

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

H. Karamouz, J. Ghiasi Ghalehkandi, H. Zadeh Adam Nazhad,
Y. Ebrahim Nezhad and N. Maheri Sis

Department of Animal Science, Islamic Azad University, Shabestar Branch,
53815-159, Shabestar, Iran

Abstract: Sucrase enzyme is an enzyme responsible for digestion and absorption of carbohydrates in the small intestine. Therefore, an experiment was conducted to study the effects of different levels of ZnO supplement on sucrase 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 sucrase 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 chicks, small intestine, sucrase and zno

INTRODUCTION

Zinc (Zn) has been known to be an essential nutrient for animals for many years. Also, Zn has multiple important functions because it is a cofactor for >200 enzymes (Sahin and Kucuk, 2003). It is required for skeleton development, growth, skin growth and integrity, appetite, reproduction, wound healing, immune competence and many biochemical processes. The recommended Zn requirement for broilers is 40 mg/kg diet for almost all countries and no difference for different stages is reported (Bettrger and O'Dell, 1993). Researcher demonstrated that Zn can accumulate in bone, liver, and intestine and subsequently be released for use during a period of Zn deficiency (Emmert and Baker, 1995). Mohanna and Nys (1999) reported increased body weight gain and food intake until the total dietary zinc content raised to 45 mg/kg. In the other study showed that growth-furthering effects of Zn have been ascribed to effects on intestinal microflora (Højberg *et al.*, 2005). On the other hand, Sucrase is a brush-border enzymes that catalyse the hydrolysis of sucrose to fructose and glucose leads to the digestion of the carbohydrates in the diet. Increases in mucosal disaccharidase (sucrose and isomaltase) activity have been described in the hatching chick, with disaccharidases exhibiting an activity peak at 2 days of age (Ghiasi ghaleh Kandi *et al.*, 2010; Uni *et al.*, 1995).

Some of researchers are hypothesized that supplementing animal diets with Zn may elevate the

prepares of arouse the synthesis of digestive enzymes in the small intestine, resulting in a better absorption of nutrients and potentially enhancing growth performance (Hedemann *et al.*, 2006). Therefore, in the current research, the effect of ZnO supplement on the sucrose enzyme activity in the small intestine of chicken 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 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

Table 1: composition of the basal experimental diet (%)

Ingredient	(22-42days)	
	(22-42days)	(1-21days)
Ground yellow corn	54.00	57.00
Soybean	29.16	27.00
Fish meal	4.00	1.50
Wheat bran	1.50	1.78
Wheat starch	7.77	8.48
Sea shell meal	1.30	1.55
De Calcium Phosphate	1.25	1.39
Vitamin Prmix ¹	0.25	0.25
Minera Permixon ²	0.25	0.25
DL-methionine	0.00	0.10
Sodium Chloride	0.25	0.25
Coccidio acetate	0.05	0.05
Fine Sand	0.22	0.40
Calculated analysis		
ME kcal/ kg	2933.6	2950.4
Crude protein (%)	20.63	18.44
Calcium (%)	1.03	1.01
Available P (%)	0.46	0.41

1: Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K; 2: Composition of mineral premix provided as follows kilogram of premix: Mn, 120.000 mg; Zn, 80.000 mg; Fe, 90.000 mg; Cu, 15.000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg

90% of the length of small intestine for analyzing the Sucrase enzyme activity were separated with specific scissors (a 8 cm sample was taken). The samples for Sucrase 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 phosphate buffer saline (pH = 7) was formed into a homogenized solution using sonic Vibracell Sonics (VCX 130 TE USA) device. The activity of sucrase was determined according to the procedure of Dahlqvist and Thamson (1963) and Teshfam (1984). For measuring the activity of sucrase, It was needed to determine total protein in which (*calorimetric*) method was used (Watanaba *et al.*, 1986). The activity level of ALP enzyme of each sample is divided into the amount of its 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 total protein. Therefore, the activity level of the enzyme, according to the IU/g protein is researched.

Statistical analysis: Results were statistically analyzed using the linear model of SAS software (2000) and Multivariate Analysis Variance. Comparative analysis of

Table 2: Sucrase 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	0.070±0.012 ^b	0.090±0.031 ^b	0.081±0.044 ^b	0.062±0.011 ^b
50 ppm ZnO	0.079±0.014 ^b	0.099±0.041 ^b	0.076±0.039 ^b	0.067±0.010 ^b
100 ppm ZnO	0.096±0.021 ^a	0.120±0.059 ^a	0.103±0.065 ^a	0.098±0.016 ^a
10% length of small intestine				
	21 Day	28 Day	35 Day	42 Day
(0.0 ppm ZnO) Control group	0.091±0.015 ^b	0.056±0.018	0.078±0.031	0.100±0.059 ^b
50 ppm ZnO	0.085±0.017 ^b	0.066±0.019	0.068±0.028	0.089±0.066 ^b
100 ppm ZnO	0.111±0.033 ^a	0.071±0.023	0.081±0.046	0.143±0.072 ^a
30% length of small intestine				
	21 Day	28 Day	35 Day	42 Day
(0.0 ppm ZnO) Control group	0.086±0.045	0.077±0.038	0.063±0.021	0.66±0.050
50 ppm ZnO	0.065±0.027	0.085±0.019	0.072±0.031	0.053±0.039
100 ppm ZnO	0.93±0.043	0.090±0.037	0.084±0.052	0.070±0.042
50% length of small intestine				
	21 Day	28 Day	35 Day	42 Day
(0.0 ppm ZnO) Control group	0.038±0.012	0.058±0.018	0.033±0.021	0.029±0.093
50 ppm ZnO	0.031±0.016	0.064±0.020	0.039±0.013	0.032±0.005
100 ppm ZnO	0.047±0.011	0.065±0.029	0.046±0.025	0.037±0.013
70% length of small intestine				
	21 Day	28 Day	35 Day	42 Day
(0.0 ppm ZnO) Control group	0.023±0.085	0.050±0.023	0.030±0.017	0.020±0.011
50 ppm ZnO	0.024±0.021	0.042±0.031	0.039±0.023	0.036±0.016
100 ppm ZnO	0.035±0.017	0.069±0.029	0.050±0.030	0.039±0.002
90% length of small intestine				
	21 Day	28 Day	35 Day	42 Day
(0.0 ppm ZnO) Control group	0.021±0.011	0.055±0.031	0.025±0.004	0.022±0.016
50 ppm ZnO	0.035±0.003	0.062±0.018	0.024±0.00	0.020±0.003
100 ppm ZnO	0.046±0.002	0.073±0.029	0.032±0.021	0.047±0.011

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

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 sucrose 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% of small intestine and also at age of 21 and 42 days in 10% of the small intestine in comparison with other groups ($p < 0.05$). Also, small intestine sucrose values slightly increased in 100 ppm ZnO supplemented group in other ages and parts of small intestine. However, it hadn't significant effect on sucrose enzyme activity. The obtained results indicated that the addition of ZnO supplement (100 ppm) to feed had significant effect on the small intestine sucrose 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 (Conway, 1994; Gao, 1998). Our results corresponded with Hedemann *et al.* (2006). They reported that disaccharidase (sucrose and maltase) activities in the mucosa from 10% (cranial small intestine mucosa) were greater in pigs fed 100 ppm of Zn than the other groups. In addition, they indicated, there was no effect of Zn on the activity of the disaccharidases in the 50%. 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. In contrast, Mavromichalis *et al.*, (2000) reported that there was no stable effect of Zn supplementation on villus height. Sell *et al.*, (1989) found the highest utmost specific activities of several disaccharidases in the proximal jejunum in a study that examined turkey mucosal disaccharidase activity at 2, 7, 14 and 28 days.

CONCLUSION

In conclusion, based on the present results and literature data, It was suggested that usage 100 ppm ZnO supplement in broilers diet can increase mucosal disaccharidase activity, redounding to better intestinal digestion and absorption of nutrients.

ACKNOWLEDGMENT

This work was funded by Islamic Azad University, Shabestar Branch, Iran.

REFERENCES

- Sahin, K. and O. Kucuk, 2003. Heat stress and dietary vitamin supplementation of poultry diets. *Nutr. Abstr. Rev. Ser. B. Livest. Feeds Feeding.*, 73: 41-50.
- Bettgrger, W.J. and B.L. O'Dell, 1993. Physiological roles of zinc in the plasma membrane of mammalian cells. *J. Nutr. Bio. Chem.*, 4: 194-207.
- Emmert, J.L. and D.H. Baker, 1995. Zinc stores in chickens delay the onset of zinc deficiency symptoms. *Poult Sci.*, 74: 1011-21.
- Mohanna, C. and Y. Nys, 1999. Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. *Br. Poult. Sci.*, 40: 108-114.
- Højberg, O., N. Canibe, H.D. Poulsen, M.S. Hedemann and B.B. Jensen, 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. *Appl. Environ. Microbiol.*, 71: 2267-2277.
- Ghiasi ghaleh Kandi, J., R. Beheshti, H. Karamouz, A.Gorbani, Y. Ebrahimmazad, K. Hatefinazhad and N. Maheri-Sis, 2010. Effect of different levels of perlite on sucrase mucosal enzymes activity in small intestine of broiler chicks. *Global Veterinaria.*, 4(2): 103-107.
- Uni, Z., Y. Noy and D. Sklan, 1995. Post hatch changes in morphology and function of the small intestines in heavy and light strain chicks. *Poult. Sci.*, 74: 1622-1629.
- Hedemann, M.S., B.B. Jensen and H.D. Poulsen, 2006. Influence of dietary zinc and copper on digestive enzyme activity and intestinal morphology in weaned pigs. *J. Anim. Sci.*, 84: 3310-3320.
- National Research Council (NRC), 1994. *Nutrient Requirements of Poultry*. 9th Rev. Edn., National Academy Press, Washington DC.
- Teshfam, M., 1984. Comparison of the effects of the high-acid milk replacer with conventional skim milk replacer. Ph.D. Thesis, University of Bristol, UK.
- Dahlqvist, A. and D.L. Thamson, 1963. Separation and characterization of two rat-intestinal amylases. *Biochem. J.*, 89: 272-277.
- Watanaba, N., S. Kamel, A. Ohkubo, M. Yamanaka, S. Ohsaws, K. Maikino and K. Tokuda, 1986. Method for assaying total protein. *Clin. Chem.*, 32: 1551-1554.
- SAS Institute: *SAS-User's Guide*, 2000. SAS Institute Inc., Cary, NC.
- Conway, P.L., 1994. The function of the gastrointestinal microflora and its regulation. *Proceedings of the 6th International Seminar on Digestive Physiology of Pig*. Sicuan Science and Technology Press, Sicuan, pp: 233-242.
- Gao, L.S., 1998. In *Digestive Physiology and Health Protection*. Curatorial Science and Technology Press, Beijing, pp: 173-230.

Mavromichalis, I., C.M. Peter, T.M. Parr, D. Ganessunker and D.H. Baker, 2000. Growth-promoting efficacy in young pigs of two sources of zinc oxide having either a high or a low bioavailability of zinc. *J. Anim. Sci.*, 78: 2896-2902.

Sell, J.L., O. Koldovsky and B.L. Reid, 1989. Intestinal disaccharidases of young turkeys: Temporal development and influence of diet composition. *Poul. Sci.*, 68: 265-277.