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Original Article

In-ovo Administration of Ghrelin and Subsequent Intestinal Alkaline Phosphatase (ALP) Activity in Broiler Chickens

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ABSTRACT
With attention to this finding and ghrelin gastrointestinal effects, aim of this study was to investigation on effect of in ovo administration of ghrelin on subsequent alkaline phosphatase (ALP) activity in broiler chickens. In this experiment 250 fertilized eggs were collected from commercial breeder flock. The eggs were divided into five experimental groups; control T1 (without injection), group T2 (in ovo injected with solution), group T3 (in ovo injected with 50 µg/egg ghrelin), group T4 (in ovo injected with 100 µg /egg ghrelin) and group T5 (in ovo injected with 150 µg /egg ghrelin). All of groups were incubated. In ovo injection was done at day 7 of incubation. In ovo administrated 50 microgram ghrelin cause ALP activity at 30, 50 and 90 percent of intestine in compare with control group (non-injected) in 21-day-old chickens. that in ovo ghrelin administration cause ALP activity in one percent of intestine segment, significantly more than control group. Also, in ovo administrated 50 microgram ghrelin cause ALP activity at one percent of intestine segment in compare with other experimental groups or at 50 percent segment was more than control group. In 42-day-old chickens, in ovo administration of 50 and 150 microgram ghrelin cause ALP activity elevation at 70 or 90 present segment of intestine in compare with control or 100 microgram injected groups. It is concluded in ovo administration of different concentrations of ghrelin caused significant intestinal ALP activity in 21- or 42-day of rearing period. In ovo administration of 50 microgram and somewhat 150 microgram ghrelin at different age (21- or 42-day-old) may cause hyperactivity of intestinal ALP at different percents of length.
Key words: *Alkaline phosphatase, Ghrelin, In ovo injection, Intestine, Chicken.*

INTRODUCTION

Alkaline phosphatase is a group of enzymes have catalytic activity for degradation of phosphate esters and separation of phosphoric acid molecules [1, 2]. In mammalians and chicken intestinal mucosal cytosole at brush border ends have considerable alkaline phosphatase activity [3]. Moog [4] reported that intestinal epithelium of chicken embryo didn't has Alkaline phosphatase earlier day 8 of embryonic life and Alkaline phosphatase become activate after day 9-18 [5,6]. Moog [7] reported, alkaline phosphatase activity can arrive to peak at 2 or 2.5 day post-hatch. Studies show that alkaline phosphatase activity indicates maturity of intestinal cells and had key role in long chain fatty acids and cholesterol digestion [8]. Two major role of ghrelin has been considered as "grow stimulatory" and "food intake regulation" [9]. In other hand, because of structural similarity with motilin, ghrelin has considerable effects in gastrointestinal motility [10]. Ghrelin receptors in gastrointestinal neurons had been identified [11]. It has been suggested; the gastrointestinal roles of ghrelin are regulated via these receptors, these findings order potential use of ghrelin and its agonists for therapy of gastrointestinal motility disorders [12]. Scientific researches in mouse small intestine showed a low number of ghrelin-containing cells in duodenal segment. But, profuse ghrelin-positive cells were found in the rat and human duodenum and proximal jejunum, and lower amounts were found in the distal jejunum, ileum and colon [13]. In chicken, *in vitro* studies demonstrated that 1µg ghrelin can induce esophagus, crop and colon contraction, but hadn't any effects on duodenum or jejunum motility [14]. *Icv*-injection of ghrelin analog (GHRP06) in 5-day-old layer chickens hadn't any considerable effect on feed remaining in crop or gizzard [15]. About effects on gastrointestinal, the stimulatory effect of luminal ghrelin on the pancreatic exocrine

functions (enzyme secretion) is documented [16]. With attention to this finding and ghrelin gastrointestinal effects, aim of this study was to investigation on effect of *in ovo* administration of ghrelin on subsequent alkaline phosphatase (ALP) activity in broiler chickens.

MATERIALS AND METHODS

In ovo injection

This study has been conducted in Islamic Azad University, Shabestar branch's research farm during June-September 2010. In this experiment 250 fertilized eggs were collected from commercial breeder flock (Ross 308). The eggs were divided into five experimental groups; control T1 (without injection), group T2 (*in ovo* injected with solution at day 7), group T3 (*in ovo* injected with 50 µg/egg ghrelin at day 7), group T4 (*in ovo* injected with 100 µg /egg ghrelin at day 7) and group T5 (*in ovo* injected with 150 µg /egg ghrelin at day 5). All of groups were incubated. Ghrelin (Sigma-Aldrich®Rat Ghrelin – USA), dissolved in 1% acetic acid solvent and proposed concentrations of ghrelin were prepared. At day 7 of incubation, *in ovo* injection was conducted for three groups of eggs in hygiene room with 37centigrade temperature (In the sterile condition and under the ventilator). At this experiment, 22G needles were used for *in albumin* injection.

Rearing

All of hatched *in ovo* injected chickens transferred to farm for rearing. The birds were kept separately in pens next to each other and on the litter. All conditions for groups were the same. Diets ingredients included; corn, soybean meal, DCP, vegetable oil, wheat, NaCl, molasses, moister powder, anti-coccidiosis supplement, vitamin/mineral mixture, phytase, methionine/lysine supplements, commercial antioxidant and salt. The feeding regime was according to table1. Six steps rations have been used for all of groups.

Table1. Nutrient compositions of fed diets (based on corn and soybean meal) for all of experimental chickens during 42-day rearing period

Nutrients	1-9 days	10-20 days	21-35 days	36-42 days
Metabolisable energy (ME) ME/Kg	2973	2995	3020	3125
Crude protein (CP)%	24	21.8	19.97	19
Crude fiber (CF)%	3.38	3.2	3	2.8
Methionine (Met)%	0.65	0.52	0.5	0.46
Lysine (Lys)%	1.37	1.34	1.19	1.1
Calcium (Ca)%	0.84	0.95	0.93	0.89
Phosphorus (p)%	0.42	0.5	0.47	0.43

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 and 42 of the rearing period, after 5 hours of starvation, 2 broilers from every group (totally 18 chickens) which weighed 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 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 [17,18].

Enzymatic assays

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 [17, 18]. The activity of ALP was determined according to the procedure of Dahlqvist and Thamson

[19], and Teshfam [18]. For measuring the activity of ALP, It was needed to determine total protein in which (calorimetric) method was used [20] . The activity level of ALP enzyme of each sample is divided into the amount of its total protein. Therefore, the activity level of the enzyme, according to the IU /gram protein is researched.

Statistical analyses

The results of the research have been statistically analyzed using the linear model of SAS software (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.

RESULTS

The table 2 and 3 are shows ALP activity in in ovo-injected broilers. In ovo administration of ghrelin caused elevation of duodenal ALP activity. In table 2 it is clear that in-ovo administration of 50 microgram ghrelin cause ALP activity at 30, 50 and 90 percent of intestine in compare with control group (non-injected) in 21-day-old chickens. Table 3 shows that in-ovo ghrelin administration causes ALP activity in one percent of intestine segment, significantly more than control group. Also, in ovo administrated 50 microgram ghrelin cause ALP activity at one percent of intestine segment in compare with other experimental groups or at 50 percent segment was more than control group. In 42-day-old chickens, in ovo administration of 50 and 150 microgram ghrelin cause ALP activity elevation at 70 or 90 present segment of intestine in compare with control or 100 microgram injected groups. With attention to tables 2 and 3 *in ovo* administration of different concentrations of ghrelin caused significant intestinal ALP activity in 21- or 42-day of rearing period. But the effects of different concentration of ghrelin were different, qua 50 microgram and somewhat 150 microgram administrated ghrelin at different age (21- or 42-day-old) caused hyperactivity of intestinal ALP at different percents of length, in compare with control group and sometimes in compare with 100 microgram group.

Table2. Comparison of ALP activity in 1, 10, 30, 50, 70 and 90 percent of chicken intestine at 21-day in experimental groups (IU/g protein)

Intestine length Groups	1 %	10%	30 %	50%	70 %	90%
Control	1495.1± 378.9	1613.7± 379.6	999.9 ^{b} ± 113.5	616.1 ^{b}± 147.3	304.1 ± 83.6	191.4 ^{b}± 77.3
***In ovo* injected; basal solusion**	1153.2 ± 144.3	1495.6 ± 274.7	924.4 ^{b} ± 169.4	500.6 ^{b}± 154.9	247.7± 71.1	132.5 ^{b}± 46.9
***In ovo injected;* 50microgram ghrelin**	1912.7 ± 229.2	2489.4 ± 534.8	1510 ^{a} ± 102.9	975.7 ^{a}± 138.6	415.6± 126.6	445.1 ^{a}± 108.9
***In ovo injected;* 100microgram ghrelin**	1358.5 ± 416.1	1534.6 ± 437.8	1123.4 ^{ab} ± 175	576.9^{ab}± 25.6	350.8± 27.7	216.4 ^{ab}± 63.4
***In ovo injected;* 150microgram ghrelin**	1699.1 ± 140.1	1385.8 ± 417.9	1064.6^{ab}± 102.9	771.3^{ab}± 310.1	317.4 ± 104.4	229.2^{ab}±138.6

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

Table3. Comparison of ALP activity in 1, 10, 30, 50, 70 and 90 percent of chicken intestine at 42-day in experimental groups (IU/g protein)

Intestine length Groups	1%	10%	30 %	50%	70 %	90 %
Control	1143.9 ^{b}± 171.8	1410.4 ^{ab}± 230	1109.8^{ab}± 162.3	426 ^{b}± 65	428.7 ^{b}± 44.8	295.1 ^{b}± 66.4
***In ovo* injected; basal solusion**	879.9 ^{b}± 181.9	1079.4 ^{b}± 422.1	805.7 ^{b} ±203.2	729.2 ^{ab}± 548	343.7 ^{b}± 167.4	162.9 ^{b}± 93.1
***In ovo injected;* 50microgram ghrelin**	1669.9 ^{a} ±359.6	1794.5 ^{a}± 435.9	1163.2 ^{a} ±342.1	893.9 ^{a}± 367.9	917.7 ^{a}± 362.1	845.2 ^{a}± 249.3
***In ovo injected;* 100microgram ghrelin**	961.3 ^{b}± 178.3	1296.2 ^{ab}± 62.9	1006.5 ^{ab}± 77.3	572.9 ^{ab}± 45.7	725.6 ^{a} ±173.6	644.1 ^{a}± 58.2
***In ovo injected;* 150microgram ghrelin**	967.1 ^{b} ± 112.6	1186.9 ^{ab}± 145	1137.7^{ab}± 292.9	613.1^{ab}± 55.6	372.6 ^{b}± 54.2	215.1 ^{b}± 12.5

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

DISCUSSION

Ghrelin stimulates gastrointestinal tissue morphogenesis and function development in animals. Ghrelin retarded gastric, intestinal and pancreatic development [21]. Another study with neonatal pigs fed with milk formula showed that repetitive intragastric administrations of ghrelin (15 µg/kg b. wt. every 8 hours during the first week of life) led to significant decline in body weight and small intestine length as compared with control piglets [22]. In contrast, the wet weight of the stomach was increased Rindi *et al*. [23] could identified ghrelin mRNA in intestine of human fetus and suggested its developmental role in intestine. According to present study, It is seem that ghrelin can elevate GH-releasing and gastric HCL [24]. May because of HCL-releasing effect, ghrelin could have role in lipid digestion, and because of GH-releasing effect, it could stimulate entrocytes maturation. Finally, matured entrocytes could have considerable ALP activity at different segment of intestine. Furthermore GH-releasing and HCL-releasing can activate ALP and improve its function for digestion and absorption of lipids at different age of broiler chickens. It is concluded**;** in ovo administrated 50 microgram ghrelin at 21- or 42-day of broiler rearing period can has maximum effect on ALP activity. In overall, *in ovo* administration of 50 microgram ghrelin is suggested. These findings are according to previously findings about stimulation of enzymes of pancreas [16]. In ghrelin-treated piglets, the crypt depth was significantly increased in the entire small intestine. Lysosomal vacuoles, markers of epithelial immaturity [25], were present in the enterocytes of ghrelin-treated piglets and were larger compared with controls. Also , present results is concordant with importance of chicken maternal ghrelin and developmental roles of chicken ghrelin that reported and reviewed by Yoshimura et al. [6] and Kaiya *et al*, [26].

SUGGESTIONS

It was suggested that *In-ovo* administration of ghrelin may promote mucosal ALP activity and finally, may be redound to better intestinal digestion and absorption of nutrients. Also, we suggest that *In-ovo* administration of ghrelin, affects the other enzymes mucosal activity test.

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