

## Effect of Different Levels of Perlite on Leucine Aminopeptidase Mucosal Enzymes Activity Small Intestinal of Broiler Chicks

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**Abstract:** An experiment was conducted to study the effects of different levels of perlite on leucine Aminopeptidase enzyme activity of the small intestine of male broilers. The experimental design was arranged as randomized complete blocks 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 treatments with the same base diet plus 2 and 4% perlite. Animals were slaughtered after 21, 28, 36 and 42 days and different segments of small intestine (at 1, 10, 30, 50, 70 and 90% of total length the small intestine) were taken from each replicates (N=2) leucine Aminopeptidase enzyme activity was measured and recorded. Data were analyzed by SAS (P<0.05). As intake of perlite, significantly increased Leucine Aminopeptidase (LAP) enzyme activity at weeks and sites of the small intestine of the broiler chicks (P<0.05), these data suggested that perlite administration did not have significant effect on control treatment comparing to treatment. As LAP enzyme is an enzyme responsible for digestion and absorption of long chain fatty acid in the small intestine and as the effect of perlite administration in the diet of broilers on this enzyme had not been investigated the following study which had been designed and performed.

**Key words:** Perlite • Leucine Aminopeptidase • Small intestine and broiler chicks

### INTRODUCTION

Perlite is one of the volcanic, Aluminum-Silicate minerals which are hydrated and clear and in it, there can be found tiny holes. Raw perlite is in 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 will change color to snow white or grey white. Perlite contains neutral pH and it was confirmed by the official congress of controlling animal's diet in the U.S. and was fit into the chemical recipe of the diet. Its usage as an additive is also confirmed in Europe. Concerning the chemical point it contains Aluminum and Silicate components [1].

These are limited number of studies on the use of perlite as an adsorbent. The removal of dyes such as

methylene blue, Doğan *et al.* [2] Methyl violet, Doğan *et al.* [3], Doğan *et al.* [4], victoria blue, Demirbas *et al.* [5] and metal ions such as copper (II), Alkan and Doğan [6] and cadmium Mathialagan and Viraraghavan [7] by perlite. Perlite is essentially a metastable amorphous aluminum silicate and has recently been used as an aflatoxin detoxicant and adsorbent in the removal of wastewater and the amount of chloride in blood serum [8]. Tangkawanit *et al.* [9] have studied analcime synthesized from perlite for its potential use as an ion exchanger for removal of the toxic metals Cu<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>. Glodek [10] experimented the use of perlite in hog feed. A comparison was made between hogs fattened with traditional feeds and those fattened with the same feeds combined with perlite.

It must be especially emphasized that the perlite fed pigs achieved a daily weight gain higher by 197g and duration of fattening lower by 23 days with the same feed utilization as the ration-fed control animals. Sakai and Nagao [11] 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, causality and food consumption were not affected by the experimental food and there was no significant change in the parameters related to biochemical components 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 percents of perlite, did not grow well; after all, one percent of perlite was reported to be the appropriate dosage for the growth of mice.

Researches on chick's performance designated the appropriate perlite level for the diet of broilers to be 1 to 3 percent and for hatching chickens to be 3 percent [1]. Leucine Aminopeptidase (LAP) is a brush-border and sitosole 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. The effect of perlite on the LAP enzyme in the small intestine of chicken broilers is being performed for the first time [12].

**MATERIAIS AND METHODS**

**Animal and Diets:** 180 male broilers of commercial hybrid (Ross 308) were divided into 3 treatment groups (0, 2 and 4 %). Each treatment group were divided into 3 replicates of 20 birds. The birds in each replicate were kept separately in cages next to each other and on litter. All conditions were the same for all replicates. Chicks' diets were formulated according to the advice of NRC (1998). The control treatment groups was fed by basal diet (with 0.0 % level of perlite) through out the experimental period. While the other two treatment groups fed by basal diet with addition of 2 and 4 % of perlite, respectively. The birds were provided by the food and water, freely (Table 2).

**Sample Collection:** In the Rearing period, all conditions such as temperature, humidity, light, ventilation and management were appropriate and similar for all treatment groups and in days 21, 28, 35 and 42 of the rearing period, after 3 hours of fasting, 2 broilers from every group (totally 18 chickens on each day of sampling) which had

Table 1: Chemical composition of perlite

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

Uluatam, 1991. (cited by Dogan *et al.*, 1999)

nearly equal to the average weight of each replicate have been chosen and slaughtered. Hastily, samples of 1, 10, 30, 50, 70 and 90 percent of the length of small intestines for analyzing the LAP enzyme were separated.

**Enzyme Assay:** In the laboratory, 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 device. For measuring the activity of LAP Nigel *et al.* [13] method was used. It goes without mentioning that for measuring the activity level of LAP. We need to measure Total protein in which Pirogarrol method was used [14]. The activity level of each enzyme of each sample is divided into the amount of its Total protein so that the activity level of the enzyme, according to the IU in liter/gram protein is reached.

**Statistical Analyses:** The results of the research have been statistically analyzed using the linear model of SAS software (2001) and Multivariate Analysis Variance [15]. Comparative analysis of the average of treatments was performed using Duncan's multifunctional method in the random of 5 percent.

**RESULTS**

According to Table 3, adding perlite to the diet of the broilers at different ages and to different parts of the small intestine caused variety of influences on the activity of LAP enzyme. In a way that the activity of LAP enzyme

Table 2: Ingredient and nutrient compositions of experimental diets

Ingredient	(1-21 Days)			(21-42 Days)		
	0%	2%	4%	0%	2%	4%
Corn	54.50	54.00	45.00	62.64	39.00	59.00
SBM (%44)	34.14	34.19	35.81	27.00	27.70	27.70
Oil	2.50	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
Vitamin-premix	0.25	0.25	0.25	0.25	0.25	0.25
Mineral-premix	0.25	0.25	0.25	0.25	0.25	0.25
DCP	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
perlite	0.00	2.00	4.00	0.00	2.00	4.00
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
<b>Nutrients</b>						
ME3 (kcal/kg)	2850.21	2850.11	2850.14	2920.54	2920.03	2920.03
Protein (Percent)	20.50	20.51	20.50	18.17	18.18	18.17
Calcium (Percent)	0.99	0.99	0.99	0.89	0.89	0.89
Phosphorus (Percent)	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	2.58	2.58

SBM<sup>1</sup> = soybean meal; DCP<sup>2</sup> = dicalcium phosphate. ME<sup>3</sup> = 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 mg gram Colin chloride. Per 2.5 kilogram vitamin supplement containing 900000 IU of vitamin A, 200000 IU of vitamin D<sub>3</sub>, 19000 IU of vitamin E, 200 mg vitamin K<sub>3</sub>, 18050 mg vitamin B<sub>1</sub>, 49000 mg vitamin B<sub>2</sub>, 9800 mg vitamin B<sub>3</sub>, 29650 mg vitamin B<sub>5</sub>, 2940 mg vitamin B<sub>6</sub>, 1000 mg vitamin B<sub>9</sub>, 15 mg vitamin B<sub>12</sub>, 100 mg biotin, 190000 mg cholin chloride, 1000 mg antioxidant

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

	1 % length of small intestine			
	21 Day	28 Day	35 Day	42 Day
Control group	3820.8±1310.7	4001.7±1335.1	2677.7±487.9	2936.8±606.5
2 % group	3004.3±723.6	3197.1±197.9	3173.3±1257.7	2165.8±553.1
4 % group	3340.4±703.5	2833.9±1021.2	3421.3±1211.1	2573.6±463.5
	10 % length of small intestine			
	21 Day	28 Day	35 Day	42 Day
Control group	4771.2±267.5 <sup>a</sup>	3823.3±791.8	2909.5±402.9	2959.1±473.9
2 % group	2994.8±758.2 <sup>b</sup>	3522.5±787.9	2922.2±647.2	2485.2±646.1
4 % group	3923.6±714.9 <sup>a</sup>	2797.9±740.4	3030.1±628.1	2383.1±761.1
	30 % length of small intestine			
	21 Day	28 Day	35 Day	42 Day
Control group	4276.8±1381.4	4414.8±518.8	2813.4±494.1	2882.9±453.3
2 % group	3919.7±731.9	4122.6±868.7	2869.6±576.4	2848.6±946.7
4 % group	4196.6±626.6	3850.2±555.4	3111.7±494.7	2158.6±844.5

Table 3: Continued

50 % length of small intestine				
	21 Day	28 Day	35 Day	42 Day
Control group	3841.8±811.6	4566.4±570.8	3064.1±862.7	2833.3±724.4
2 % group	4428.1±676.5	4058.8±1265.9	2960.1±521.1	2840.1±958.4
4 % group	4384.2±777.8	3374.6±630.9	2885.3±318.8	2461.2±837.1
70 % length of small intestine				
	21 Day	28 Day	35 Day	42 Day
Control group	4393.2±847.8	3971.8±1452.6	3260.9±522.6	3528.4±1220.8
2 % group	5420.7±786.1	4226.9±1304.1	3125.8±751.4	3690.3±1198.1
4 % group	4674.8±1135.9	4242.1±1166.9	3268.8±803.7	2308.7±759.3
90 % length of small intestine				
	21 Day	28 Day	35 Day	42 Day
Control group	3562.8±1128.9	4584.7±1655.2	4216.9±1316.4	3602±669.7
2 % group	4401.7±1295.5	3967.4±537.9	4676.2±765.7	3438.2±1388.4
4 % group	4020.7±582.3	4927.5±1035.9	4214.7±1208.6	2855.1±877.2

a, b Means in the same row that do not have common superscripts differ, P<0.05. (X±SD)

demonstrated a significant increase only at the age of 21 days after sampling as 10% of the length of the small intestine in control treatments and 4% perlite group comparing to 2 % perlite was witnessed (P <0.05). perlite, hadn't significant effect on LAP activity In different ages and segments of small intestine.

### DISCUSSION

Researches have been conducted on the role of perlite in different animals in a way that perlite is responsible for dissection of excretion and its absorption through transmission 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 scorch and callus in the breast [16]. The swine which were fed daily by perlite, were heavier (197 gram) comparing to the control treatment and it resulted in the reduction of the fattening period [10]. Three levels of perlite (1, 10 and 20%) were used for 8 weeks for feeding 21 male and 21 female mice and concluded that the mice's behavior, causality and food consumption were not affected by the experimental food and there was no significant change in the parameters related to biochemical components 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 levels of perlite, did not grow well; after all, level 1 of perlite was reported to be the appropriate dosage for the growth of mice [11].

The usage of perlite in the diet of broilers had rendered to decrease of the toxicity of Aflatoxin in the body and the amount of chloride in blood serum [8]. In a research on chicks' turnover, it was designated that the appropriate perlite level for the diet of broilers was 1 to 3 percent and 3 percent for hatching chickens [1]. Considering the present results, adding perlite to the diet of the broilers did not demonstrate a significant increase in the activity of LAP enzyme in different parts and different days and only the activity of LAP enzyme at the age of 21 days after sampling as 10% of the length of the small intestine in control treatments and 4% perlite group comparing to 2 % perlite was witnessed (P <0.05). It seems like, perlite has no effect on the optimum conditions of the activity of LAP enzyme of the small intestine and therefore perlite does not have a significant role in digestion and absorption of proteins. In conclusion, perlite supplementation has not significant effects on LAP activity in broiler chicks.

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