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Effect of Different Levels of Zinc Oxide Supplement on Mucosal Lucine Aminopeptidase Enzyme Activity in Small Intestine of Male Broiler Chicks

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Abstract: Lucine Aminopeptidase (LAP) enzyme is an enzyme responsible for hydrolyzes the smaller peptides and amino acids at the end of the long chain peptide in the small intestine. Therefore, an experiment was conducted to study the effects of different levels of ZnO supplement on LAP 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 LAP enzyme activity at the age of 21and 42 days at proximal small intestine mucosa in comparison with other groups ($p<0.05$).

Key words: Broiler chicks, small intestine, lucine aminopeptidase and 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 (Underwood and Suttle, 1999). Moreover, It is required for skeleton development, growth, skin health and appetite, reproduction, wound healing, immune competence and many biochemical processes (Chunshan *et al*., 2006). In the other study showed that growth-furthering effects of Zn have been ascribed to effects on intestinal microflora (Højberg *et al*., 2005). Zn has multiple important functions because it is a cofactor for>200 enzymes (Sahin and Kucuk, 2003). 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, Leucine Aminopeptidase (LAP) is a brush-border and situsole enzyme which hydrolyzes the smaller peptides and amino acids at the end of the long chain peptide and peptidase activity leads to the digestion of the proteins in the diet (Benajiba and Maroux, 1980). In the current research, the effect of inorganic zinc supplement on activity of LAP as index of mucosal functional in small intestine of male broilers was investigated.

MATERIALS AND METHODS

Chicks and diets: This study was carried out prospectively in an eastern Azerbaijan industrial farm (Northwest of Iran) in May 2008. 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 (1994), 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 h of fasting, 6 broilers from every group (totally 18 chickens on each day of sampling)

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

Calculated analysis	F(22-42 days)	(1-21 days)
ME kcal/ kg	2933.6	2950.4
Calcium (%)	20.63	18.44
Crude Protein (%)	1.03	1.01
Available P (%)	0.46	0 .41

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

	21 Day	28 Day	35 Day	42 Day
1% Length of small intestine				
(0.0 ppm ZnO) Control group	3275.02±146.10	4145.65±135.20	4235.54±162.33	785.21±133.04 [a]
50 ppm ZnO	3321.12±152.33	985.06±142.01	4158.78±165.74	561.20±138.11 [a]
100 ppm ZnO	3423.33±168.71	3900.17±152.11	4239.08±164.07	4621.98±145.9 [b]
10% length of small intestine				
(0.0 ppm ZnO) Control group	3564.87±172.04	4156.55±152.08	4001.31±190.23	4600.01±222.04 [a]
50 ppm ZnO	3256.08±181.504	263.09±142.09	4050.47±199.10	4758.81±250.81[a]
100 ppm ZnO	3856.27±179.11	4289.12±185.11	4089.88±210.01	5000.24±254.04 [b]
30% length of small intestine				
(0.0 ppm ZnO) Control group	4652.07±282.60[a]	3984.00±187.05	4187.45±152.13	3984.11±171.11
50 ppm ZnO	4749.25±275.05 [a]	4029.99±162.11	4226.42±164.04	3894.25±182.02
100 ppm ZnO	5125.01±292.11 [b]	4156.55±189.10	4368.91±173.20	4002.33±184.66
50% length of small intestine				
(0.0 ppm ZnO) Control group	3151.11±126.25	4006.16±122.63	983.90±144.08	4441.01±142.09
50 ppm ZnO	3200.14±132.05	3958.21±155.11	3995.30±152.11	4559±138.11
100 ppm ZnO	3222.10±149.90	3875.44±172.09	4010.11±161.16	367.61±155.52
70% length of small intestine				
(0.0 ppm ZnO) Control group	3551.88±210.01	2955.53±310.14	159.40±290.05	3354.09±325.12
50 ppm ZnO	3623.07±232.42	3121.75±332.05	4228.39±282.06	3321.88±305.45
100 ppm ZnO	3600.95±265.11	3200.06±352.88	4151.81±300.03	3370.07±331.82
90% length of small intestine				
(0.0 ppm ZnO) Control group	4535.31±351.11	4679.12±332.32	4333.80±325.22	3100.77±298.25
50 ppm ZnO	4566.04±359.02	4561.55±300.05	4259.11±315.14	3161.04±290.12
100 ppm ZnO	4571.11±372.05	4490.36±342.14	4459.44±316.10	3225.08±308.05

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

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 LAP enzyme activity were separated with specific scissors (a 8-cm sample was taken). The samples for LAP 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 LAP was determined according to the procedure of Dahlqvist and Thamson (1963), and Teshfam (1984). For measuring the activity of LAP, It was needed to determine total protein in which (*calorimetric*) method was used (Watanaba *et al*., 1986). The activity level of LAP enzyme of each sample is divided into the amount of its total protein. Therefore, the activity level of the enzyme, according to the IU /g protein is researched.

Statistical analyses: Results were statistically analyzed using the linear model of SAS Institute (2000) and Multivariate Analysis Variance. Comparative analysis of the average of treatments was performed using Duncan's multifunctional method in the random of 5%.

RESULTS AND DISCUSSION

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

The obtained results indicated that the addition of ZnO supplement (100 ppm) to feed had significant effect on the small intestine LAP activity, such as 21 and 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 (Gao, 1998; Conway, 1994). On the other hand, researchers 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 (Hedemann *et al*., 2006). In another research conducted by Karamouz *et al*. (2011), on the mucosal enzymes activity in small intestine of male broiler, it was revealed that the zinc oxide supplement

increased the concentration of sucrase in small intestine. In contrast, Mavromichalis *et al.* (2000), reported that there was no stable effect of Zn supplementation on villus height. In similar research, some researchers reported that adding Zn supplement to diets, resulting in increase of some intestine digestive enzymes (Sreedhar *et al*., 2004). 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^o) from Hydrogen Peroxide (H_2O_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 (Powell, 2000). 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 LAP activity and finally, redound to better intestinal digestion and absorption of nutrients.

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