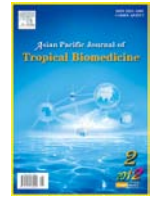




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Effect of intracerebrovascular injection of ghrelin on some of erythropoietic indicators in 42-day-old broiler chicken

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ABSTRACT

Objective: To evaluate the erythropoietic indicators in 42-day-old broiler chicks following intracerebrovascular injection of exogenous rat ghrelin at 21 day of age. **Methods:** A total of 90 male chicks were divided into three experimental groups including three replicates (10 chicks for each replicate): group 1 without any ghrelin injection, group 2 subjected to intracerebrovascular injection of 0.5 mg ghrelin/kg b.w. and group 3 subjected to intracerebrovascular injection of 1.0 mg ghrelin/kg b.w. at 21 days of age. **Results:** Intracerebrovascular injection of 0.5 or 1.0 mg ghrelin/kg b.w. at 21 days of age did not have any significant effect on the measured erythropoietic indicators including red blood cell, hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. **Conclusions:** Intracerebrovascular injection of exogenous rat ghrelin does not have any considerable effect on erythropoietic activity of broiler chickens.

1. Introduction

From discovery of ghrelin peptide by Kojima *et al.*[1] to now, numerous bio-functions have been identified for this regulatory peptide, but many studies in the last decade had focus on two main acts of ghrelin: growth hormone (GH)-releasing and appetite regulation[2,3]. Furthermore, its effects on cardiovascular system were investigated in a group of rats following myocardial infarction, and ghrelin administration has shown to increase body weight, cardiac output, and diastolic thickness of the noninfarcted posterior wall, as well as to inhibit left ventricular enlargement, and intravenous or subcutaneous ghrelin injection increases cardiac output, improves cardiac contractility, and causes a significant decrease in mean arterial pressure, without changing heart rate[4].

Kaiya *et al.*[5] who could isolate ghrelin from chicken

gastrointestinal tract had introduced ghrelin as a 26 amino acid peptide hormone in avian species. Moreover, 80%–90% of peripheral ghrelin is acylated with medium chain fatty acid naturally[6]. Ghrelin is the strength GH-stimulator peptide with stimulation of somatotroph proliferation in chicken, which has been documented via *in vitro* or *in vivo* studies[5,7]. Other functions of chicken ghrelin such as food intake regulation[8], endocrine stimulation of prolactin-release[9] and antioxidative effect[10] have been reported via *in ovo* or intracerebrovascular injection of ghrelin during embryonic or post-hatch life. Intracerebrovascular injection of ghrelin in chickens decreased food intake through glutamatergic system[11]. In addition, Ghazanfari *et al.* conducted a study on *ghrelin* gene mRNA expression in relation with dietary protein and energy contents, and they did not find any considerable effect of these macronutrients on *ghrelin* mRNA expression[12].

Another discussed effect of ghrelin is erythropoietic effect that was suggested by Taati *et al.*[13], but it was not observed in Narin and Çetin[14] studies on mammalian. In Taati *et al.* study, red blood cell (RBC)

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number and hematocrit had significant increases following intracerebrovascular injection of ghrelin in rat[13]. For identification of possible erythropoietic effects of ghrelin, the aim of the present study was to evaluate erythropoietic indicators in 42-day-old broiler chicks following intracerebrovascular injection of exogenous rat ghrelin at 21 days of age.

2. Materials and methods

2.1. Chicken grouping

In present experiment, 1-day-old broiler chicks were obtained from commercial broiler breeders farms (Ross 308). A total 90 male chicks were divided into three experimental groups includes three replicate (10 chicks for each replicate): group 1 without any ghrelin injection, group 2 subjected to intracerebrovascular injection of 0.5 mg/kg b.w. and group 3 subjected to intracerebrovascular injection of 1 mg/kg b.w. ghrelin at 21 day of age. The birds had normal broiler rearing period to 42 day of age and fed with standard grower and finisher rations formulated according to NRC (1994). At 21 day of age, ghrelin injection was done for groups using 22G needles.

2.2. Detection of erythropoietic indicators

Table 1

Erythropoietic measures of broiler chicks following intracerebrovascular injection of exogenous ghrelin.

Group	Ghrelin dosage (mg/kg b.w.)	RBC ($\times 10^6$)	Hematocrit (%)	Hemoglobin (mg/dL)	MCV (fL)	MCH (pg)	MCHC (%)
1	0	6.2	37.0	12.5	60.4	20.3	35.2
2	0.5	6.0	36.3	12.1	60.1	20.1	34.0
3	1.0	6.1	36.7	12.2	60.0	20.0	33.4

No significant difference is found between the groups at the 0.05 level. MCV, MCH and MCHC represent mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, respectively.

4. Discussion

Past reports had shown that anemia suffered patients have low level of peripheral ghrelin[15], and it is proposed that decreases in ghrelin release cause incidence of anemia[16]. Akarsu *et al.* had suggested positive correlation between peripheral ghrelin and hemoglobin concentrations[15]. Ghrelin addition on bone marrow cells causes stimulation of cell proliferation[17]. In present study, intracerebrovascularly injected ghrelin did not have any considerable effect on hematopoiesis in chicken. Present findings are according to those of Narin and Çetin[14] who also did not find any significant effect of rat ghrelin on RBC, Hb or hematocrit rates. GH can stimulate erythropoiesis in mammalian model[18,19] and Taati *et al.* shows similar effect for rat ghrelin (as GHS)[13], but the report of Narin and Çetin[14] is different

At 42 days old (end of rearing period), whole blood samples were collected via wing venous and filled to EDTA included tubes. RBC number, hematocrit, hemoglobin (Hb) and relative erythropoietic indicators were determined by hematocytometer after hematocrit centrifuge.

2.3. Statistical analysis

Data obtained from hematological assays were subjected to statistical analysis with one way ANOVA by SAS software ver. 9.1. Duncan multiple range test was applied for detection of possible significant difference between mean value of experimental groups ($P < 0.05$).

3. Results

Erythropoietic indicators of the experimental groups are presented in Table 1. Intracerebrovascular injection of 0.5 or 1.0 mg ghrelin/kg b.w. at 21 days of age did not have any significant effect on the measured erythropoietic indicators including RBC number, Hb, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

from that of Taati *et al.*[13]. It seems that rat ghrelin in chickens does not affect erythropoiesis, whereas its erythropoietic effects in rodent has debates[13–15]. It was concluded that intracerebrovascular injection of exogenous rat ghrelin does not have any considerable effect erythropoietic activity of broiler chickens. Further investigations with purified chicken ghrelin in chickens on these measures are suggested.

Conflict of interest statement

We declare that we have no conflict of interest.

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