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

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ABSTRACT

Lipase is an enzyme responsible for digestion and absorption of lipids in the small intestine and effect of perlite in the diet of broilers on lipase enzyme had not been investigated. This study had been designed and performed to evaluate effect of different levels of perlite on lipase enzyme activity in small intestine of male broiler chicks. The experiment design was arranged as randomized complete blocks in 4×2 factorial arrangement of treatment. The 180 male broilers of Ross 308 commercial hybrid was designated into 3 groups (0, 2 and 4%). Three 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). Lipase enzyme activity was measured and recorded. Data were analyzed by SAS ($p < 0.05$). Data showed that intake of perlite, significantly increased Lipase enzyme activity at different weeks and sites of the small intestine of the broiler chicks ($p < 0.05$). These data suggested that 2% perlite administration had significantly affected lipase activity as compared with control treatment.

Key words: Perlite, lipase, small intestine, broiler chicks, enzyme

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). Dietaru addition of inorganic compounds such as natural zeolite and minerals caused considerable morphological and enzymatic changes in intestine of broiler chickens (Incharoen *et al.*, 2009; Ruttanavut and Yamauchi, 2010). 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 icons such as copper (II) and cadmium

(Demirbas *et al.*, 2002; Dogan and Alkan, 2003a, b; Dyer *et al.*, 2004). 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 (Talebali and Farzinpour, 2006). Tangkawanit *et al.* (2005) have studied analcime synthesized perlite for its potential use as an ion exchanger for removal of the toxic metals such as Cu^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} . In other study the use of perlite in swine feed were experimented (Talebali and Farzinpour, 2006). 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 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 one % of perlite was reported to be the appropriate dosage for the growth of mice. Alkan and Dogan (2001) and Sheila (1990) reported that perlite is responsible for breakdown of feces and absorbent of moisture and it acts like a damper between the earth and birds and increases growth along with decreasing the respiratory diseases, thigh bruise and bump in the breast.

Lipase is one of the most importance enzymes that play a role in fat digestion. This enzyme facilitates hydrolysis of bonds between 1-monoglycerid 3-monoglycerid But its action on 2-monoglycerid is very slowly, therefore, major products of this enzyme is free fatty acids and monoglycerid. This enzyme acts on fats which is previously emulsified (Duke, 1996).

Lipolysis for first time was established by Shif. Amylase, saccharidase, peptidase and lipase are secreted by intestinal mucosa. Studies on Depancreatized dogs reported that intestinal mucosa is able to hydrolyze fat, its activity increased by biliary salts (Duke, 1996; Kihara and Sakata, 1997). The activity of intestinal mucosal lipase is approximately same with pancreatic lipase activity. Thus, from quantity aspect, this enzyme, intestinal lipase, has importance role on hydrolyzing and fat absorption. In fact about 50% of absorbed fat from gastrointestinal is mediated by intestinal lipase. Because after obstruction of pancreatic duct, about 50% of fat hydrolyzed and absorbed (Kihara and Sakata, 1997). Optimum pH for activity of intestinal lipase is 9. Duodenal lipase activity was moderated for 24 h at 37°C in pH 7 and in pH more than 10 and less than 5 activity of lipase decreased less than 10 min. EDTA caused the decreasing of lipase activity (Osman, 1982). This study had been designed and performed to evaluate effect of different levels of perlite on lipase enzyme activity in small intestine of male broiler chicks.

MATERIALS AND METHODS

Birds and diet: A total number of 180 male broilers of commercial hybrid (Ross 308) were divided into 2 experimental groups (0, 2 and 4% perlite). Each treatment group was divided into 3 replicates of 20 birds. Birds in each replicate were kept in cages separately next to each other and on litter. All conditions were same for all replicates. Chicks' diets were formulated according to NRC (1994). The control treatment group was fed basal diet (with 0.0% level of perlite) throughout the

Table 1: Chemical composition of perlite Dogan *et al.* (1999)

Constituent	Percentage
SiO ₂	71-75
Al ₂ O ₃	12.5-18
Na ₂ O	2.9-4.0
K ₂ O	4.0-5.0
CaO	0.5-2.0
Fe ₂ O ₃	0.1-1.5
MgO	0.03-0.5
TiO ₂	0.03-0.2
MnO ₂	0.0-0.1
SO ₃	0.0-0.1
FeO	0.0-0.1
Ba	0.0-0.1
PbO	0.0-0.5
Cr	0.0-0.1

experimental period. The other two treatment groups were fed diets supplemented with 2 and 4% of perlite respectively. Food and water were provided *ad-libitum* (Table 1).

Sample collection: In rearing period, all conditions such as temperature, humidity, light, ventilation and management were appropriate and similar for all broilers at the age of 21, 28, 3 and 42, 2 broilers from every group were slaughtered after 5 h of starvation, (18 chickens for each sampling). Abdominal cavity was opened and the entire gastrointestinal tract was removed. Small intestine was isolated and its length was measured. The positions at 1, 10, 30, 50, 70 and 90% of the length of small intestine for analyzing the lipase enzyme activity were separated with specific scissors (8 cm sample was taken). The samples for lipase determination were cut open lengthwise, rinsed carefully with phosphate buffer saline (pH = 7), blotted dry, enveloped in vacuum pack and stored at -80°C until enzyme analysis (Teshfam, 1984).

Enzyme assay: After thawing, all of vacuum packed samples were opened and then using a sensitive scale, 0.05 g 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. Enzyme activity of Lipase was measured according to the procedure (calorimetric method) (AOAC, 1995). For detection of enzyme activity it was needed to measure total protein by Pirogallol (calorimetric) method (Teshfam, 1984; Watanaba *et al.*, 1986). Enzyme activity level of each sample was divided into the amount of its total protein so that activity level of the enzyme is calculated according to the IU in liter/gram protein.

Statistical analysis: The results of the study have been statistically analyzed using the linear model of SAS software (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

T_i = 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 \pm Standard deviation (X \pm SD). When significant difference among means was found, means were separated using Duncan's multiple range tests.

RESULTS

According to Table 2, adding different levels of perlite to broilers diet at different ages caused different effects on activity of Lipase enzyme on different parts of the small intestine. Adding 2% perlite to the diet at the ages of 21, 28, 35, 42 days demonstrated a significant increase in 1, 10, 30, 50, 70 and 90% of small intestine in comparison with other treatment (4%) and control groups ($p < 0.05$). Results showed that perlite had significant effect on Lipase activity in other ages and segments of small intestine. In part of 1% of small intestine in days 21, 35 and 42, there is a significant increase in 2% treatment than control and 4% treatments. But in part of 10% of small intestine in days 21, 35 and 42, there is a significant increase in 2% treatment than control group and in control than 4% treatment. In part of 30% of small intestine in day 21, there is a significant increase in 2% treatment than control and in control than 4% treatment whereas, in days 28 and 35, there isn't significant increase among control and 4% treatment. In part of 50% of small intestine in day 21, there is a significant increase in 2 and 4% treatments than control group whereas, in days 35 and 42, there is a significant increase in control and 2% treatment than 4% treatment. In part of 70% of small intestine in days 21 and 28, there is a significant increase in 2% treatment than control and 4% treatments. Finally, in part of 90% of small intestine in days 21 and 35, there is a significant increase in 2% treatment than control and 4% treatment.

Table 2: Comparison of average lipase activity between treatments in different periods and 1, 10, 30, 50, 70 and 90 percent segments of small intestine in broiler chicks (IU g⁻¹ protein)

Treatment	Small intestine (%)	Day			
		21	28	35	42
Control group	1	2331.1 \pm 518.5 ^b	4376.9 \pm 646.3	2968.9 \pm 298.7 ^b	4313.9 \pm 521.8 ^b
2% group		4107.4 \pm 1454.3 ^a	3997.7 \pm 732.4	4382.9 \pm 721.6 ^a	4403.2 \pm 519.6 ^a
4% group		3046.1 \pm 506.6 ^b	4286.8 \pm 675.9	2616.7 \pm 370.9 ^b	4440.8 \pm 593.9 ^a
Control group	10	4049.7 \pm 1090.3 ^b	3316.0 \pm 659.9	1947.2 \pm 72 ^b	3639.9 \pm 292.6 ^b
2% group		5565.3 \pm 660.1 ^a	3543.6 \pm 625.9	3054.7 \pm 165.1 ^a	5153.9 \pm 571.1 ^a
4% group		3013.5 \pm 654.4 ^c	2959.3 \pm 423.4	2365.3 \pm 349.1 ^b	2527.0 \pm 253.1 ^c
Control group	30	2878.0 \pm 680.7 ^b	1995.9 \pm 260.9 ^b	1876.5 \pm 141.8 ^b	2655.3 \pm 364.6
2% group		4572.3 \pm 903.6 ^a	3159.5 \pm 301.7 ^a	2575.4 \pm 254.3 ^a	2557.4 \pm 297.5
4% group		2064.9 \pm 615.6 ^c	1923.8 \pm 361 ^b	1537.6 \pm 181.5 ^b	2934.8 \pm 413.3
Control group	50	2200.0 \pm 588.8 ^b	2749.6 \pm 413.9 ^b	3020.3 \pm 210.2 ^a	4611.9 \pm 743.3 ^a
2% group		3338.8 \pm 338.9 ^a	3701.1 \pm 553.7 ^a	2740.8 \pm 314.1 ^a	4609.1 \pm 468.6 ^a
4% group		3731.7 \pm 420.2 ^a	2200.3 \pm 463.9 ^b	1586.9 \pm 311.9 ^b	2890.6 \pm 298.8 ^b
Control group	70	2988.9 \pm 549.6 ^b	2349.2 \pm 211.4 ^{ab}	2904.2 \pm 343.3 ^a	2708.1 \pm 357.9
2% group		3741.6 \pm 490.3 ^a	2825.9 \pm 311.1 ^a	2512.6 \pm 365.1 ^a	2608.9 \pm 401.9
4% group		2596.4 \pm 566.3 ^b	1502.0 \pm 348.5 ^b	1835.8 \pm 304 ^b	2958.1 \pm 429.2
Control group	90	2904.9 \pm 88.4 ^b	2992.4 \pm 798.4	1716.6 \pm 210.2 ^b	2508.3 \pm 395.4
2% group		4168.6 \pm 891.8 ^a	3225.7 \pm 228.6	3281.3 \pm 459.3 ^a	2992.6 \pm 310.8
4% group		2727.6 \pm 404.3 ^b	3395.2 \pm 663.3	1979.3 \pm 178.4 ^b	2984.0 \pm 545.9

Means in the same column with different superscripts differ significantly X \pm SD ($p < 0.05$)

DISCUSSION

Researchers showed role of perlite on different animal species in breakdown of feces through transmission of moisture and it acts like a damper between the earth and the birds and increased the growth and decreased respiratory diseases, thigh bruises and bumps in the breast (Alkan and Dogan, 2001; Sheila, 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 (Talebali and Farzinpour, 2006). 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 (Talebali and Farzinpour, 2006). In a study on chicks' performance, it was concluded that the appropriate perlite level for broiler diet was 1 to 3 percent and for laying hens was 3 percent (Talebali and Farzinpour, 2006).

In this study, the level of mucosal enzyme activity per IU g⁻¹ protein was calculated in different regions of small intestine broiler chicken. Lipase is of the most important enzymes in fat digestion which decomposes it to fatty acids and glycerol. The activity of intestinal mucosal lipase is approximately same with pancreatic lipase. Researches shown that secretion of lipase in hen to six days of age is low and increased from 7 to 21 days of age. Optimum pH for intestinal lipase is 9 and EDTA causes decreasing of lipase activity (Kihara and Sakata, 1997; Osman, 1982). In other research were done by Hulan and Bird (1972) revealed that enhancement of carbohydrates and fat receiving through diet, increases the activity of amylase and lipase enzymes.

With Adding 2% perlite to broiler diet has significance increased on lipase activity in several parts of small intestine. Perlite absorbs hydrogen ions instance other buffers and causes increasing of gastrointestinal pH. Increasing of intestinal pH is supplies sufficient environment for most enzymes activity especially intestinal amylase and lipase.

CONCLUSION

In conclusion based on result of present study and literatures data, it was suggested that adding of 2% of perlite can increase broiler performance and had stimulatory effects on intestinal lipase activity in broilers.

REFERENCES

- AOAC, 1995. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
- Alkan, M. and M. Dogan, 2001. Adsorption of copper(II) onto perlite. *J. Colloid Interface Sci.*, 243: 280-291.
- Demirbas, O, M. Alkan and M. Dogan, 2002. The removal of Victoria blue from aqueous solution by adsorption on a low-cost material. *Adsorption*, 8: 341-349.
- Dogan, M. and M. Alkan, 2003a. Adsorption kinetics of methyl violet onto perlite. *Chemosphere*, 50: 517-528.
- Dogan, M. and M. Alkan, 2003b. Removal of methyl violet from aqueous solution by perlite. *J. Colloid Interface Sci.*, 267: 32-41.

- Dogan, M., M. Alkan and Y. Onganer, 1999. Adsorption of methylene blue from aqueous solution onto perlite. *Water Air Soil Pollut.*, 120: 229-248.
- Duke, G.E., 1996. Avian Digestion. In: *Physiology of Domestic Animals*. 11th Edn., Swenson, M.J. and W.O. Reece (Eds.). Cornell University Press, Ithaca, New York, pp: 428-435.
- Dyer, A., S. Tangkawanit and K. Rangsiwatananon, 2004. Exchange diffusion of Cu^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} into analcime synthesized from perlite. *Microporous and Mesoporous Mater.*, 75: 273-279.
- Hulan, H.W. and F.H. Bird, 1972. Effect of fat level in isonitrogenous diets on the composition of avian pancreatic juice. *J. Nutr.*, 102: 459-468.
- Incharoen, T., O. Khambualai and K. Yamauchi, 2009. Performance and histological changes of the intestinal villi in chickens fed dietary natural zeolite including plant extract. *Asian J. Poult. Sci.*, 3: 42-50.
- Kihara, M. and T. Sakata, 1997. Fermentation of dietary carbohydrates to short Chain fatty acids by gut microbe and its influence on intestinal morphology of a detritivorous teleost tilapia. *Comp. Biochem. Biophys.*, 118: 1201-1207.
- NRC, 1994. *Nutrient Requirement of Poultry*. National Academy Press, Washington DC.
- Osman, A.M., 1982. Amylase in chicken intestine and pancreas. *Comp. Biochem. Physiol. B: Comp. Biochem.*, 73: 571-574.
- Ruttanavut, J. and K. Yamauchi, 2010. Growth performance and histological alterations of intestinal villi in broilers fed dietary mixed minerals. *Asian J. Anim. Sci.*, 4: 96-106.
- SAS Institute, 2001. *SAS State Software*. SAS Institute Inc., Cary, NC., USA..
- Sakai, T. and S. Nagao, 1985. Twenty-eight week toxicity study of perlite powder in mice. *J. Toxicol. Sci.*, 10: 83-93.
- Sheila, E.S., 1990. Perlite for litter management and treatment for broilers. Department of Poultry Science. North Carolina State University. <http://www.schundler.com/poultry.htm>.
- Talebali, H. and A. Farzinpour, 2006. Effect of different levels of perlite on performance of broiler chicks. *Int. J. Poult. Sci.*, 5: 432-435.
- Tangkawanit, S., K. Rangsiwatananon and A. Dyer, 2005. Ion exchange of Cu, Ni, Pb and Zn in hydroxides analcime (ANA) synthesized from Thai. *Microporous Mesoporous Mater.*, 79: 171-175.
- 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.
- Watanaba, N., S. Kamel, A. Ohkubo, M. Yamanaka, S. Ohsawa, K. Maikino and K. Tokuda, 1986. Method for assaying total protein. *Clin. Chem.*, 32: 1551-1554.