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Effect of Different Levels of Perlite on Mucosal Amylase Enzymes Activity in Small Intestine of Broiler Chicks

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Abstract: Amylase enzyme is an enzyme responsible for digestion and absorption of carbohydrates in the small intestine and effect of perlite in the diet of broilers on amylase enzyme had not been investigated. This study had been designed and performed to evaluate effect of different levels of perlite on amylase enzyme activity in small intestine of male broiler chicks. The experiment design was arranged as randomized complete bocks in 4×2 factorial arrangement of treatment. 180 male broilers of Ross 308 commercial hybrid was designated into 3 groups (0, 2 and 4 %). 3 replicates of 20 birds were assigned to each treatment. Control treatments were fed base diet and treatment groups with the same base diet plus 2 or 4% perlite. Animals were slaughtered at 21, 28, 36 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). Amylase enzyme activity was measured and recorded. Data were analyzed by SAS ($p<0.05$). Data showed that intake of perlite, significantly increased Amylase enzyme activity at different weeks and sites of the small intestine of the broiler chicks ($p<0.05$). These data suggested that perlite administration had significantly affected amylase activity as compared with control treatment.

Key words: Amylase, perlite, small intestine and broiler chicks

INTRODUCTION

Perlite is one of the volcanic, Aluminum- Silicate minerals which are hydrated and clear in color and there can be found tiny holes inside. Raw perlite is transparent and light grey or gloss black and if it is put in the temperature of 871 degrees centigrade will increase 4 to 20 times in volume and its color will change to snow white or grey white. Perlite has neutral pH and it was confirmed by the official congress of controlling animals as a feed additive in U.S. Its usage as an additive is also confirmed in Europe. Concerning the chemical constituent, it contains Aluminum and Silicate components (Talebali and Farzinpour, 2006). There are limited number of studies on the use of perlite as an adsorbent for removal of dyes such as methylene blue, Methyl violet victoria blue and also removal of metal ions such as copper (II) and cadmium (Do¢gan *et al*., 1999; Do¢gan and Alkan, 2003a; Do¢gan and Alkan, 2003b; Demirbas *et al*., 2002; Alkan and Do¢gan, 2001; Mathialagan and Viraraghavan, 2002). Perlite is essentially a metastable amorphous aluminum silicate, and has recently been used as an aflatoxin binder and adsorbent or controlling of wet litter and also decrease level of chloride in blood serum (Scheideler, 1993). Tangkawanit *et al*. (2005) have studied analcime synthesized perlite for its potential use as an ion exchange for removal of the toxic metals such as Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}. Glodek (1980) experimented the use of perlite in swine feed. A comparison was made between pigs fattened with traditional feeds and those fattened with the same feeds combined with perlite. He concluded that perlite fed pigs achieved a daily weight gain higher (197 g) and duration of breeding period lower by 23 days with the same feed utilization as the ration-fed control animals. Sakai and Nagao (1985) used three levels of perlite (1, 10, 20%) for 8 weeks for feeding 21 male and 21 female mice and concluded that the mice's behavior, mortality and food consumption were not affected by the experimental food and there was no significant change in the biochemical parameters of the blood and urine, the weight of the limbs, autopsy findings and pathology of tissue, However the male mice fed by 10 and 20% of perlite did not grow well but 1% of perlite was reported to be the appropriate dosage for the growth of mice. Alkan and Do¢gan (2001) and Scheila (1990) reported that perlite is responsible for breakdown of feces and absorbent of moisture and it acts like a damper between the earth and the birds and increases the growth along with decreasing the respiratory diseases, thigh bruise and bump in the

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breast. α-Amylase is an enzyme that hydrolyses alpha-bands of large alpha-linked polysaccharides such as starch and glycogen, yielding glucose and maltose. It is the major form of amylase enzyme found in mammals and broiler (Gracia *et al*., 2003; Ma and Guo, 2008). Despite of rat pancreatic juice has very high amylase activity, it seems some part of the amylase activity in the intestinal mucosal homogenates originates from the pancreas and all of amylase activity has not been removed by washing the mucosa with 0-9% sodium chloride solution. It has been found that rats can digest starch after the removal of the pancreas and salivary glands (Mc Geachin *et al*., 1958), and the small-intestinal mucosa of these rats had a high amylase activity (Mc Geachin and Ford, 1959). Therefore a considerable amount of amylase seems to be synthesized in the small-intestinal mucosa itself (Mc Geachin *et al*., 1958 and Mc Geachin and Ford, 1959) .In our study amylases with both origins have studied in the washed particles from the mucosal homogenates. Researches on perlite effects on chick's performance designated for evaluation of appropriate perlite level for the diet of broilers which it is specified to be 1 to 3% in broiler and 3% in layer (Talebali and Farzinpour, 2006). In the current research, the effect of perlite on the Amylase enzyme activity in the small intestine of chicken broilers was investigated.

MATERIALS AND METHODS

Birds and diet: This study was carried out prospectively in an eastern Azerbaijan industrial farm (Northwest of Iran) in May 2008. A total number of 180 male broilers of commercial hybrid (Ross 308) were divided into 3 treatment groups: 0, 2 and 4% perlite (Table 1). Each treatment group was divided into 3 replicates of 20 birds. Birds in each replicate were kept separately in cages next to each other and on litter. All conditions were same for all replicates. Experimental diets, formulated according to NRC (1994). The control treatment group was fed by basal diet (with 0.0% level of perlite) throughout the experimental period. The other two treatment groups fed by basal diet with addition of 2 and 4% of perlite respectively (Table 2). Food and water were provided adlib.

Sample collection: In the Rearing period, all conditions such as temperature, humidity, light, ventilation and management were appropriate and similar for all broilers and in days 21, 28, 35 and 42 of the rearing period, after 5 h of starvation, 2 The samples for ALP determination were cut broilers group (totally 18 chickens on each day of sampling) which weighed nearly equal to the average weight of each replicate have been chosen and slaughtered. The abdominalcavity was opened, and the entire gastrointestinal tract was removed.

Table 1: Chemical composition of perlite

Constituent	Percentage present
xSiO	271-75
Al	203 12.5-18
Na	20 2.9-4.0
K	20 4.0-5.0
CaO	0.5-2.0
X Fe	203 0.10.03-0.2
MnO	20.0-0.1
XSO	30.0-0.1
FeO	0.0-0.1
Ba	0.0-0.1
X PbO	0.0-0.1
x Cr	0.0-0.1

Do¢gan *et al*. (1999)

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 (Hedemann *et al*., 2006; Teshfam, 1984).

Enzyme assay: After thawing, all of vacuum packed were opened and then using a sensitive scale, 0.05 g 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. Enzyme activity of Amylase was measured according to the procedure (calorimetric method) of pars Azmoun kits (Lorentz, 1998). For detection of enzyme activity it was needed to measure total protein which Pirogallol (calorimetric) method was used (Watanaba *et al*., 1986). The level of activity of enzyme of each sample is divided into the amount of its total protein so the activity level of the enzyme is calculated according to the IU in L/g protein.

Statistical analyses: The results of the research have been statistically analyzed using the linear model of SAS Institute (2001).
Analysis of variance according to the model:

$$x_{ij} = \mu + T_j + e_{ij}$$

where,
x_{ij} = All dependent variable
μ = Overall mean
Ti = The fixed effect of RRO levels (i = 1, 2, 3)
e_{ij} = The effect of experimental error

Values of different parameters were expressed as the mean±standard deviation (X±SD). When significant difference among means was found, means were separated using Duncan's multiple range tests.

Table 2: Ingredient and nutrient compositions of experimental diets

	1-21 Days			21-42 Days		
Ingredient	0% perlite	2% perlite	4% perlite	0% perlite	2% perlite	4% perlite
Corn	54.50	54.00	45.00	62.64	39.00	59.00
Soybean Meal	34.14	34.19	35.81	27.00	27.70	27.70
Oil	2.5	2.50	2.50	2.50	2.50	2.50
Methionine	0.60	0.60	0.80	0.60	0.60	0.60
Lysine	0.00	0.00	0.00	0.20	0.20	0.20
Vit-Permix	0.25	0.25	0.25	0.25	0.25	0.25
Min-Permix	0.25	0.25	0.25	0.25	0.25	0.25
Dcp[1]	1.60	1.60	1.62	1.13	1.13	1.13
Oyster	1.44	1.40	1.33	1.48	1.44	1.39
Salt	0.28	0.28	0.28	0.28	0.28	0.28
Perlit	0.00	2.00	4.00	0.0	02.0	04.00
Starch	1.06	1.41	7.37	0.00	2.60	2.60
Fine Sand	3.38	1.46	0.07	3.67	2.05	0.10
Nutrients						
Me[2]	2850.21	2850.11	28050.14	2920.54	2920.03	2920.03
Protein%	20.50	20.51	20.50	18.17	18.18	18.17
Calcium%	0.99	0.99	0.99	0.89	0.89	0.89
Phosphorus%	0.44	0.44	0.44	0.34	0.34	0.34
Me/Protein	139.00	138.96	139.03	160.69	160.64	160.64
Calcium/Phosphorus	2.23	2.23	2.23	2.56	2.54	2.58

DCP[1]: dicalcium phosphate; ME[2]: Metabolisable energy. Per 2.5 kg mineral supplement containing 99200 mg magnesium, 84700 mg zinc, 50000 mg iron, 10000 mg copper, 990 mg Iodine, 200 mg selenium, 250000 mL/g Colin chloride. Per 2.5 kg vitamin supplement containing 900000 IU of vitamin A, 200000 IU of vitamin D_3, 19000 IU of vitamin E, 200 mg vitamin K_3, 18050 mg vitamin B_1, 49000 mg vitamin B_2, 9800 mg vitamin B_3, 29650 mg vitamin B_5, 2940 mg vitamin B_6, 1000 mg vitamin B_9, 15 mg vitamin B_{12}, 100 mg biotin, 190000 mg cholin chloride, 1000 mg antioxidant

Table 3: Comparison of average amylase activity between treatments in different periods and segments of small intestine in broiler chicks (IU/g protein)

	21 Day	28 Day	35 Day	42 Day
1% Length Of Small Intestine				
Control Group	4798.8±157.2[b]	3511.9±443.7[b]	2498.1±298.7[b]	1804.5±312.1[b]
2% Group	6352.6±527.7[a]	4975.1±123.9[a]	7472.3±931.7[a]	3795.4±312.5[a]
4% Group	5454.5±970.3[ab]	4306.4±459.1[a]	3768.5±662.2 [b]	3210.7±333.7a
10% length of small intestine				
Control Group	5959±379.2 [c]	6312.4±468.8	7158.5±758.4[a]	[b]6758.4±640.4
2% Group	14269.5±540.2[a]	6398.6±236.7	8460.9±964.7[a]	6247.3±974.8
4% Group	1078.8±1359.2 [b]	6580.4±228.9	6309.9±850.5 [b]	5985.2±513.3
30 % length of small intestine				
Control Group	8710.8±1433.7[a]	3724.3±310.4 [c]	4037.9±853.1[b]	3486.7±617.2[b]
2% Group	9918.7±528.9[a]	6187±1230.3[a]	9541.3±881.3[a]	6820.6±644.2[a]
4% Group	6070.1±837.8[b]	4404.9±453.5[b]	4990.9±690.4[b]	3324.2±129.9b
50% length of small intestine				
Control Group	9990.9±1345.6[a]	5535.8±433.1[a]	5119.9±999.9	5403.9±2103.1
2% Group	9056.5±494.6[a]	6047.3±1004.1[a]	4261.7±822.3	6190±946.5
4% Group	3862.2±521.2[b]	3456.9±281.4[b]	4170.8±464.9	4908.9±189.1
70% length of small intestine				
Control Group	7037.3±524.7	4785.6±566.6[b]	3780.4±464.7	5521.3±637.7[a]
2% Group	7779.7±758.5	6864.9±510.1[a]	3609.4±542.9	6063.7±934.5[a]
4% Group	8039.6±1069.336	56.5±473.9 [c]	4438.2±694.9	2958.1±249.2b
90% length of small intestine				
Control Group	6570±674.2[a]	5689.8±171.6	3994.9±548.6[b]	3154.4±649.5[b]
2% Group	7098.4±465.3[a]	5571.6±641.8	8459.7±712.5[a]	5456.6±252.4[a]
4% Group	4378±1003.1[b]	4959.6±848.9	3365.1±630.2[b]	3356.6±1027.4b

**: a, b Means in the same column with different superscripts differ significantly X ± SD ($p<0.05$)

RESULTS AND DISCUSSION

According to Table 3, adding different levels of perlite to the diet of the broilers at different ages cause different effects on activity of Amylase enzyme on different parts of the small intestine. Adding 2% perlite to the diet of the birds at the ages of 21, 28, 35, 42 days demonstrates a significant increase in 1, 10, 30, 50, 70 and 90% of small intestine in comparison with other treatment (4%) and control groups ($p<0.05$). Effects on activity of Amylase enzyme on different parts of the small intestine. Adding 2% perlite to Results showed that perlite had significant effect on Amylase activity in other ages and segments of small intestine.

Researchers showed role of perlite on different animals species in breakdown of feces through transmission of moisture and it acts like a damper between the earth and the birds and increases the growth

and decreasing the respiratory diseases, thigh bruises and bumps in the breast (Alkan and Do¢gan, 2001 and Scheila, 1990). The swine which were fed daily by perlite, were heavier (197 g) comparing with the control treatment and it resulted in the reduction of the fattening period (Glodek, 1980). Three levels of perlite (1, 10 and 20%) were used for 8 weeks for feeding 21 male and 21 female mice and results showed that the mice's behavior, mortality and food consumption were not affected by the experimental food and there was no significant change in the biochemical parameters of blood and urine, the weight of the limbs, autopsy findings and pathology finding of tissue. However the male mice fed by 10 and 20 levels of perlite, did not grow well and level 1 of perlite was reported to be the appropriate dosage for the growth of mice (Sakai and Nagao, 1985). The usage of perlite in the diet of broilers can decrease toxicity of Aflatoxin in the body and the amount of chloride in blood serum (Scheideler, 1993). In a research on chicks' performance, it was concluded that the appropriate perlite level for broiler diet was 1 to 3% and for laying hens was 3% (Talebali and Farzinpour, 2006).

Some of the different action mechanisms of aluminosilicates (zeolite and perlite) on animals still are in ambiguous and researchers according to various characteristics of aluminosilicates have mentioned several mechanisms which are followed (Osman, 1982). Efficient utilization mechanism of nutrients in animals can be due to zeolites ion exchange and absorption properties. If zeolite placed in an acidic environment have exchanged their structural hydrogen ions and act as buffering agent and caused increase in rumen pH and improve in growth of cellulolytic bacteria. Zeolite due to its own physical and chemical properties has ability to bind with magnesium, calcium, potassium and ammonium ions and under specific conditions release these bonds. Nutrients temporally attached to the zeolite and thus their passes through digestive tract are slow, so nutrients more time spent in this place and by this mean will be more exposed under gastrointestinal tract enzymes. One of the most important action mechanisms of zeolites is its ability to moves the intestinal enzymes that this act in turn causes increase in enzyme activity and stability and is involved in better nutrients absorption. Zeolite mechanically stimulate intestinal and stomach epithelial cells and by this way increases the blood perfusion to the intestinal mucosa and leads to increased coverage of gastrointestinal mucosa, height of the villus and depth of intestinal crypts and consequently increased secretory activity of these cells. Zeolite decreased grains excretion in the feces. This action was due to increased intestinal pH induced by the zeolite and is provided more suitable environment for the digestion of starch by pancreatic amylase. Zeolite has effect on the osmotic pressure of intestinal lumen and prevented from digestive disorders such as metabolic acidosis. Zeolite apparently helps to slow down respiration rate (which is a sign of heat stress) and allows to animals to be convenient at high environmental temperatures. Zeolite to create a stable complex combined with aflatoxin and by this way absorption of aflatoxin decreased from the gastrointestinal tract (Cabuk *et al*., 2004; Edwards *et al*., 1992; Leach *et al*., 1990).

In this study, the level of mucosal enzyme activity per IU/g protein in the different regions of the small intestine broiler chicken was calculated. The average a-amylase activity in the mucosal derived from the first three quarters of small intestines was 23% of that found in the fourth quarter. These differences are highly significant. Noy and Sklan (1995) reported that daily net secretion of amylase into the duodenum was in lowest level at 4 and steadily increased up to 21 day. Amylase A is responsible for the major part of the amylase activity of the mucosal homogenates. Amylase A has the properties of an endoamylase (α-amylase) and can cause hydrolyzing starch to maltose and higher oligosaccharides. The random hydrolysis of α-(1-4)-glucosidic linkages in the starch molecules accounts for the rapid decrease in viscosity of the rice-starch solutions. This enzyme is activated by chloride ions, inhibited by EDTA and does not hydrolyse disaccharides and its optimum from pH is 6 to 7 (Dahlqvist and Thamson, 1963).

CONCLUSION

Results of this study on the effect of perlite on the activity of Amylase enzyme in the small intestine of broilers demonstrates that adding 2% of perlite to the broiler's diet significantly increases the activity of Amylase enzyme on different days and different parts of the small intestine. It seems perlite can provide the neutral pH condition and necessary atmosphere (absorbent of some ions) for the activity of Amylase in the intestine. This causes better digestion of carbohydrates and finally causes the improvement of performance in 2% treatments, comparing with control group and 4% treatment group. In conclusion, based on result of present study and literature data, it was suggested that adding of 2% of perlite can be increase broiler performance.

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