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EFFECT OF HIGH LEVELS OF ZINC ON PLASMA METALLOENZYMES ACTIVITY AND BLOOD PARAMETERS IN BROILER CHICKS

SUMMARY

Alkaline phosphatase is a metalloenzyme containing approximately 0.15 % of zinc. One hundred and forty-four one-day-old Ross 308 broiler chicks were used in the experiment. Three zinc levels (40, 120 and 200 mg/kg) were added to the basal diet to establish the treatments. On d 21 and 42 average body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were obtained by pen. At 42 d heterophil/lymphocyte ratio (H/L), an indicator of stress in birds, were counted and also plasma was used for measurement of plasma minerals concentration (Ca, P, and Cu), cholesterol, and serum enzyme activity of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) by an automated chemistry analyzer. No dietary treatment significantly altered ADFI, BWG and FCR at days 21 and 42. Both 120 and 200 mg Zn/kg diet resulted in a significant reduce in H/L ratio ($p < 0.05$). Adding additional Zn resulted in significant decrease of blood P, Cu and cholesterol concentration ($p < 0.05$). Supplementation the diet of broiler chicks with additional levels of zinc resulted in significant ($p < 0.05$) increase in blood plasma ALP and LDH. The overall results of this study show that levels of 120 and 200 mg Zn/kg diet could be effective in decrease of H/L ratio and cholesterol and increase of ALP and LDH without any harmful effect on performance traits.

Keywords: alkaline phosphatases, Lactate dehydrogenase, Performance, plasma minerals, zinc

INTRODUCTION

The alkaline phosphatases (AP) are a class of cell-surface zinc metalloenzymes that hydrolyse phosphate ester groups at an alkaline pH in vitro (Bakst and Akuffo, 2007). Alkaline phosphatase plays a key role not only in calcification but also in bone resorption (Russell et al, 1969). Mathies (1958) studied a highly purified enzyme preparation from swine kidney and found that alkaline phosphatase is a metalloenzyme containing approximately 0.15 % of zinc. The activity of the enzyme in serum is decreased in patients suffering from Zn deficiency due to acrodermatitis enteropathica and rises during oral Zn therapy (Neldner and Hambidge, 1957). Lactate dehydrogenase (LDH) is an oxidoreductase which takes part in the process of the carbon hydrates glycolysis.

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Extracellular activity of this intracellular enzyme is increased under the condition of oxidative stress, due to the integrity of cell membrane destroying in the course of lipid peroxidation. This leads to an increase of lactic acid concentration (Jovanovic et al, 2010). Wang et al (2003) found out that the addition of zinc enriched the serum ALP and LDH activity in the piglets. In other hand, various studies conducted in different animal species have shown that Zn is antagonistic to copper (Latimer et al, 1989) calcium and phosphorus (Greger, 1989). It has been found that the absorption of Cu by the rat is reduced by Zn administration. Increase in liver Zn content causes a redistribution of hepatic Cu, with an increase in the amount bound to metallothionein, which is thought to be involved in the storage and detoxication of Cu and other heavy metals (Bremner et al, 1976). Also Sahin et al (2001) reported that dietary chromium and zinc decreased serum cholesterol concentration. The present study was conducted to evaluate if the high levels of zinc have effect on plasma metalloenzymes activity, copper, calcium, phosphorous and cholesterol in broiler chicks.

MATERIAL AND METHODS

Birds and treatments - One hundred and forty-four one-day-old Ross 308 broiler chicks were used in the experiment. The study was carried out according to a completely randomized design, with three dietary zinc ($Zn-SO_4$) levels and four replicates of 12 birds. The experimental diets were manufactured from a basal diet (Table 1), which was formulated to meet the nutrient requirements of broiler chickens (NRC, 1994). Three zinc levels (40, 120 and 200 mg/kg diet) were added to the basal diet to establish the treatments. Zinc contents in starting, finishing basal diets and potable water were 72, 70 and 5 mg/kg respectively, as measured by atomic absorption analysis. Birds were kept in floor pens, and diets and fresh water were provided *ad libitum* from day one. The lighting program used was 24 hours of artificial light during the entire experimental period, which lasted 42 days.

Performance parameters - Birds were weighed on d 1, 21 and 42 as a group. Average body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were obtained by pen on d 21 and 42.

H/L ratio - The blood was collected (via wing vein) in tubes containing EDTA as anticoagulant. Heterophil/lymphocyte (H/L) ratios, an indicator of stress in birds, were counted to a total of 100 cells. For each bird two slides were counted and the mean was calculated.

Blood parameters and enzymes activities - At the end of the experimental period (42 d of age), blood samples were collected from wing vein using sterile lancet and centrifuge tubes containing EDTA and then centrifuged at 3000 rpm for 15 min. Plasma was separated and used for measurement of plasma minerals concentration (Ca, P, and Cu), cholesterol, and serum enzyme activity of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). The experiments were performed by an automated chemistry analyzer of Zest Shimi Kit (Ziest Chem., Diagnostica, and Cat No.10-508. 5256).

Table 1.: Ingredients and calculated composition of the starter and finisher diets.

Ingredients	Starter (%)	Finisher (%)
Corn	53.55	59.57
Soybean meal 44%CP	38.93	33.34
Monodibasic Phosphate	1.43	1.21
Limestone	1.35	1.38
Vegetable oil	3.84	3.51
Salt	0.41	0.43
DL-methionine	0.207	0.214
L-Lysine HCl	0.129	0.197
Choline HCl 60%	0.06	0.05
Mineral-vitamin premix ¹	0.1	0.1
Total	100	100
<u>Calculated Nutrients</u>		
Crude protein %	22	20
ME, kcal/kg	3,050	3,100
Calcium, %	0.9	0.85
Available phosphorus, %	0.4	0.35
Digestible Lys, %	1.15	1.07
Digestible Met., %	0.49	0.48
Digestible Met+Cys %	0.81	0.77
Choline, mg/kg	1,420	1,300

¹Composition (per kg): manganese, 75,000 mg; iron, 50,000 mg; copper, 8,000 mg; iodine, 750 mg; vitamin A, 8,000 kIU; vitamin D3, 2,000 kIU; vitamin K3, 1,800 mg; vitamin B1; 1,800 mg; vitamin B2, 6,000 mg; vitamin B6, 2,800 mg; vitamin B12, 12,000 µg; pantothenic acid, 10,000 mg; niacin, 40,000 mg; folic acid, 1,000 mg; biotin, 60,000 µg; selenium, 0.3 mg/kg. Basal diets Zn measured by atomic absorption spectrometer and Zinc contents were 74 and 72 mg/kg in starting and finishing basal diets.

Statistical Analysis - Statistical analyses were conducted using the ANOVA general linear models procedure of SAS software (1997). When ANOVA revealed significant effects, means were separated by Duncan's multiple range tests. The values were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The influence of dietary treatments on performance traits are shown in table 2. No dietary treatment significantly altered ADFI, BWG and FCR at days 21 and 42. Lack of effect of dietary zinc on ADFI, BWG and FCR was in accordance with Kidd et al (1994) that observed no differences in BW and feed conversion of broilers supplemented with 140 or 164 µg zinc/g diets. Also these results was similar to observations by Pimental et al (1991) that found no

differences in feed intake and growth of broilers fed up to 88 μg zinc/g diet. These results could be due to reallocation of energy toward immune development resulting in inefficient nutrient utilization for growth.

Table 2.: Average daily feed intake (ADFI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers fed different levels of zinc.

Treatment	0-21			0-42		
	ADFI (g/bird)	BWG (g/bird)	FCR (g/g)	ADFI (g/bird)	BWG (g/bird)	FCR (g/g)
40 mg	30.33	18.18	1.67	67.15	36.13	1.86
120 mg	29.78	18.56	1.6	63.95	35.9	1.87
200 mg	31.35	19.48	1.68	70.4	36.3	1.94
MSE	1.33	1.097	0.074	5.22	2.58	0.01

Figure 1 shows the effect of different treatments on H/L ratio. Adding Zn to diet had a significant effect on H/L ratio ($p < 0.05$). Although there was no significant difference between the levels of 120 and 200 mg Zn/kg diet, but both levels decreased H/L ratio in compare with the control suggesting that supplementation of Zn was useful in reducing stress. Sunder et al (2008) reported that supplementation of Zn was useful in reducing H/L ratio in young broilers. Zinc is required for the normal development of lymphocytes, peripheral T-cell numbers and T-cell helper functions (Kidd et al, 1996). Probably additional Zn was efficient to support optimum development of lymphocytes, which alleviated stress, as observed from the present study.

The effects of different levels of Zn on blood parameters of Ca, P, Cu and cholesterol are presented in table 3. Increase of zinc level resulted in significant decrease of blood P, Cu and cholesterol concentration ($p < 0.05$) whereas did not significantly affect on blood Ca amount. Albeit reduction of blood P and Cu in level of 120 mg was much more lower than level of 200 mg. Parak and Strakova (2011) reported a significant decrease in the level of total cholesterol in the blood plasma of breeding cocks fed high levels of Zn, whereas blood plasma Ca in the experimental groups did not reveal any significant changes. Decreasing of P and Cu can be due to depression in their uptake in the stomach and duodenum and higher fecal excretion of them because of antagonistic interactions between Zn and other minerals.

In figure 2 blood serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) of all groups are given. Results of this experiment revealed that supplementation the diet of broiler chicks with additional levels of zinc (120 and 200 mg Zn/kg diet) resulted in significant ($p < 0.05$) increase in blood plasma ALP and LDH. Increase in blood plasma ALP and LDH was agree with the finding of Al-Daraji and Amen (2011) that showed addition of zinc to the diet of broiler breeder chickens (75 and 100 mg Zn kg diet) resulted in increase in blood ALP activity in comparison with control group. Also Fu-yu et

al (2007) reported the increase in the activity of LDH with zinc additive in serum of bulls. This increase may be attributed to the role of zinc in sex and steroid hormones synthesis and its action on the metabolism of sex steroids together with prostaglandins (Brown and Pentland, 2007). Increase in the corticosteroids hormones secretion, epinephrine and norepinephrine lead to increase of ALP activity, but mechanism is not quietly clear (Al-Darraji, 2008).

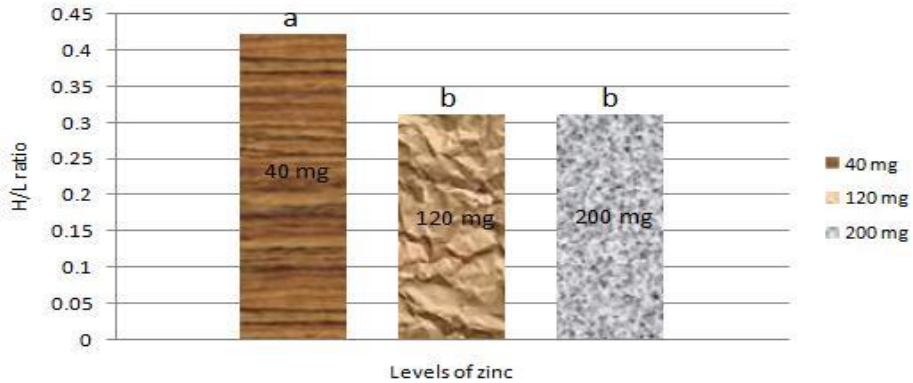


Figure 1.: H/L ratio of broilers fed different levels of zinc^{a,b} Columns that do not share the same letters differ significantly ($p < 0.05$)

Table 3.: Effects of different treatments on blood Ca, P, Cu and cholesterol in broilers.

Treatment	Ca	P	Cu	Cholesterol
40 mg	9.45	6.43 ^a	0.38 ^a	138.75 ^a
120 mg	9.45	6.2 ^b	0.33 ^b	127.75 ^b
200 mg	9.35	5.93 ^c	0.29 ^c	113.25 ^c
MSE	0.15	0.08	0.018	4.45

^{a,b,c} Columns that do not share the same letters differ significantly ($p < 0.05$)

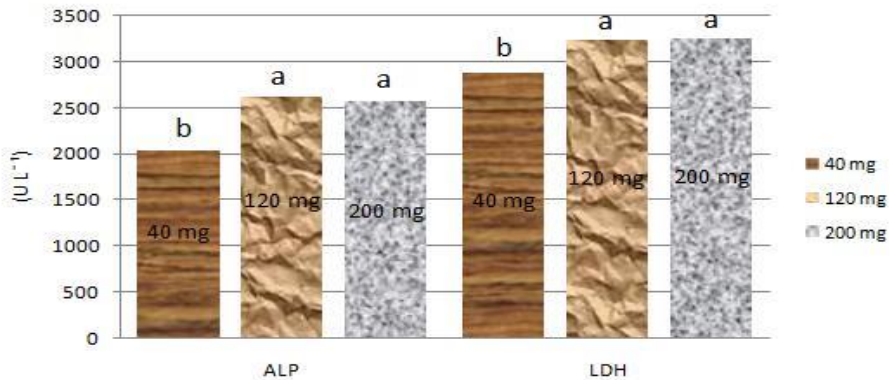


Figure 2.: ALP and LDH in plasma of broilers fed different levels of zinc^{a,b} Columns that do not share the same letters differ significantly ($p < 0.05$)

CONCLUSIONS

The overall results of the current study showed that levels of 120 and 200 mg Zn/kg diet could be used in decrease of H/L ratio and cholesterol and increase of ALP and LDH in blood of broiler chicks without any decline in performance traits. Also reduction of blood P and Cu, caused by additional levels of zinc, especially 120 mg/kg diet were not so great that could create any problem.

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UTICAJ VISOKIH NIVOVA CINKA NA AKTIVNOST METALOENZIMA PLAZME I PARAMETRE KRVI KOD PILIĆA BROJLERA

SAŽETAK

Alkalna fosfataza je metaloenzim koji sadrži otprilike 0,15 % cinka. U eksperimentu je korišćeno 308 pilića Ross brojlera starih 141 dan. Tri nivoa cinka (40, 120 i 200 mg/kg) dodavani su baznoj ishrani kako bi se utvrditil tretmani. Na dan 21 i 42, prosječni prirast tjelesne mase (BWG), prosječan dnevni unos hrane (ADFI) i odnos konverzije hrane (FCR) izračunati su ručno. Na dan 42, izračunat je odnos heterofila/limfocita (H/L), koji je indikator stresa kod ptica, a plazma je iskorišćena za mjerenje koncentracije minerala u plazmi (Ca, P, and Cu), holesterola, i aktivnosti enzima seruma alkalne fosfataze (ALP) i laktat dehidrogenaze (LDH) automatskim hemijskim analizatorom. Ni jedan tretman kroz ishranu nije značajno izmijenio ADFI, BWG i FCR u dane 21 i 42. Ishrana koja je sadržala i 120 i 200 mg Zn/kg imala je za rezultat značajno smanjenje odnosa H/L ($p < 0.05$). Dodavanje dodatnog Zn za rezultat je imalo značajno smanjenje koncentracija P, Cu i holesterola u plazmi ($p < 0.05$). Dopuna ishrane pilića brojlera dodatnim nivoima cinka za rezultat je imala značajno ($p < 0.05$) povećanje ALP i LDH u plazmi krvi. Generalno, rezultati ove studije pokazuju da bi nivoi od 120 i 200 mg Zn/kg u ishrani mogli biti efikasni u smanjenju odnosa H/L i holesterola i rasta ALP i LDH bez ikakvih štetnih efekata na rezultate uzgoja.

Ključne riječi: alkalna fosfataza, laktat dehidrogenaza, rezultati uzgoja, minerali plazme, cink