Livestock Science 154 (2013) 158–164

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effect of *in ovo* ghrelin administration on hatching results and post-hatching performance of broiler chickens

A. Lotfi [a,*], H. Aghdam Shahryar [a], H. Kaiya [b]

[a] Department of Animal Science, Shabestar Branch, Islamic Azad University, 53815-159 Shabestar, Iran
[b] Department of Biochemistry, National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka 565-8565, Japan

ARTICLE INFO

Article history:
Received 22 July 2012
Received in revised form
8 March 2013
Accepted 20 March 2013

Keywords:
Ghrelin
Hatching weight
Hatchability
In ovo injection
Chickens

ABSTRACT

The aim of this study was to investigate the effects of *in ovo* ghrelin administration on hatching weight, hatchability, and post-hatch performance of broiler chickens. One thousand fifty fertilized eggs were divided into 7 treatments (150 eggs/treatment): treatment C (control, intact without injection), treatment G50d5 (50 ng ghrelin/egg at day 5), treatment G100d5 (100 ng ghrelin/egg at day 5), treatment G50d10 (50 ng ghrelin/egg at day 10), treatment G100d10 (100 ng ghrelin/egg at day 10), treatment G0d5 (vehicle without ghrelin at day 5), and treatment G0d10 (vehicle without ghrelin at day 10). Hatchability in all of the injected treatments was lower than C ($P<0.01$), indicating an influence of injection that was augmented. Hatchability of treatments G50d10 and G100d10 were greater than treatment G0d10 ($P<0.01$). Hatching weight was greater in all injected treatments than intact. Healthy hatched chicks from each treatment were allocated to 21 floor pens with three replicate pens pretreatment and 7 chicks per pen. Chicks were fed common starter and grower diets, and BW and feed intake were recorded weekly. At day 42 post-hatch, one chick from each replicate that had BW close to the mean was selected. Blood samples were collected, and, then, chicks were harvested to evaluate carcass quality. There was a decrease in feed intake and feed conversion ratio (FCR) for treatments subjected to *in ovo* ghrelin ($P<0.01$). There were increases in thigh, breast, and gizzard weights, and abdominal fat deposition in 42-d old chickens on treatment G100d10 ($P<0.01$). The results indicated that *in ovo* administration of exogenous ghrelin can improve hatching weight and post-hatch performance, and *in ovo* administration of 100 ng ghrelin/egg, can be efficient in increasing breast and thigh yields. When suitable hatching weight, performance, and carcass yield are the main focus of broiler production, *in ovo* injection of 50 ng ghrelin/egg can be beneficial.

1. Introduction

Ghrelin is a multifunctional regulatory peptide that was discovered in the rat stomach by Kojima et al. (1999). Numerous studies have demonstrated various functions of ghrelin, such as its effect on growth hormone (GH)-releasing activity (Hashizume et al., 2005), food intake, weight gain and energy balance (Nakazato et al., 2001; Toshinai et al., 2003), and some endocrine/paracrine functions (Dezaki et al., 2008).

A lot of evidence supports reproductive effects of ghrelin in mammalian models; ghrelin injection to pregnant rat cause greater body weight of offspring (Nakahara et al., 2006) or detection of ghrelin and growth hormone secretagogue receptor 1a (GHS-R1a) mRNA expression in sheep placenta (Harrison et al., 2007), with its increasing with developmental stage of embryo, as well as indicated effects of maternal ghrelin in reproduction.

* Corresponding author. Tel.: +98 914 3060 782.
E-mail address: arlotfi@gmail.com (A. Lotfi).

1871-1413/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2013.03.020

Chicken ghrelin consisted of 26 amino acids and is shorter than human or rat ghrelin with 28 amino acids (Kaiya et al., 2002). Ghrelin acts as a GH-releasing factor in chicken (Ahmed and Harvey, 2002). Ghrelin gene expression in avian embryo indicates developmental effects of chicken ghrelin (Chen et al., 2007; Shao et al., 2010; Sirotkin et al., 2006). *In ovo* ghrelin has been identified in albumen and yolk of fertile chicken egg (Yoshimura et al., 2009), and its gene expression was observed during embryonic life, especially after day 5 of incubation (Gahr et al., 2004). Also, ghrelin mRNA and expression have been identified in follicles (Sirotkin et al., 2006), pancreatic cells of chicken (Richards et al., 2006), and oviduct of quail (Yoshimura et al., 2005).

Based on the reported findings about maternal or *in ovo* ghrelin and GH-releasing effect of ghrelin, it seem that *in ovo* ghrelin may improve growth and cause greater hatching indices, including increased hatching weight and success of hatching rate. It was proposed that ghrelin as a GH-releasing peptide may cause growth stimulation in chicken embryo and subsequent greater initial body weight for hatched chickens that is an important factor for broiler production. In past studies, administration of growth stimulatory peptides such as insulin-like growth factor-1 (IGF-1; Kocamis et al., 1998) and peptide YY (Coles et al., 1999) via *in ovo* injection bio-technique had increased growth and improved feed conversion ratio in broiler chickens. The aim of the present study was to investigate the effect of exogenous *in ovo* ghrelin on hatching weight, post-hatching performance (feed intake, weight gain, and feed conversion ratio), and carcass characteristics of broiler chickens.

2. Materials and methods

2.1. In ovo injection procedure

In this experiment, 1050 fertilized eggs were collected from commercial breeder flock (Ross 308). The eggs were divided into 7 treatments (150 eggs/treatment): control (C; without injection), treatment G50d5 (*in ovo* injected with 50 ng ghrelin/egg at day 5 of incubation), treatment G100d5 (*in ovo* injected with 100 ng ghrelin/egg at day 5), treatment G50d10 (*in ovo* injected with 50 ng ghrelin/egg at day 10), treatment G100d10 (*in ovo* injected with 100 ng ghrelin/egg at day 10), treatment G0d5 (*in ovo* injected with solvent, without ghrelin at day 5), and treatment G0d10 (*in ovo* injected with solvent, without ghrelin at day 10). All eggs were incubated with normal incubation condition (37.8 °C and 60% relative humidity). The lyophilized rat ghrelin was obtained (Sigma-Aldrich Co., St. Louis, MO, US), dissolved in 1% acetic acid solvent, and prepared to desired concentrations. The injected volume was 500 μL/ egg for all of injected eggs. At day 5 of incubation, *in ovo* (in albumen) injection was done for treatments G50d5, G100d5, and G0d5. At day 10, the same *in ovo* injection were done for treatment G50d10, G100d10, and G0d10 at day 10 at a hygiene dark partition with 37 °C. Before injection, egg shells were marked for identification of air cell position and optimum injection point via candling. The 22 G needles were used for in albumen injection. Needle angle to the injection point was around 45° and it was 5 mm away from air cell, without any possible damage to air cell. Each sterilized needle was used for individual injection. Embryos and their macroscopic development were observed during incubation process by candling. At day 21 (the end of incubation), hatchability (hatched eggs/total fertile eggs) and hatching weight of chicks were determined for all treatments. Experimental procedure was in according to recommendations of Islamic Azad University—Veterinary department Animal ethics committee.

2.2. Post-hatch housing

This part of study took place at poultry research station of Iranian Agricultural Research Center (eastern Azerbaijan province, Iran) in 2010. The healthy hatched chicks from each treatment were allocated in equal numbers in 21 floor pens (three pens for each treatment and seven chicks per pen). Diets formulated according to NRC (1994) recommendations (Table 1) were used for preparation of starter and grower diets for all birds. Body weight and feed intake were recorded weekly. At the end of experiment or day 42 post-hatch and after determination of feed intake, body weight, and feed conversion ratio (FCR), one chick from each pen of each treatment with body weight close to the mean of each pen was selected. Blood samples were collected from wing veins using sterilized syringes. Birds were then harvested to evaluate carcass quality.

Table 1
Ingredients and nutrient specifications of experimental diets for broiler chickens.

Item	Starter (0–21 d)	Grower (22–42 d)
Ingredient (%)		
Corn	60.36	65.44
Soybean meal	34.12	28.62
Calcium carbonate	1.22	1.19
Dicalcium phosphate	1.83	1.80
Vegetable oil	1.49	1.91
Lys	0.11	0.16
DL-Met	0.17	0.18
Vitamin–mineral premix[a]	0.50	0.50
Salt	0.20	0.20
Compositions (calculated)		
MEn (kcal/kg)	2901	2987
Crude protein (%)	20.30	18.30
Ca (%)	1.00	0.96
Available P (%)	0.50	0.48
Lys (%)	1.20	1.10
Met+Cys (%)	0.46	0.44

[a] Supplied per kilogram of diet: 6050 μg vitamin A (retinyl acetate +retinylpalmitate), 55 μg vitamin D_3, 22.05 μg vitamin E (alpha-tocopheryl acetate), 2.0 mg vitamin K_3, 5 mg vitamin B_1, 6.0 mg vitamin B_2, 60 mg vitamin B_3, 4 mg vitamin B_6, 0.02 mg vitamin B_{12}, 10.0 mg pantothenic acid, 6.0 mg folic acid, 0.15 mg biotin, 0.625 mg ethoxyquin, 500 mg CaCO, 80 mg Fe, 80 mg Zn, 80 mg Mn, 10 mg Cu, 0.8 mg I, and 0.3 mg Se.