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In ovo Administration of Ghrelin and Subsequent Intestinal *Leucine aminopeptidase* (LAP) Activity in Broiler Chickens

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Abstract: Aim of this study was to investigation on effect of in ovo administration of ghrelin on subsequent Leucine Aminopeptidase (LAP) 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* administration of 150 μg/egg ghrelin in embryonic period, could stimulate LAP activity at 21-day- old chicks in 10, 30 and 50% of intestine with 3520.4, 266.9, 4595.6 IU/g protein, also *in ovo* injected 50 and 150 μg/egg ghrelin could stimulate LAP activity in 1, 50 and 70% of intestine with 3071.4, 4779.3 and 5013.4 IU/g. In 42-day-old chicks, in ovo injected 50 μg/egg ghrelin could stimulate LAP activity in 1, 10, 30, 40, 70, and 90% percent of intestine. These findings demonstrated stimulatory effects of ghrelin in low doses (50 μg) in chicken intestine LAP activity.

Key words: Chicken, ghrelin, *In ovo* injection, intestine, Leucine Aminopeptidase (LAP)

INTRODUCTION

Nowadays, after more than one decade from discovery of ghrelin (Kojima *et al*., 1999), limited information about chicken ghrelin is available. Ghrelin is a regulatory peptide with acylation on *serin-3* (in many animals such as mammals and avian) with 28 amino acids in human or rat (Kojima *et al*., 1999, 2008) and 26 amino acids in chicken (Kaiya *et al*., 2002), that its acylation structural modification is necessary for biological activity of ghrelin (Kojima *et al*., 2008).

Two major roles of ghrelin have been considered as "grow stimulatory" and "food intake regulation" (Kojima and Kangawa, 2005). In other hand, because of structural similarity with motilin, ghrelin has considerable effects in gastrointestinal motility (Masuda *et al*., 2000). Ghrelin receptors in gastrointestinal neurons had been identified (Broglio *et al*., 2004). 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 (Poitras *et al*., 2005).

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 (Kitazawa *et al*., 2007). *Icv*-injection of ghrelin analog (GHRP06) in 5-day-old layer chickens hadn't any considerable effect on feed remaining in crop or gizzard (Khan *et al*., 2006).

About effects on gastrointestinal organs, the stimulatory effect of luminal ghrelin on the pancreatic exocrine functions (enzyme secretion) is documented (Nawrot-Porabka *et al*., 2007). 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 Leucine Aminopeptidase (LAP) activity in broiler chickens.

MATERIALS AND METHODS

***In ovo* injection:** This study has been conducted in university 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.

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

Nutrients	1-9 d	10-20 d	21-35 d	36-42 d
ME (Metabolisable energy) 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

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 37ºC temprature. 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 Table 1. Six steps rations have been used for all of groups.

Nutrient compositions of fed diets (based on corn and soybean meal) for all of experimental chickens during 42-day rearing period (Table 1).

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 h 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. Hastily, samples of 1, 10, 30, 50, 70 and 90% of the length of small intestines for evaluation of LAP enzyme activity.

Enzymatic assays: In the laboratory, using a sensitive scale, 0.05 g of the mucosal intestine was weighed and along with 10 mL 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.* (1964)'s 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 (Watanaba *et al.*, 1986). The activity level of 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 L/g 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. Comparative analysis of the average of treatments was performed using Duncan's multifunctional method in the random of 5%.

RESULTS AND DISCUSSION

Obtained data from LAP assays are presented as Table 2 and 3. Table 2. Mean LAP activity in 1, 10, 30, 50, 70 and 90% of intestine in 21-day-old chickens (IU/g protein) Table 3. Mean LAP activity in 1, 10, 30, 50, 70 and 90% of intestine in 42-day-old chickens (IU/g protein).

Leucine Aminopeptidase (LAP) is a brush-border and situsolic enzyme which hydrolyzes the smaller peptides and Amino acids at the end of the long chain peptides and its peptidase activity leads to the digestion of the ptoteins in the diet (Benajiba and Maroux, 1980; Bundit, 2005). The effect of ghrelin on the LAP enzyme in the small intestine of chicken broilers is being performed for the first time.

Table2. Mean LAP activity in 1, 10, 30, 50, 70 and 90% of intestine in 21-day-old chickens (IU/g protein)

Intestine length	1% of intestine	10% of intestine	30% of intestine	50% of intestine	70% of intestine	90% of intestine
control	395.5 ±2864.4	855.9 ± 3092.7	407.3 ± 2141.3	7/354 ±3981.2^{ab}	442.4 ± 5371.0	770.9 ± 5113.2
Solution	415.3 ± 2798.9	140.7 ± 2922.0	371.6 ± 2123.3	511.4 ±3586.9^{ab}	415.9 ± 4346.5	222.7 ± 5188.9
50 µg ghrelin	101.0 ± 3071.4	88.11 ± 3048.3	60.0 ± 1985.9	617.2 ± 4779.3^{a}	1266.7 ± 5013.4	309.4 ± 4781.7
100 µg ghrelin	498.0 ± 2715	533.1 ± 2719.1	62.6 ± 2000.5	417.5 ±3343.8^{b}	743.9 ± 3850.2	1056.3 ± 4426.3
150 µg ghrelin	119.3 ± 2626.7	809.1 ± 3520.4	491.3 ± 2666.9	515.8 ± 4595.6^{a}	144.0 ± 4789.2	230.1 ± 4316.6

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

Table3. Mean LAP activity in 1, 10, 30, 50, 70 and 90% of intestine in 42-day-old chickens (IU/g protein)

Intestine length	1% of intestine	10% of intestine	30% of intestine	50% of intestine	70% of intestine	90% of intestine
control	1/377±2728.5^{b}	5/197 ± 2960	562.0 ±3702.4^{ab}	470.0 ± 3279.1^{b}	1301.6 ± 3956.6	923.1 ± 5064.6
Solution	503.3 ± 2627.0^{b}	485.1 ± 2494.3	196.1 ± 3103.3^{b}	229.5 ±3475.8^{b}	315.0 ± 4850.8	656.6 ± 4588.5
50 µg ghrelin	391.9 ± 3713.0^{a}	686.5 ± 3579.6	149.0 ± 3820.3^{ab}	501.3 ±4856.9^{a}	697.1 ± 5407.2	1259.8 ± 5448.3
100 µg ghrelin	428.1 ± 2475.1^{b}	292.4 ± 2952.1	58.2 ± 3958.8^{ab}	151.4 ± 4501.1^{ab}	1197.6 ± 5372.9	927.4 ± 4273.6
150 µg ghrelin	388.4 ± 2573.7^{b}	566.5 ± 3431.9	577.4 ± 4664.9^{a}	393.9 ± 4117.4^{ab}	220.1 ± 4469.4	643.3 ± 4764.4

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

Ghrelin stimulates gastrointestinal tissue structure and function development in animals. Ghrelin retarded gastric, intestinal and pancreatic development (Kotunia and Zabielski, 2006). Rindi *et al.* (2002) could identified ghrelin mRNA in intestine of human fetus and suggested its developmental role in intestine.

In present study, *in ovo* administration of ghrelin or in other words, 150 μg/egg ghrelin in avian embryonic period, could stimulate Lap activity at 21-day- old chicks in 10, 30 and 50% of intestine with 3520.4, 266.9, 4595.6 I U/g protein, *in ovo* injected 50 150 μg/egg ghrelin could stimulate LAP activity in 1,50 and 70% of intestine with 3071.4, 4779.3 and 5013.4 IU/g (Table 2).

In 42-day-old chicks, *in ovo* injected 50 μg/ egg ghrelin could stimulate LAP activity in 1, 10, 30, 40, 70 and 90% of intestine (Table 3). It seems that ghrelin may stimulate gastric acid secretion in gastrointestinal tract (Korbonits *et al.*, 2001) and further acceleration and efficiency of LAP activity for protein or amino acids absorption.

CONCLUSION

In summary, these findings demonstrated stimulatory effects of ghrelin in low doses (50 μg) in chicken intestine LAP activity. Moderate increases resulted by administration of higher doses (100 or 150 microgram) is eccording to previously findings about stimulation of enzymes of pancreas (Nawrot-Porabka *et al.*, 2007). Also, a present result is concordant with importance of chicken maternal ghrelin and developmental roles of chicken ghrelin that reported and reviewed by Yoshimura *et al.* (2009) and Kaiya *et al.* (2007).

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