

Effects of peripheral ghrelin on growth performance, selected blood biochemical parameters and thyroid hormone in Japanese quail (*Coturnix coturnix japonica*)

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Summary: The aim of present study was to investigate the effects of peripheral administration of ghrelin on performance and some serum parameters of Japanese quail. One hundred twenty 21 day-old male Japanese quails were divided into three groups for a 35 days experimental rearing period. The control group (G0) included no injection as an intact, the second group (treatment 1/G50) received 50 ng ghrelin/kg body weight (BW), and the third group (treatment 2/G100) was given 100 ng ghrelin/kg BW. Ghrelin was administrated (I. P. administration) at the onset of the experimental rearing period on 21-day old birds. Blood samples were taken at two different times: 1) 12 hrs after the injection, and; 2) at the end of the rearing period (on day 35). The effects of ghrelin were evaluated during experimental rearing periods. There were no changes in feed intake and body weight gain (BWG) following ghrelin administration at different dosages (50 or 100 ng/kg BW), whereas a minor decrease in feed intake was observed in G100 group. Feed conversion ratio (FCR) is significantly decreased in G50 and G100, compared to control ($P < 0.05$). Thyroxin (T_4) was increased in ghrelin-administered groups in both samples (short/long-term). In conclusion, ghrelin administration in Japanese quails may improve FCR, and stimulate secretion of thyroid hormones, without severe effects on feed intake. However, it did not have considerable effects on carcass characteristics and serum biochemical measures in short and long-terms in Japanese quails.

Keywords: Biochemistry, carcass quality, feed conversion ratio, Japanese quail, thyroid hormones.

Introduction

Since ghrelin was discovered in animals (12), numerous studies have been conducted in relation to its physiological and metabolic functions in different animal species. These reports have shown its various regulatory functions namely; growth hormone - releasing activity (7), appetite regulation, weight control and energy metabolism (15, 23, 24) and reproductive effects (5), whereas, the regulatory roles of ghrelin peptide are not limited to the effects mentioned. Ghrelin was identified in birds by Kaiya et al. (8); in chicken (*Gallus gallus domesticus*) where it formed with 26-amino-acids. In total, pre-proghrelin coding sequences have been identified in 31 species of birds such as chicken, emu, turkey, goose, duck, pigeon and Japanese quail (20). The impacts of ghrelin on growth performance of chickens and goose have been studied in numerous experiments (2, 13, 14). However, it has been documented that ghrelin has GH-releasing activity in vertebrates such as chicken, it might also cause particular and different functions such as feed intake inhibition in birds (9). Hypothetical role of ghrelin on bird feed intake is an interesting research subject which has created a lot of debates among interested scholars. Some studies (4, 18) have revealed that central-injection of

ghrelin inhibits food intake in chickens. Studies have indicated that the peripheral ghrelin in broiler chickens has an inhibitory effect on feed intake (3, 6, 17), whereas this effect has not been shown in layer chickens, as other species of birds (10). Interestingly, in a study on Japanese quail conducted by Shousha et al. (21), infusion of peripheral (exogenous) ghrelin caused an increase in feed intake, whereas central (endogenous) ghrelin may inhibit feeding. Shousha et al. (22) also stated that endogenous ghrelin may reduce water intake in Japanese quail. In goose (as other species of birds), infusion of exogenous ghrelin caused a temporary increase in feed intake during the growing period but was not stable during the entire rearing period (2).

A review of the related literature reveals that, so far, the effect of peripheral ghrelin on feeding has been studied on three species of birds; chicken, goose and quail. Shousha et al. (21, 22) studied feed and water intake of Japanese quail, however, effects of ghrelin on growth performance, carcass characteristics and blood biochemical indices are somewhat unknown. Therefore, the aim of the present study was to investigate possible effects of peripheral ghrelin on growth performance (feed intake, body weight, and feed conversion ratio), carcass

quality and selected hemato-chemical measures of Japanese quail, as a complete and comparative study with attention on previous related reports on other poultry species.

Materials and Methods

Present study was conducted in poultry station of Iranian Agricultural Research Center in Eastern Azerbaijan Province in northwest of Iran. Materials of the study included one hundred twenty 21 day-old male Japanese quails (*Coturnix coturnix japonica*) (BW: 64g ± 1); they were selected randomly and further assigned into three groups, based on complete randomization design (CRD). Each group included four replicates and each replicate (as floor pen) included ten birds. Floor pens were used as the context for housing. Moreover, the condition of the experiment made feed available as *ad libitum*.

Lyophilized rat ghrelin was purchased from Sigma-Aldrich Co. (USA), dissolved in 1% acetic acid solvent according to manufacturer's instructions and diluted with distilled water to the desired amount (0.5 ml for each injection sample/each bird). Pre-experimental tests showed that administration of acetic acid (as a solvent) injection did not have any biological effects on animals' health, performance and related parameters (14). Ghrelin was administered peripherally (I.P.: intraperitoneal injection) at the onset of the experimental rearing phase (21-day old birds). Injected doses were as follows: control (G0): intact without any injection; treatment 1 (G50): 50 ng ghrelin/kg body weight (BW), and treatment 2 (G100): 100 ng ghrelin/kg BW.

Diet and performance: Diets formulated according to NRC (16) recommendation (Table 1) were used for all birds during the experiment (from days 21 to 35). Body weight, feed intake and mortality rate were recorded during the experimental rearing period (21 to 35 days) to determine and evaluate growth performance. The mean mortality rate of whole experimental rearing period was 1.49% for G0, G50 and G100 groups, and there were not any considerable differences between groups (injected and non-injected).

Carcass yield and serum biochemical assays: On day 21 (12 h after the administration of ghrelin) and at the end of the rearing period (on day 35), two birds from each replicate of the experimental groups with a body weight (basic weight) close to the average weight of the replicate were selected. Then, blood samples were taken from wing veins, centrifuged (1200 X g, 8 min, 18° C), and serum was obtained for measuring selected biochemical and hormonal parameters of blood (glucose, total cholesterol, triglyceride, total protein, Triiodothyronine-T₃, and thyroxin-T₄) by using Alcyon 300 auto analyzer (Abbott Park, IL., USA) and its commercial ELISA specific kits (Pars Azmoon kits, Pars Azmoon Inc., Tehran). Analysis

procedure was conducted immediately after blood sampling.

Table1. Ingredients and specifications of experimental diets for Japanese quail during experimental rearing period (d 21 to 35).

Ingredients	Control
Yellow Corn	53.31
Soybean Meal	39.69
Corn Gluten Meal	3.07
Vegetable Oil	1.00
Oyster Mushroom	-
Oyster Shell	1.22
Di Calcium Phosphate	0.77
L- Lysine	0.06
DL-Methionine	0.12
Mineral-Vitamin Premix*	0.5
Sodium Chloride	0.25
Calculated analysis	
ME (Kcal/Kg)	2900
CP (%)	24.00
Calcium (%)	0.80
Phosphor (%)	0.29
Sodium (%)	0.11
Lysine (%)	1.30
Methionine + Cycteine (%)	0.89

*Supplemented for kg of the diets: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3mg; B12, 0.03 mg; Biotin, 0.1 mg; Choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu, 12 mg; I, 1 mg; Se, 0.2 mg, and adequate antioxidant.

To determine the characteristics of carcass yields, 35 day-old birds were slaughtered (3 birds from each experimental group which were not subjected to blood sampling). Carcass characteristics including carcass weight, pectoral muscle, thigh, liver and abdominal fat content were measured and calculated by dividing the weight of each mentioned organ into individual final BW (day 35). The effects of ghrelin administration on feed intake, body weight gains (BWG), and feed conversion ratio (FCR) were determined for experimental rearing period. The experimental procedures of this study were conducted regarding the recommendations of the Animal Ethics Committee of the Veterinary Studies of Islamic Azad University (Reg. no.7884).

Statistical analyses of the results: In the present study, experiment was designed depending on complete randomization (CRD) of the sample birds. Statistical analysis was carried out using the GLM procedure of SAS Statistical Analysis Software Ver. 9.1 (19). The probability of difference between the experimental groups was detected by the Tukey's range test, and the probability value was set at P<0.05 for checking the statistical

significance of difference between the independent treatments. The statistical model is presented below:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where,

Y_{ij} : all dependent variable

μ : overall mean

T_i : the effect of ghrelin dosages ($i = 1, 2, 3$)

E_{ij} : the random effect of residual

Results

Measured growth performances (feed intake, BWG, and FCR) are presented in Table 2. There were no significant changes in feed intake and body weight gain

(BWG) following ghrelin administration in different dosages (50 or 100 ng/kg BW; Table 2), whereas a minor (not significant) decrease in feed intake was observed in G 100 group. FCR was significantly affected by ghrelin dosages (50 or 100 ng/kg BW), and decreased significantly in G50 and G100 groups when compared to control ($P < 0.05$; Table 2).

Table 3 illustrates the effects of administrated ghrelin on carcass characteristics in Japanese quail. It had no significant effects on final body weight, and carcass characteristics including carcass yield, thigh, breast muscle, liver and abdominal fat.

Table 2. Performance of Japanese quails subjected to peripheral administration of ghrelin (d 21-35).

Groups	Injection dosage (ng/kg BW)	Feed intake (g)	BWG ¹ (g)	FCR ²
Intact (control)	0	459.1	108.4	4.23 ^a
G50	50	453.4	111.2	4.07 ^a
G100	100	448.1	112.3	3.99 ^b
<i>P</i> -value		0.53	0.08	0.04
<i>SEM</i>		3.96	2.02	0.05

^{a-b} Means with different letters are different ($P < 0.05$).

¹ BWG: body weight gain.

² FCR: feed conversion ratio.

G50: groups that received 50 ng/BW exogenous ghrelin.

G100: groups that received 100 ng/BW exogenous ghrelin.

Table 3. Carcass characteristics in Japanese quails subjected to peripheral administration of ghrelin.

Groups	Injection dosage (ng/kg BW)	Carcass yield (%)	Breast muscle (%)	Thigh (%)	Abdominal fat (%)	Liver (%)
Intact (control)	0	56.69	25.12	12.55	1.02	2.59
G50	50	56.82	25.10	12.58	0.99	2.61
G100	100	57.11	25.44	12.60	0.97	2.65
<i>P</i> -value		0.34	0.26	0.31	0.12	0.30
<i>SEM</i>		0.57	0.53	0.55	0.09	0.41

¹ Carcass characterizations are presented with percent of live weight.

G50: groups that received 50 ng/BW exogenous ghrelin.

G100: groups that received 100 ng/BW exogenous ghrelin.

Table 4. Serum biochemical and hormonal parameters in 21d old Japanese quails subjected to peripheral administration of ghrelin (12 h after ghrelin administration).

Groups	Injection dosage (ng/kg BW)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	T ₃ (mg/dl)	T ₄ (mg/dl)
(control)	0	209.20 ^b	202.60 ^a	89.80	2.62	12.04 ^b
G50	50	213.20 ^a	203.40 ^a	85.20	2.96	13.08 ^a
G100	100	214.20 ^a	182.40 ^b	90.80	2.90	13.62 ^a
<i>P</i> -value		0.011	0.001	0.101	0.66	0.013
<i>SEM</i>		1.31	2.72	2.31	0.16	0.09

^{a-b} Means with different letters are different ($P < 0.05$).

G50: groups that received 50 ng/BW exogenous ghrelin.

G100: groups that received 100 ng/BW exogenous ghrelin.

Table 5. Serum biochemical and hormonal parameters in 35d old Japanese quails subjected to peripheral administration of ghrelin.

Groups	Injection dosage (ng/kg BW)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	T ₃ (mg/dl)	T ₄ (mg/dl)
(control)	0	197.0	190.40	78.20	2.58	12.02 ^b
G50	50	198.60	189.00	79.20	2.62	12.18 ^b
G100	100	194.20	186.20	82.60	2.63	13.34 ^a
<i>P</i> -value		0.132	0.08	0.102	0.401	0.001
<i>SEM</i>		1.85	1.35	1.21	0.03	0.02

^{a-b} Means with different letters are different ($P < 0.05$).

G50: groups that received 50 ng/BW exogenous ghrelin.

G100: groups that received 100 ng/BW exogenous ghrelin.

Tables 4 and 5 illustrate the effects of ghrelin administration on serum biochemical and hormonal parameters in Japanese quail. Table 4 shows that there were no significant effects on biochemical measurements including glucose, total cholesterol, triglyceride, and total protein following ghrelin administration ($P < 0.05$), whereas total cholesterol decreased in ghrelin-administered groups, especially in G100 group ($P < 0.01$). Table 4 shows that there were no significant effects on biochemical measures (including glucose, total cholesterol, and triglyceride) following ghrelin administration.

T₄ of 21-day old birds was increased in both ghrelin-administered groups (G50 and G100). Effects of ghrelin on thyroid hormones of 35 day old birds were only observed in T₄ (Table 5) and also in long-term samples (Table 5), whereas these effects were not significant in triiodothyronine (T₃).

Discussion and Conclusion

As earlier stated, findings on the effects of ghrelin in quail performance are limited. Yoshimura et al. (25) found that ghrelin-producing cells exist in the reproductive organs of Japanese quail, especially in the mucosal epithelium of the oviduct, and ghrelin-producing/releasing activity is associated with the development of this organ. In a comparative study, Kitazawa et al. (11) reported that ghrelin has contractile effects on upper and lower segments of gastrointestinal tract and it stimulates motility of middle intestine in quail. Shousha et al. (21) who had reported initial results on peripheral and central effects of rat ghrelin showed that different methods and dosages of ghrelin administration might have different effects on feed intake in Japanese quail, and low dosages of peripheral ghrelin may act as hunger signal in birds.

In the present study, peripheral ghrelin improved feed conversion ratio with minor decreases in feed intake (Table 2). This result is in accordance with Shousha et al.

(21), who reported that an increased dosage of peripheral ghrelin decreases feed intake in Japanese quail. In addition, our findings are in agreement with Geelissen et al. (6) and Lotfi et al. (14) who reported that infusion of exogenous ghrelin may cause decreased feed intake and improved FCR in broiler chickens.

It seems that increased serum T₄ levels following ghrelin administration (short-term effect) indicate increased metabolic rate due to ghrelin infusion as reported by Buyse et al. (3) in chickens. In a previous study (1), a severe increase in T₄ was observed following *in ovo* infusion of higher dosages of exogenous ghrelin. Present findings on thyroid hormones of quails (Table 4) are in agreement with Buyse et al. (3) and Aghdam Shahryar and Lotfi (1) and it may indicate that ghrelin administration in Japanese quails causes increased metabolic rate similar to chickens. On the other hand, avian ghrelin is a stimulator of hypothalamo-pituitary-adrenal axis (17) which is an alternative mechanism for high metabolic rates (with cooperation of thyroid hormone) in birds such as Japanese quails. It seems that present effect of ghrelin may not significantly affect feed intake, body weight and carcass characteristics on the long-term (Tables 2 and 3).

In conclusion, ghrelin administration in Japanese quails may improve feed conversion ratio under rearing conditions and stimulate thyroid hormones release, as a short-term effect. However, it has no considerable effects on carcass characteristics and serum biochemical measures. It can thus be suggested that ghrelin has somewhat similar functions in Japanese quails, in terms of metabolic effects, when compared to chicken or mammalian ghrelin.

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