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EFFECT OF ZINC ON METALLOENZYMES ACTIVITY AND SOME BLOOD PARAMETERS IN BROILER CHICKS VACCINATED AGAINST COCCIDIOSIS

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In this study one-day-old broiler chicks were used with three dietary zinc doses (40, 120 and 200 mg/kg). On the 7th day, all broilers were vaccinated against coccidiosis (Livacox Q®, Merial) via oral administration. At the end of the experimental period, concentration of plasma minerals (Ca, P, and Cu), cholesterol, and serum enzyme ALP and LDH were measured. Adding additional Zn resulted in significant decrease of blood P, Cu and cholesterol concentration ($p < 0.05$). However it did not significantly affect blood Ca amount. In addition, the supplementation of the diet of broiler chicks by different levels of zinc resulted in significant ($p < 0.05$) increase in blood plasma ALP and LDH.

Blood parameters – broiler – coccidiosis – metalloenzymes – zinc

Մեր կողմից կատարված հետազոտությունում մեկ օրական բրոյլեր ճտերին կերակրել ենք ցինկ պարունակող կերային հավելումներով՝ միկրոտարրի 40, 120 և 200 մգ/կգ բաժնեչափով: 7-րդ օրը բոլոր ճտերը վակցինացվել են կոկցիդիոզի նկատմամբ վակցինայով՝ ներքին ճանապարհով: Փորձաշրջանի վերջում արյան (Livacox Q®, պլազմայում որոշվել է հանքային տարրերի՝ կալցիումի, ֆոսֆորի և պղնձի, խոլեստերինի պարունակությունը, ինչպես նաև հիմնային ֆոսֆատազ և լակտատդեհիդրոգենազ ֆերմենտների ակտիվությունը: Ցինկի հավելումների կիրառումը հանգեցրել է արյան մեջ ֆոսֆորի, պղնձի և խոլեստերինի պարունակության զգալի նվազմանը, մինչդեռ դա գրեթե չի ազդել արյան մեջ կալցիումի պարունակության վրա: Ցինկի հավելումները բերել են նաև արյան պլազմայում հիմնային ֆոսֆատազ և լակտատդեհիդրոգենազ ֆերմենտների ակտիվության զգալի բարձրացմանը:

Արյան ցուցանիշներ – բրոյլեր – կոկցիդիոզ – մետաղաֆերմենտ – ցինկ

В проведенных нами исследованиях суточным цыплятам скормливали цинк в дозах 40, 120 и 200 мг/кг. На 7-й день все цыплята были вакцинированы против кокцидиоза вакциной ®, введенной внутрь. К концу экспериментов определяли содержание минеральных элементов (кальция, фосфора и меди), холестерина и активность ферментов – щелочной фосфатазы и лактатдегидрогеназы в плазме крови. Добавление цинка в рацион приводит к существенному снижению концентрации фосфора, меди и холестерина в плазме крови, существенно не влияя на содержание в крови кальция. Добавление цинка также приводит к существенному повышению активности ферментов – щелочной фосфатазы и лактатдегидрогеназы в плазме крови.

Показатели крови – бройлер – кокцидиоз – металлоэнзимы – цинк

The alkaline phosphatases (AP) are a class of cell-surface zinc metalloenzymes that hydrolyzes phosphate ester groups at an alkaline pH in vitro [3]. Mathies studied a highly purified enzyme preparation from swine kidney and found that alkaline phosphatase is a metalloenzyme containing approximately 0.15% of zinc [8]. The activity of the enzyme in serum is decreased in patients suffering from Zn deficiency due to acrodermatitis enteropathica and has increased during oral Zn therapy [9]. Lactate dehydrogenase (LDH) is involved in the process of the carbon hydrates glycolysis. Extracellular activity of this intracellular enzyme is increased under the condition of oxidative stress. Wang et al. found out that the addition of zinc enriched the serum ALP and LDH activity in the piglets [12]. Also various studies conducted in different animal species have shown that Zn is antagonistic to copper [7] calcium and phosphorus [6]. The present study was conducted to evaluate if different levels of zinc have effect on plasma metalloenzymes activity, copper, calcium, phosphorous and cholesterol in broiler chicks during a coccidiosis challenge.

Materials 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 (tab.1), which was formulated to meet the nutrient requirements of broiler chickens (NRC, 1994). Three zinc levels (40, 120 and 200mg/kg) 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. On the 7th day all broilers were vaccinated against coccidiosis (®, Merial) via oral administration. 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.

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
Calculated Nutrients		
Crude protein %	22	20
ME, kcal/kg	3,050	3,100
Calcium, %	0.9	0.85
Available phosphorus, %	0.4	0.35
Sodium, %	0.2	0.21
Chloride, %	0.27	0.29
Digestible Lys, %	1.15	1.07
Digestible Met., %	0.49	0.48
Digestible Met+Cys %	0.81	0.77
Digestible Thr, %	0.78	0.71
Choline, mg/kg	1,420	1,300

1 - Composition (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.

Blood parameters and metalloenzymes activities.

At the end of the experimental period (42 days 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).

Statistical Analysis.

Experimental data normality was verified, and then data were submitted to analysis of variance, using SAS (1997) software package. Means were compared using Duncan multiple test.

Results and Discussion. *Blood parameters (Ca, P, Cu and cholesterol).* The effects of different levels of Zn on blood parameters of Ca, P, Cu and cholesterol are presented in tab. 2. Adding additional Zn resulted in significant decrease of blood P, Cu and cholesterol concentration ($P < 0.05$) whereas did not affect significantly on blood Ca amount. Albeit it is notable that decrease of blood P and Cu in level of 120 mg was much more lower than level of 200 mg. Parak and Strakova 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 [10]. 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.

TABLE 2. Effects of different levels of zinc on blood Ca, P, Cu and cholesterol of broilers.

Zn mg/kg	Ca mg/dL	P g/dL	Cu μg/mL	Cholesterol mg/dL
40	9.48	6.53 ^a	0.39 ^a	137.5 ^a
120	9.48	6.2 ^b	0.34 ^b	128.25 ^b
200	9.35	5.93 ^c	0.29 ^c	111.25 ^c
MSE	0.15	0.08	0.018	4.45

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

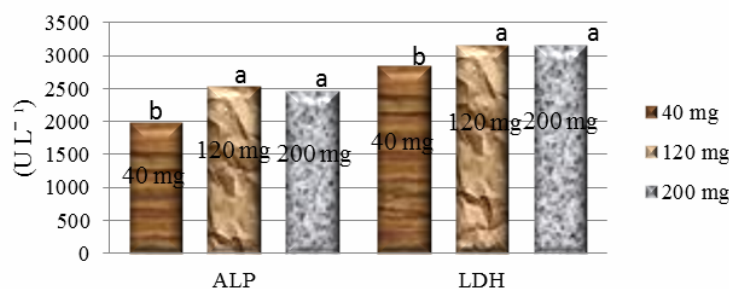


Fig.1. Blood metalloenzymes activity of broilers fed different levels of zinc

Metalloenzymes activities. In Fig. 1 the values of blood serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) for all groups are presented. Results of this experiment revealed that supplementation the diet of broiler chicks with different levels of zinc (120 and 200 mg kg⁻¹) resulted in significant ($P < 0.05$) increase in blood plasma ALP and LDH. It was agree with the finding of Al-Daraji and Amen that showed addition of zinc to the diet of broiler breeder chickens (75 and 100 mg/kg) resulted in increase

in blood ALP activity in comparison with control group [1]. Also Fu-yu et al. reported the increase in the activity of LDH with zinc additive in serum of bulls [5]. 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 [4]. Increase in the corticosteroids hormones secretion, epinephrine and norepinephrine lead to increase of ALP activity, but mechanism was not quietly clear [2].

The overall results of this study showed that although supplementation the diet with additional Zn decreased the amount of blood cholesterol and increased metalloenzymes, but at the same time it has resulted to significant decrease in blood P and Cu that can be harmful for the birds. Albeit level of 120 mg Zn kg⁻¹ decreased blood P and Cu is much more lower than other treatment and may be considered as a better level of Zn to be used in broiler diet in compared with 200 mg kg⁻¹.

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