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RESEARCH ARTICLE

SERUM BIOCHEMICAL INDICES OF BROILER BIRDS FED ON INORGANIC, ORGANIC AND NANO ZINC SUPPLEMENTED DIETS

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ABSTRACT

A total of 280 unsexed broiler chicks were distributed randomly in seven dietary treatments (D). The diets were D_1: basal diet + mineral mixture without Zn; D_2: D_1 + 15ppm inorganic Zn; D_3: D_1 + 15ppm organic Zn; D_4: D_1 + 7.5ppm organic Zn; D_5: D_1 + 0.3ppm nano Zn; D_6: D_1 + 0.06ppm nano Zn; D_7: D_1 + 0.03ppm nano Zn. Blood samples were collected from four birds of each group on 21st and 42nd day of the study. The different serum biochemical indices were spectrophotometrically measured using the chemical kits prepared by Crest Biosystems. The lowest serum cholesterol was found in group D_6 which differed significantly ($P<0.05$) from D_1 and D_7. The highest level of SGPT was found in D_7 which varied significantly ($P<0.05$) from D_1, D_2 and D_4 and the lowest level of SGPT was observed in group D_1 which varied significantly ($P<0.05$) with all the other treatments except D_2. The highest average level of ALP was observed in treatment D_5 which varied significantly ($P<0.05$) with all the other treatments except D_6 whereas its lowest level was observed to be present in D_1 which was significantly ($P<0.05$) different from all the remaining dietary groups. Organic and nano zinc supplemented @ 15 ppm and 0.06 ppm, respectively to the basal diet altered the serum cholesterol, SGPT and ALP levels significantly ($P<0.05$).

INTRODUCTION

Zinc (Zn) is an indispensible trace mineral for the over all functioning of the animal body (McDowell, L.R., 2003). It influences the immune system, nucleic acid synthesis, cell proliferation, protein synthesis, and enzymic activities in the living system (Ferreira *et al.*, 2002). NRC (1994) estimated the Zn requirement for broiler chickens to be 40 mg/kg in the diet. Supplementation of Zn in its inorganic or chelated form marginally improved the cholesterol, glucose, albumin and total protein levels whereas significantly reduced the level of SGOT and SGPT (Shamsudeen, P. and Shrivastava, H. P., 2013). Herzig *et al.* (2009) also observed a significant decrease of plasma cholesterol when broilers were fed with high amounts of zinc in diet. In recent years, the use of nano-scale materials as nanoparticles of oxidized elements like zinc oxide nanoparticles (ZnO NPs) are being used in different fields of applied sciences (Handy *et al.,* 2008). ZnO NPs can be effectively used as feed additive in the poultry diets. Due to their extreme small size and unique physical properties, the nanoparticles are likely to be different when compared to the inorganic and organic forms of zinc. Nanoparticles can effectively fulfill the mineral requirements in the animal body, promote growth rate and feed efficiency (Oberdörster, G. *et al.,* 2005). As a feed additive, these are expected to have better bioavailability, small dose rate and stable interaction with other components.

The aim of this comparative study was to evaluate the effect of inorganic, organic and nano zinc supplemented diets on different blood metabolites in broiler chickens.

MATERIALS AND METHODS

A total number of two hundred eighty day old unsexed broiler chicks were weighed, wing banded and distributed randomly into seven dietary treatment groups with two replicates having 20 chicks in each replicate. Seven dietary treatments (D) were: D_1: Basal diet + mineral mixture without any zinc, D_2: D_1 + 50% of Zn present in basal diet through inorganic zinc ($ZnSO_4$) [15ppm], D_3: D_1 + 50% of Zn present in basal diet through organic zinc (Zn-Met) [15ppm], D_4: D_1 + 25% of Zn present in basal diet through organic zinc (Zn-Met) [7.5ppm], D_5: D_1 + nano ZnO @ 1/100th of Zn content in basal diet [0.3ppm], D_6: D_1 + nano ZnO @ 1/500th of Zn content in basal diet [0.06ppm], D_7: D_1 + nano ZnO @ 1/1000th of Zn content in basal diet [0.03ppm]. Basal diet for broiler starter and finisher was prepared as per BIS (1992) specification. The broilers were reared in deep litter system and fed with ad libitum feed and water for 42 days. Experimental feed samples were analyzed for proximate composition according to the AOAC (Association of official Analytical Chemists, 2000). The ingredient and proximate composition of the experimental basal diet for the birds is presented in Table 1. Zinc concentration of the basal diet was estimated to be 30 ppm by atomic absorption spectrophotometer.

Blood samples were collected from four birds of each dietary group on 21st and 42nd day of the experiment. Birds were bled by wing vein puncture using sterile syringes and needles and 3 ml of blood was collected using a 24 gauge needle with

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minimum disturbance. The blood samples after collection were transferred into sterile tubes without any addition of anticoagulant and kept for 3 hours in slanting position. Samples were centrifuged at 5000 rpm for 10 minutes at 4ºC. Sera were collected by one ml auto-pipette. The collected sera samples were stored in deep freeze at -20°C in properly capped and labeled tubes for serum biochemical studies. Biochemical parameters viz., glucose, cholesterol, triglyceric acid, total protein, albumin (A), globulin (G), A/G ratio, urea, Blood urea nitrogen (BUN), Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxalate transaminase (SGOT), Alkaline phosphatase (ALP), calcium and phosphorus were estimated by atomic absorption spectrophotometry using the kits prepared by Crest Biosystems®.

Table 1 Ingredients and proximate composition of broiler basal ration on dry matter basis

Ingredients	Starter (%)	Finisher (%)	Proximate composition	Starter mash	Finisher mash
Maize	57	64	Crude protein	23.02	20.04
Soyabean meal	38	30	Ether extract	4.94	5.06
De oiled rice bran	2	3	Crude fibre	3.95	3.7
Mineral mixture#	2.7	2.7	Total ash	7.78	7.94
Common salt	0.3	0.3	Acid insoluble ash	3.17	3.2
Total	100	100	Nitrogen-free extract	60.29	63.27
Feed additives	0.09	0.04	Calcium	1.87	1.84
			Total phosphorus	0.49	0.52
			ME (kcal/kg)*	2800	2900

Mineral mixture without zinc * Calculated value

Statistical Analysis

Data obtained from the experiment were subjected to statistical analysis wherever required. Analysis of Variance was obtained according to the method of Snedecor and Cochran (1998).

RESULTS

Serum biochemical parameters viz., glucose, cholesterol, triglyceric acid, total protein, albumin, globulin, urea, BUN, SGPT, SGOT, ALP, calcium and phosphorus of six week old broiler birds are presented in the Table 2. The levels of glucose, triglyceric acid, total protein, urea, albumin, globulin, BUN, SGOT, Ca and P in blood serum of the broiler birds varied insignificantly (P>0.05) between the treatments whereas, cholesterol, SGPT and ALP levels in the blood serum showed significant (P<0.05) differences. Graph 1. shows significant variations (P<0.05) in the serum cholesterol, SGPT and ALP profiles of broiler birds under different dietary treatments The highest level of serum cholesterol was observed in group D_1 (98.27 ± 4.73 mg/dl) and it was found to be differed significantly (P<0.05) from D_3, D_5 and D_6. The lowest serum cholesterol was observed in group D_6 (82.53 ± 4.69 mg/dl) which was observed to be differed significantly (P<0.05) from D_1 and D_7. The highest level of SGPT was found in treatment D_7 (15.55 ± 1.34 U/L) which varied significantly (P<0.05) from D_1, D_2 and D_4 and the lowest level of SGPT was observed in group D_1 (8.09 ± 0.79 U/L) which varied significantly (P<0.05) with all the other treatments except D_2. The highest average level of ALP was observed in treatment D_5 (134.21 ± 5.30 U/L) which varied significantly (P<0.05) with all the other treatments except D_6 whereas its lowest level was observed to be present in treatment D_1 which was significantly (P<0.05) different from all the remaining treatment groups.

DISCUSSION

The serum glucose, total protein, urea, blood urea nitrogen, albumin, globulin, albumin and globulin ratio, SGOT, calcium and phosphorus levels of all the treated groups of broiler birds showed no significant variations. Parák and Straková (2011) reported non-significant levels of serum glucose, serum calcium and phosphorus while comparing feeding of inorganic with organic zinc in breeding cocks.

Idowu *et al.* (2011) also reported that serum glucose levels between control and zinc-proteinate groups did not differ significantly. An experiment on feeding of organic zinc in broilers showed non-significant variation in serum albumin level Feng *et al.* (2010). In contrast to these results, Al-Daraji and Amen (2011) reported significantly (P<0.05) higher levels of serum cholesterol, serum calcium and phosphorus on increasing zinc concentration in the diet by addition of 100 mg pure zinc/ kg of diet than that of control (basal diet) in broiler breeders from 58-66 weeks of age.

In our study, the serum cholesterol, SGPT and ALP levels of all the treated groups of broiler birds differed significantly (P<0.05). A significant variation in the serum cholesterol level of broiler birds was found in case of zinc supplemented groups than the control. Herzig *et al.* (2009) proved that there was a significant decrease of plasma cholesterol when broilers were fed with high amounts of zinc in diet. It was also observed by Parák and Straková (2011) while comparing feeding of inorganic with organic zinc in breeding cocks. The plasma cholesterol levels were influenced by ZnO supplementation when fed 80 mg/day, either alone or in combination with copper or vitamins (Gensler *et al.*, 2002). Zinc showed an anti-atherogenic effect in hypercholesterolemic rabbits (Ren *et al.*, 2006; Rashtchizadeh *et al.*, 2008). Mice with zinc-deficient diets exhibited increased cholesterol and triglycerides levels in their blood plasma (Reiterer *et al.*, 2005). Bolkent *et al.* (2006) proved the protective effect of zinc supplementation on lipid metabolism indices in laboratory rats. Zinc deficiency caused increased plasma lipid levels in LDL recipient mice. Our results along with the different research findings confirm the positive impact of zinc on lipid metabolism. Aksu *et al.* (2010) also reported decreased total and LDL cholesterol, and increased HDL cholesterol in the blood plasma of chickens, when the feed mixtures were supplemented with organic complexes of zinc, copper and manganese. However, Kucuk *et al.* (2008) reported no significant changes in the total cholesterol, triglycerides and glucose levels when supplementing 30 mg of Zn per 1 kg of a feed mixture. The serum SGPT level of organic zinc fed birds were found to be significantly higher than the inorganic zinc fed and control groups.

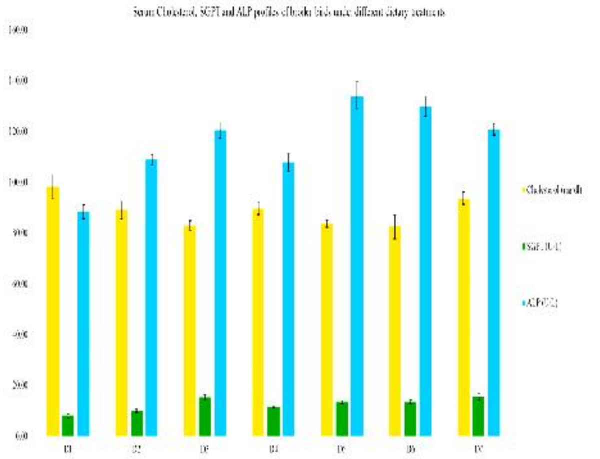
A significant difference in SGPT level was found in birds of D_3 and D_4, fed with two different levels of Zn-Met. Osman and Ragab (2007) also observed significant effects (P<0.05) of Zn-Met supplemented @ 30 ppm, 40 ppm and 50 ppm on serum

Table 2 Serum biochemical profile of the broiler birds under different dietary treatments

Parameters	Treatments							P value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	
Glucose (mg/dl)	256.29±15.37	250.95±5.47	244.60±4.91	252.70±9.02	247.01±5.61	241.26±8.41	253.18±5.89	0.877
Cholesterol (mg/dl)	98.27^{a} ±4.73	89.09^{abc} ±3.48	83.06^{c} ±1.92	89.84^{abc} ±2.52	83.65^{c} ±1.44	82.53^{c} ±4.69	93.71^{ab} ±2.24	0.017
Triglyceric acid (mg/dl)	69.33±1.70	65.90±2.87	62.45±1.93	67.53±2.45	64.75±3.10	63.10±3.38	67.53±4.06	0.609
Total Protein (g/dl)	2.68±0.16	2.76±0.05	3.15±0.20	3.10±0.22	2.95±0.06	3.20±0.06	3.03±0.09	0.096
Albumin (g/dl)	1.80±0.04	1.87±0.09	1.90±0.07	2.13±0.08	1.77±0.14	2.00±0.06	1.95±0.04	0.081
Globulin (g/dl)	0.87±0.19	0.89±0.12	1.25±0.17	0.97±0.17	1.17±0.09	1.20±0.09	1.08±0.09	0.344
A/G ratio	2.52±0.76	2.32±0.53	1.60±0.20	2.36±0.31	1.56±0.26	1.70±0.17	1.85±0.16	0.458
Urea (mg %)	8.41±0.25	8.65±0.10	8.67±0.27	8.63±0.36	8.51±0.18	9.13±0.25	8.68±0.16	0.497
BUN (mg %)	1.96±0.16	2.05±0.38	2.34±0.08	2.13±0.20	2.24±0.14	2.27±0.12	2.04±0.13	0.791
SGPT (U/L)	8.09^{f} ±0.79	10.02^{ef} ±0.58	15.22^{ab} ±1.01	11.38^{cde} ±0.29	13.40^{abcd} ± 0.66	13.49^{abc} ±0.80	15.55^{a} ±1.34	1.76E-05
SGOT (U/L)	102.72±1.84	106.88±1.62	108.85±1.37	105.07±2.16	102.14±2.30	102.40±2.61	101.40±1.89	0.119
ALP (U/L)	88.51^{f} ±2.80	109.01^{cde} ±2.08	120.41^{bcd} ±3.02	107.79^{e} ±3.52	134.21^{a} ±5.30	130.04^{ab} ±3.97	120.84^{bc} ±3.97	9.54E-08
Ca (mg/dl)	7.82±0.24	8.41±0.57	9.89±0.32	8.64±0.49	8.34±0.58	8.39±0.33	8.08±0.81	0.177
P (mg/dl)	3.37±0.13	4.17±0.33	4.26±0.43	4.02±0.16	3.84±0.31	3.33±0.06	3.61±0.16	0.095

Values bearing different superscripts in a row differ significantly (P<0.05)

SGPT level. The ALP level in the serum of zinc supplemented birds was found to be significantly different (P<0.05) from the control. Idowu *et al.* (2011) also observed significant difference in the levels of serum protein, uric acid, SGOT and ALP and serum zinc concentrations between control and zinc proteinate groups with higher levels in zinc proteinate and reported that due to zinc binding capacity of serum, alkaline phosphate acts as good indicator of zinc status. Al-Daraji and Amen (2011) reported significantly higher levels of serum protein and ALP levels on increasing zinc concentration in the diet by addition of 100 mg pure zinc/ kg of diet than that of control (basal diet) in broiler breeders from 58-66 weeks of age.



Graph 1. Significant variations (P<0.05) in the serum cholesterol, SGPT and ALP profiles of broiler birds under different dietary treatments

The increase in ALP level on zinc supplementation might be due to increase in corticosteroid hormone secretion, epinephrine and nor-epinephrine (Al-Daraji and Amen, 2011). In contrast to this, non-significant level of serum ALP in organic zinc fed groups was reported by Parák and Straková (2011). As far as groups of zinc nanoparticles fed birds are considered, a significant difference was observed (P<0.05) in the levels of serum cholesterol, SGPT and ALP than the inorganic and organic zinc supplemented groups.

Ahmadi (2009) also observed different serum biochemical parameters of broiler chicks such as SGPT, SGOT, ALP, total protein, albumin, gamma-globulin, triglyceride, and cholesterol were significantly affected (P<0.05) when their diets were supplemented with silver nano particles. In his report it was mentioned that these effects might be related to oxidative stress that caused peroxidation of fat and release of free radicals in the body. These reasons can be possibly assumed to be responsible for the significant differences in the levels of different serum metabolites of nano zinc fed birds.

CONCLUSION

Zinc plays an important role in improving different serum metabolite levels. ZnO NPs and Zn-Met were found to be more effective when compared to $ZnSO_4$ on the serum biochemical indices. It can be concluded that organic and nano zinc supplemented @ 15 ppm and 0.06 ppm, respectively to the basal diet of broiler birds altered the serum cholesterol, SGPT and ALP levels significantly (P<0.05) when compared to its inorganic form. Further study is needed to explore its metabolic pathway, absorption and assimilation mechanism and ideal dose rate to supplement in poultry ration.

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