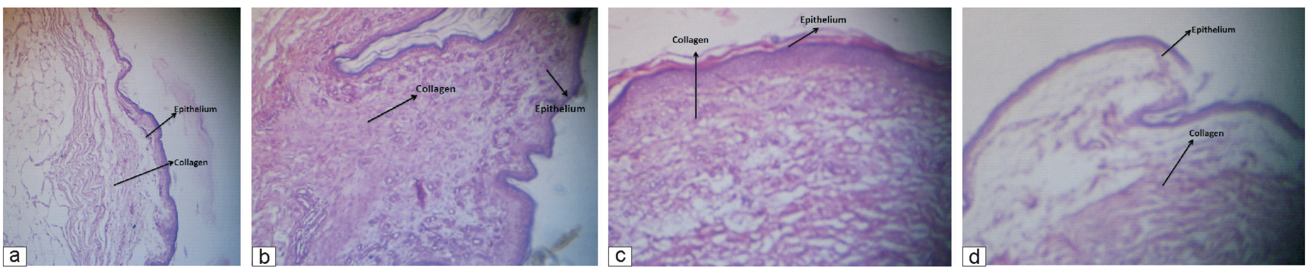

Fig. 2 (A-D). Photomicrograph of skins showing epithelium and collagen in broiler fed on experimental diets (H&E, 100x). A: 40 ppm Zn from $ZnSO_4$; B: 30 ppm Zn from Zn-gly; C: 20 ppm Zn from Zn-gly; D: 10 ppm Zn from Zn-gly.

of Zn. Similarly, several researchers (Rossi *et al.* 2007; Salim *et al.* 2011) observed improvement in skin quality with supplementation of organic Zn.

Based on the results, it could be concluded that dietary Zn concentration could be reduced to 20 ppm when supplemented as Zn glycinate without affecting hematological and serum biochemical constituents. However, replacement of 40 ppm Zn from inorganic source with 30 ppm Zn supplemented from organic Zn significantly improved serum globulin concentration, alkaline phosphatase (ALP) activity and skin quality in broilers compared to those fed 40 ppm Zn as $ZnSO_4$.

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