

Effect of Intracerebroventricular Injection of Ghrelin Peptide On Some of Blood Biochemical in Female Broiler

Habib. Aghdam Shahryar¹⁺, Majid Toghyani², Abolfazl Ghorbani¹ and Alireza. Lotfi¹

¹Department of Animal Science, Shabestar branch, Islamic Azad University, Shabestar, Iran

²Department of Animal Science, Khorasgan branch, Islamic Azad University, Esfahan, Iran

Abstract. Ghrelin is a peptide and ghrelin is brain-gut peptide with growth hormone- releasing inducing activities. In this experiment, following intracerebroventricular (i.c.v) injection of the ghrelin on cholesterol, triglyceride, Ca, P concentrations and alkaline phosphatase activity level of female broiler chickens were evaluated. 144 female one-day-old chicks in the three treatments and four replications were kept in the same terms. In day 21, after weighting, chicks were divided into three groups included: 1) control 2) *i.c.v.* injection of 50 ng/kg body weight in day 21, 3) *i.c.v.* injection of 100 ng/kg body weight in day 21. 12 hours after injection and also in day 42, blood samples were taken and cholesterol, triglyceride, Ca, P and alkaline phosphatase activity level were measured. Data showed that, in female chicks with injected of 50 ng/kg ghrelin, cholesterol, triglyceride (P<0.05) levels in day 21 than day 42 were decreased and P concentrations (P<0.05) and alkaline phosphatase activity level in day 21 than day 42 were increased. Results indicate that *i.c.v.* injection of 50 ng/kg ghrelin can affect on some of blood biochemical and could be blood lipids in female broiler decreased.

Keywords: Ghrelin, intracerebroventricular injection, blood biochemical and broiler

1. Introduction

Ghrelin is a multifunctional regulatory peptide that it was discovered by Kojima *et al.* [1] in rat for first time. Many relative studies were conducted on mammalian species. But functions of ghrelin in chicken are less clear in comparison with mammals. For the first time, Kaiya *et al* [2] could isolate ghrelin peptide from proventriculus of chicken. Chicken ghrelin includes 26 amino acids and is shorter than human or rat ghrelin with 28 amino acids [2]. Ghrelin acts as GH-releasing factor in chicken. Currently, ghrelin has also been identified to be present in albumen and yolk of fertile chicken egg [3]. In fact avian ghrelin is a “stimulator of growth hormone (GH) release” that is well documented with *in vivo* and *in vitro* studies [2, 4 and 5].

In a study, serum GH elevation was observed only minutes after *icv*-injection of human ghrelin [4].

Also, ghrelin mRNA and expression have been identified in follicles [6] and pancreatic cells of chicken [7] and also oviduct of quails [8].

Regardless to ghrelin affects on serum concentration of GH [2] and Prolactin [9]. Many aspects of chicken ghrelin in serum biochemical measures (such as plasma lipids, Ca and P concentrations) are not clear. The aim of this study was to investigation on Influence of intracerebroventricular injection of ghrelin peptide on serum total cholesterol, triglyceride, Ca and P concentrations and alkaline phosphatase activity in female broiler chicks.

2. Material and Methods

⁺Corresponding author. Tel.: +(984712223016); fax: +(984712223016)
E-mail address: (ha_shahryar@yahoo.com)

An experimental, 144 female one-day-old chicks (Ross- 308) in the completely randomized design with three treatments and four replications were kept in the same terms. Chicks fed control diets in experiment period and diets formulated basal on Ross- 308 catalog. All birds received ad libitum access to feed and water. In day 21, after weighting, chicks were divided into three groups with total same weight included: 1) control 2) *i.c.v.* injection of 50 ng/kg body weight in day 21, 3) *i.c.v.* injection of 100 ng/kg body weight in day 21. 12 hours after injection and also in day 42, blood samples were collected from chicks, immediately samples were centrifuged and serum was obtained for determination of cholesterol, triglyceride, Ca, P concentrations and Alkaline phosphatase activity level with Alcyon 300 auto analyzer (Abbott Park, IL., USA) and its commercial specific kits for these measures.

Data obtained by hatching results and laboratory analysis of blood serum were evaluated with SAS software (Ver. 9.1) and the differences between groups were detected with Duncan multiple tests, $P < 0.05$.

3. Results

Serum biochemical is showed in Table. 1. Intracerebroventricular injection of the ghrelin peptide in 50 and 100 ng/kg body weight in 21 and 42d of rearing didn't has any significant effect on total cholesterol, but with injection of 50 ng/kg gherlin, cholesterol concentration was decreased.

Triglyceride (TG) level was significant in day- 21 and day 42. Higher TG level obtained in day 42 ($P < 0.05$). Injection of 50 ng/kg gherlin, lower TG concentration was showed ($P < 0.05$).

Serum Ca concentrations were not significant between groups, but different between time measured ($P < 0.05$). No significant difference between P concentrations in time measured was obtained. Injection of 50 ng/kg gherlin group showed higher P concentration than other groups ($P < 0.05$). There were not any significant differences between Alkaline Phosphatase (ALP) activity rates in time measured and injection levels among experimental groups.

Interactions between time measured and injection levels on cholesterol, TG and ALP were no significant, for Ca and P characters were significant ($P < 0.01$).

Table.1: Effect of injection of ghrelin peptide on some of blood biochemical in female broiler

| Items | Cholesterol (mg/dl) | Triglyceride (mg/dl) | Calcium (mg/dl) | Phosphor (mg/dl) | Alkalin Phosphatase (U/L) |
|----------------------------|------------------------|-------------------------|--------------------|---------------------|---------------------------------|
| <i>Time (T)</i> | | | | | |
| 21day | 148.00 | 66.83 ^b | 9.905 ^a | 8.05 | 3154.2 |
| 42day | 153.11 | 74.83 ^a | 9.172 ^b | 8.25 | 2828.4 |
| SEM | 4.31 | 2.049 | 0.29 | 0.27 | 183.24 |
| P value | 0.187 | 0.014 | 0.025 | 0.432 | 0.221 |
| <i>I.C.V injection (I)</i> | | | | | |
| Control | 142.17 | 70.83 ^a | 9.00 | 5.46 ^b | 2419.6 |
| 50 ng/kg BW | 128.33 | 65.66 ^b | 9.22 | 8.30 ^a | 3293.5 |
| 100 ng/kg BW | 145.67 | 70.66 ^a | 9.38 | 6.28 ^b | 2468.0 |
| SEM | 9.582 | 1.49 | 0.63 | 0.504 | 324.9 |
| P value | 0.097 | 0.035 | 0.090 | 0.030 | 0.319 |
| T × I | 0.699 | 0.075 | 0.002 | 0.007 | 0.358 |

Different letters (a or b) show significant difference.

Serum total cholesterol was not affected by intracerebroventricular injection of ghrelin peptide. Triglyceride concentration was lower in injection of 50 ng/kg gherlin group in comparison with other groups.

It was reported that circulating human ghrelin can decrease blood lipids and is a key factor for prevention of hyperlipidemia after dietary fat intake [10]. The high cholesterol or artherosclerosis cause higher concentration of circulating ghrelin in comparison with healthy condition [11]. Buyse et al. [12] follow

ghrelin injection to chickens, reported decreasing fatty acid synthesis and anti-lipogenic effect of ghrelin. Present results (decreasing serum triglyceride and cholesterol following intracerebroventricular injection of ghrelin peptide) are in accordance with [12] findings and partially are in agreement with Egecioglu *et al.* [10] reports in human. It is suggested, ghrelin has regulatory effect for lipid metabolism in chickens, similar with anti-lipogenic effect of human ghrelin.

The effect of chicken ghrelin on serum Ca, P and ALP is not studied clearly in the published researches. In present study intracerebroventricular injection of ghrelin peptide hadn't any significant effect on ALP activity. Role of ghrelin on ALP activity is limited to some information obtained from mammalian species. Fukushima *et al.* [13] suggested that osteogenesis role of ghrelin is ALP-mediated and ghrelin cause osteogenesis by stimulation of ALP.

Our findings about ALP are opposite to Maccarinelli *et al.* [14] report in mammalian model. No difference between groups in Ca levels. Pérez-Castrillón *et al.* [15] declared that ghrelin hadn't any important role in calcium metabolism and serum Ca hadn't significant correlation with circulation human ghrelin. Increasing serum Ca with injection ghrelin in 21 day is opposite to ghrelin osteogenic effect and osteogenic-related Ca decreases [13 and 16]. It seems that intracerebroventricular injection 100 ng ghrelin in day-21 could stimulate uptake of calcium for cartilage and bone formation that is occurred mainly after day-21, such as tibia formation [17].

4. Conclusion

Based on present results it is concluded that intracerebroventricular injection of 50 and 100 ng/kg body weight ghrelin peptide in day 21, have considerable effects on serum triglyceride, P and minor effect on serum Ca and cholesterol levels in female broiler chicks. Injection of 50ng ghrelin/kg body weight could decrease serum triglyceride and cholesterol levels and increased P and ALP concentration in female chicks. Further studies with other methods and other poultry species is need. In future experiments can help to clarifying various effects of injection maternal or *in ovo* ghrelin in avian species.

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6. References

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