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# Effect of Inorganic Zinc Supplement on Activity of Alkaline Phosphatase Enzyme as an Index of Mucosal Functional in Small Intestine of Male Broilers

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**Abstract:** Alkaline Phosphatases (ALPs) enzyme are constantly creating an alkaline pH by dividing of phosphorus (an acidic mineral) and are greatly distributed in animal and plant tissues. Besides, ALP enzyme as an index of mucosal functional in small intestine of male broilers. Therefore, an experiment was conducted to study the effects of different levels of ZnO supplement on ALP enzyme activity of the small intestine in male broilers from 1 to 42 days. On hundred eight male broiler chicks (*Ross -308 strain*) were randomly assigned into 3 groups with 3 replicates of 12 birds per group. Control group was fed base diet and other groups with the same base diet plus 50 or 100 ppm ZnO. Broilers were slaughtered after 21, 28, 35 and 42 days and different segments of small intestine (at 1,10,30,50,70 and 90% of total length of the small intestine) were taken from each replicates (N=2). Results revealed that intake of ZnO supplement (100 ppm) significantly increased ALP enzyme activity at the age of 21,28,35 and 42 days at proximal small intestine mucosa in comparison with other groups ($P<0.05$).

**Key words:** Broiler · Alkaline Phosphatase · Intestine · ZnO

## INTRODUCTION

Zinc (Zn) has been known to be an essential nutrient for animals for many years. The recommended Zn requirement for broilers is 40 mg/kg diet for almost all countries and no difference for different stages is reported [1]. Moreover, It is required for skeleton development, growth, skin health and appetite, reproduction, wound healing, immune competence and many biochemical processes [2]. Cunningham- Rundles *et al*. [3], indicated that Zn acts as antioxidant reducing the cell membrane damage due to radicals, which in turn according to Powell, [4] alters the immunological status of the animal. It has been suggested that Zn increases the synthesis of metallothionein, a cysteine-rich protein, which acts as a free radical scavenger [3]. In the other study showed that growth-furthering effects of Zn have been ascribed to effects on intestinal microflora [5]. Zn has multiple important functions because it is a cofactor for >200 enzymes [6]. On the other hand, the digestive enzymes of the small intestinal mucosa play an significant role in the overall digestion process, From among of various enzymes, Alkaline Phosphatases (ALPs) are constantly creating an alkaline pH by dividing of phosphorus as an sacidic mineral [7]. It is exhibited that the type of enzyme is greatly distributed in animal and plant tissues [8]. Moreover, Alkaline Phosphatases (ALPs) are a group of enzymes found chiefly the liver and bone (isoenzyme ALP-1 and isoenzyme ALP-2, respectively). There are also small amounts produced by cells lining the intestines (isoenzyme ALP-3), the placenta and the kidney (in the proximal convoluted tubules), what is measured in the blood is the total amount of ALPs released from these tissues into the blood [7]. Furthermore, it is demonstrated that during foetal life, the ALP activity started to be faintly positive to the 11th day of incubation, becoming incrementally stronger afterwards and after hatching in the small intestine [8]. In the current research, the effect of inorganic zinc supplement on activity of ALP as index of mucosal functional in small intestine of male broilers was investigated.

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Table 1: Calculated analysis of fed diets (based on corn and soybean meal) for all of experimental chickens during rearing period

| Calculated analysis | (1-21 days) | (22-42 days) |
|---|---|---|
| ME kcal/ kg | 2933.60 | 2950.40 |
| Crude protein (%) | 20.63 | 18.44 |
| Calcium (%) | 1.03 | 1.01 |
| Available P (%) | 0.46 | 0.41 |

Calculated analysis is not enough. Table containing the ingerients used in the trial should be mentioned clearly

## MATERIALS AND METHODS

**Chicks and Diets:** One hundred eight 1-day-old male broiler chicks (*Ross- 308 strain)* were randomly assigned to 3 groups consisting of 3 replicates of 12 birds. Utmost care was taken to provide equal physical and environmental housing conditions (namely size of units, light, temperature and aeration). Stainless-steel feeders and plastic waterers were used. Feed and water were supplied *ad libitum*. Experimental diets, formulated according to NRC [9], included following levels of ZnO: A) control diet (no ZnO), B) 50 ZnO ppm C) 100 ZnO ppm. Birds were fed with experimental diet for starter (1-21 d) and grower (22- 42 d) periods (Table 1).

**Sample Collection:** In days 21, 28, 35 and 42 of the rearing period, after 3 hours of fasting, 6 broilers from every group (totally 18 chickens on each day of sampling) which had nearly equal to the average weight of each replicate have been chosen and slaughtered. The abdominal cavity was opened and the entire gastrointestinal tract was removed. The small intestine was isolated and the length of intestine was determined by a graduate ruler. The positions at 1, 10, 30, 50, 70 and 90 % of the length of small intestine for analyzing the ALP enzyme activity were separated with specific scissors (a 8-cm sample was taken). The samples for ALP determination were cut open lengthwise, rinsed carefully with phosphate buffer saline (pH=7), blotted dry, then samples envelop in vacuum packed and stored at -80°C until enzyme analysis.

**Enzyme Assay:** After thawing, all of vacuum packed were opened and then using a sensitive scale, 0.05 gram of the mucosal small intestine was weighed and along with 10 ml liter phosphate buffer saline (pH=7) was formed into a homogenized solution using sonic Vibracell Sonics (*VCX 130 TE USA*) device. The activity of ALP was determined according to the procedure of Dahlqvist and Thamson [10] and Teshfam [11]. For measuring the activity of ALP, It was needed to determine total protein in which (*calorimetric*) method was used [12]. The activity level of ALP enzyme of each sample is divided into the amount of its total protein. Therefore, the activity level of the enzyme, according to the IU /gram protein is researched.

**Statistical Analyses:** Results were statistically analyzed using the linear model of SAS [13] and Multivariate Analysis Variance. Comparative analysis of the average of treatments was performed using Duncan's multifunctional method in the random of 5 percent.

## RESULTS AND DISCUSSION

According to Table 2, adding different levels of ZnO supplement to the diet of broiler chicks at different ages and parts of the small intestine caused variety of influences on the activity of ALP enzyme. Adding ZnO supplement (100 ppm) to the diet of the birds at the age of 21,28,35 and 42 days demonstrates a significant increase in 1% and 10% of small intestine and also at age of 21 and 28 days in 30% of the small intestine in comparison with other groups ($P<0.05$). Also, small intestine ALP values slightly increased in 100 ppm ZnO supplemented group in other ages and parts of small intestine. However, it hadn't significant effect on ALP enzyme activity.

The obtained results indicated that the addition of ZnO supplement (100 ppm) to feed had significant effect on the small intestine ALP activity, such as 21,28,35, 42 days at the proximal of small intestine mucosa. These results possibly because of the effect of Zn on the intestinal microflora (such as inhibit the growth of *coliform bacteria*). This type of bacterium may damage the villi of intestinal mucosa and inhibit the secretion of digestive enzymes [14, 15]. Another opinion about our results, creation a pH optimum between 9.0 and 9.6 to the purpose of ALP enzyme activity by adding Zn supplement to the diet of the broilers. This opinion invigorated with the findings Knits [16]. He reported that the inactivated ALP at pH 4 to 6 is rapidly reactivated on addition of Zn ions. In another study, researchers showed that disaccharidase (sucrose and maltase) activities in cranial small intestine mucosa were greater in pigs fed 100 ppm of Zn than the other groups. On the other hand, they comprehended that pigs fed diets including 100 ppm of Zn had longer villi in the 10% of the length of small intestine than pigs the diet with 2500 ppm added Zn [17]. In contrast, Mavromichalis *et al*. [18], reported that there was no stable effect of Zn supplementation on villus height. In similar research, some researchers reported that

Table 2: Alkaline phosphatase activity between groups in different periods and segments of small intestine in male broiler chicks (IU/g protein)

| | 1 % length of small intestine | | | |
|---|---|---|---|---|
| | 21 Day | 28 Day | 35 Day | 42 Day |
| (0.0 ppm ZnO) Control group | 402.24±36.98[a] | 328.98±76.98[a] | 398.03±52.11[a] | 435.14±63.02[a] |
| 50 ppm ZnO | 497.02±44.00[b] | 412.55±66.23[b] | 400.65±82.94[a] | 433.88±77.22[a] |
| 100 ppm ZnO | 556.01±57.98[c] | 514.09±91.30[c] | 479.02±45.92[b] | 609.19±79.05[b] |
| | 10 % length of small intestine | | | |
| | 21 Day | 28 Day | 35 Day | 42 Day |
| (0.0 ppm ZnO) Control group | 434.01±54.88[a] | 444.67±44.72[a] | 593.92±81.63[a] | 527.73±59.65[a] |
| 50 ppm ZnO | 502.66±69.43[b] | 433.77±79.02[a] | 591.54±80.75[a] | 505.12±63.00[a] |
| 100 ppm ZnO | 589.42±72.09[c] | 594.52±81.09[b] | 663.39±89.94[b] | 615.84±67.73[b] |
| | 30 % length of small intestine | | | |
| | 21 Day | 28 Day | 35 Day | 42 Day |
| (0.0 ppm ZnO) Control group | 223.41±32.41[a] | 265.13±30.09[a] | 325.68±55.32 | 331.96±33.21 |
| 50 ppm ZnO | 239.63±30.81[a] | 258.09±43.66[a] | 315.43±78.94 | 303.53± 39.49 |
| 100 ppm ZnO | 302.49±35.76[b] | 337.17±55.04[b] | 345.76±69.01 | 382.25±40.03 |
| | 50 % length of small intestine | | | |
| | 21 Day | 28 Day | 35 Day | 42 Day |
| (0.0 ppm ZnO) Control group | 213.42±70.92 | 325.18±39.21 | 426.11±81.03 | 321.36±89.04 |
| 50 ppm ZnO | 235.55±69.08 | 300.57±30.06 | 403.07±80.03 | 320.00±77.02 |
| 100 ppm ZnO | 239.41±70.06 | 353.03±55.33 | 462.72±85.05 | 367.61±74.07 |
| | 70% length of small intestine | | | |
| | 21 Day | 28 Day | 35 Day | 42 Day |
| (0.0 ppm ZnO) Control group | 198.31±29.93 | 321.02±47.61 | 204.95±61.11 | 234.04±25.33 |
| 50 ppm ZnO | 211.03±31.03 | 308.09±40.00 | 214.31±68.73 | 219.09±30.54 |
| 100 ppm ZnO | 223.50±35.67 | 344.43±58.07 | 253.26± 59.93 | 278.98±44.19 |
| | 90 % length of small intestine | | | |
| | 21 Day | 28 Day | 35 Day | 42 Day |
| (0.0 ppm ZnO) Control group | 156.32±19.83 | 163.76±15.98 | 199.09±23.44 | 136.32±30.44 |
| 50 ppm ZnO | 176.01±28.56 | 134.22±34.83 | 194.46±26.98 | 152.87±35.99 |
| 100 ppm ZnO | 179.22±39.78 | 181.23±39.60 | 202.37±30.55 | 168.04±40.08 |

Mean ± Standard deviation, Means with different superscripts within the same column and for the same parameter are significant (P<0.05)

adding Zn supplement to diets, resulting in increase of some intestine digestive enzymes, namely ALP [19]. They reported that current results possibly because of the increase of epithelial turnover and decreases death mucosal cell by protective effect of zinc. Also, It is conceivable that Zn supplement reduction in labile active molecules such as iron (redox active molecule) by improving the anti-oxidant defense system. Generally, zinc's anti-oxidant mechanism is divided into two: A. Chronic effects, B. Acute effects. Chronic effect indicates the gradual activity of zinc in the tissue which leads to the stimulation of anti-oxidant compounds such as metallothioneins. In fact metallothioneins take the responsibility for providing the necessary amount of zinc in the process of oxidation and by banding the redox-active transition metals like iron and cupper and preventing them from producing free radicals. Acute effect includes the protection of the sulfhydryl group in proteins or contribution in the decrease of the formation of hydroxyl radicals (OH°) from Hydrogen Peroxide ($H_20_2$). This operation takes place as a result of the completion among Zinc and other redox-active transition metals like iron and cupper in banding sulfhydryl groups [4]. In conclusion, based on the present results and literature data, It was suggested that usage 100 ppm ZnO supplement in broilers diet may promote of mucosal ALP activity and finally, redound to better intestinal digestion and absorption of nutrients.

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