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Effect of ghrelin peripheral administration on growth performance, carcass quality, and selected serum parameters in broiler chickens

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ABSTRACT. The aim of present study was to investigate the short-term and long-term effect of the peripheral administration of ghrelin on the growth performance (feed intake, weight gain, and feed conversion ratio), carcass quality, and selected serum biochemical (glucose, total cholesterol, triglyceride, and total protein) and hormonal (T₃, T₄, and corticosterone) indices in broiler chickens. 240 one-day-old broiler chickens were selected, and allocated into three treatment groups (control and two experimental groups). On day-21 of the rearing period, ghrelin was peripherally administered to three experimental groups. The control group contained birds without any administration of peptide or solution, groups G50 and G100; included birds with Ip-injection of 50 and 100 (ng/100g BW) ghrelin peptide, respectively. The peripheral administration of exogenous ghrelin did not affect feed intake, body weight gain (BWG), feed conversion ratio (FCR) and carcass characteristics in broiler chickens. In short-term samples taken 12h after ghrelin infusion, the glucose level was increased in ghrelin-treated groups (162 and 151 mg/dl in G50 and G100 compared with 117 mg/dl in control; P< 0.01) and there were significant declines for TC, triglyceride, and TP in the ghrelin-treated groups (G50 and G100) compared with the control. In addition, long-term glucose level has a greater value in G50 and G100 (182 and 200.66 mg/dl) compared with control (133.60 mg/dl) group (P< 0.01). A significant decline was also observed for TC and triglyceride content in the ghrelin-treated groups (P<0.05). There was no significant difference among groups for TP in short-term and long-term samples. There was a significant increase for T₄ in ghrelin-treated groups (G50 and G100) compared with the control (4.55 and 4.57 ng/ml vs 4.20 ng/ml respectively; P< 0.05) in long-term samples. In conclusion, the peripheral administration of ghrelin in broiler chickens, during the commercial rearing period did not affect the overall growth performance, carcass quality and feed conversion ratio. The infusion of exogenous ghrelin may increase the levels of serum glucose, decrease total cholesterol and triglyceride, and T₄ levels are increased in the long-term (and not in the short-term or 12h after administration).

Keywords: Commercial growth performance, ghrelin, metabolism, regulatory peptide, broiler chicken.

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INTRODUCTION

Ghrelin is one of most discussed regulatory peptide in the recent two decades (Kojima et al., 1999; Benso et al., 2013; Pradhan et al., 2013). Due to its multifaceted properties, ghrelin is the one of multifunctional peptides with strong effects on endocrine axes (Kluge et al., 2010; Spencer et al., 2012), oxidative system, appetite (Inui et al., 2004; Klok et al., 2007), and growth performance (Ukkola and Pöykkö., 2002; Dimaraki and Jaffe, 2006), although not limited to these functions. Ghrelin has general (same) and specific functions in various species of animals (mammalian and non-mammalian), especially in birds (Kaiya et al., 2009, 2013; Tachibana and Tsutsui., 2016). In addition, the peptide structure of ghrelin can be different in animal species (Kojima et al., 2008).

The identification of chicken ghrelin by Kaiya et al. (2002) resulted in several scientific debates, due to the different effects of ghrelin on feed intake in avian species (Saito et al., 2002; Kaiya et al., 2013; Tachibana and Tsutsui., 2016). Ghrelin gene expression, density of ghrelin immunopositive cells, and the peripheral ghrelin level in chickens vary according to age (Yu et al., 2016) and feed additives (Poorghasemi et al., 2018). Ghrelin is known to serve as biological signal of energy utilization and is involved in energy homeostasis in broiler chickens (Song et al., 2018).

Administration of ghrelin with peripheral and central methods in chicken is evident in numerous studies (Geelissen et al., 2006; Oclon and Pietras., 2011; Zendejdel and Hassanpour., 2014). it generally reduces feed intake (hypophagia) in broiler chickens, however, in none of these studies, the performance and carcass quality of chicken were investigated. In a study with *in ovo* (pre-hatching) administration of ghrelin in chicken, a significant decrease in post-hatching feed intake was observed (Lotfi et al., 2013). However, after a post-hatch treatment the results were different (Kaiya et al., (2007), since plasma ghrelin levels in laying hens were not affected by its administration. Also in other study, the plasma level of ghrelin was not correlated with the laying-performance of layer-type birds (Höhne et al., 2017).

On the other hand, the effects of peripheral ghrelin administration in poultry species is not completely similar; for example in domestic geese (*Anser anser domesticus*) results in an increase of feed intake during the growing period (Aghdam Shahryar and

Lotfi., 2015), a finding that is in contrast with that of Oclon' and Pietras, (2011) and Lotfi et al., (2013) in broiler chickens.

Interestingly, the administration of ghrelin antagonist ([D-Lys3]-GHRP-6), decreased feed intake, and increased plasma T_4 in chicken (Aghdam Shahryar and Lotfi., 2016). Taking into consideration the different effects of ghrelin in birds, it seems that several mechanisms involved in ghrelin activity, and this peptide affects endocrine-axes, such as hypothalamo-pituitary-adrenal and hypothalamus-pituitary-thyroid axis (Oclon' and Pietras, 2011; Kluge et al., 2010).

In literature, the effects of ghrelin on hormonal parameters of poultry species are relatively similar, and there is no considerable difference among poultry species. In detail, the administration of ghrelin causes temperate insulin decreases in geese (hyperglycemic effect; Aghdam Shahryar et al., 2014), and in newly-hatched broiler chicks (Lotfi et al., 2011), and it may cause an increase of thyroid hormones levels in poultry species, especially in chickens and domesticated turkey (Aghdam Shahryar and Lotfi., 2013; 2017).

In overall, although several studies have been conducted examining the effects of ghrelin in poultry species, the effect of peripheral administration of exogenous ghrelin on broilers' growth performance parameters such as feed intake and carcass quality have not been fully assessed. It seems that acknowledge of the effect of ghrelin on broilers' performance is necessary for completing ghrelin "puzzle" in poultry. Aim of the present study was therefore to investigate the short-term and long-term effects of peripheral administration of ghrelin on growth performance (feed intake, weight gain, and feed conversion ratio), carcass quality, and selected serum biochemical (glucose, total cholesterol, triglyceride, and total protein) and hormonal (T_3 , T_4 , and corticosterone) indices in broiler chickens.

MATERIALS AND METHODS

The experiment was conducted at Poultry Farm of Islamic Azad University, Shabestar Branch (North West of Iran) in summer 2016.

Grouping, Feeding and Housing

In the present study, 240 one-day-old male broiler chicks (Ross 308) were selected, and assigned into 3 treatment groups (one control and two experimental

groups). The treatment included 5 replicates and there were 16 chickens per replicate. A completely randomized design (CRD) was used in this experiment. Diets included three formulas (Table 1), in accordance to NRC (1994). The diets and fresh water offered *ad libitum* to birds. The ambient temperature was gradually decreased from 32 °C on day 1 to 24 °C on day 20 and was then kept constant. The lighting program was provided as 23h L:1 h D throughout the study.

Table 1. Ingredients and nutrient specifications of experimental diets

Item <i>Ingredient, %</i>	Starter 1-10 d	Grower 11-24 d	Finisher 25-42 d
Corn (cp =8.5%)	53.82	54.50	56.08
Soybean meal (cp= 44%)	40.00	39.00	37.00
Soybean oil	1.80	2.80	3.50
DCP	1.45	1.18	1.22
Oyster meal	1.70	1.47	1.31
Vitamin - mineral premix ¹	0.50	0.50	0.50
DL-Methionine	0.32	0.21	0.14
Lysine	0.16	0.09	-
Salt	0.25	0.25	0.25
Compositions (calculated)			
ME (kcal/kg)	2854	2950	3000
Crude Protein%	21.36	21	21
Ca %	0.99	0.86	0.70
P available %	0.49	0.425	0.414
Met + Cys %	1.02	0.89	0.80
Lysine %	1.34	1.19	1.13

¹Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12,000 IU; cholecalciferol 1,500 IU; vitamin E, 30 mg; vitamin K3, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 5 mg; vitamin B12, 30 µg; Ca-D- panthotenate, 10 mg; folic acid, 0.75 mg; D-biotin, 0.08 mg; Mn, 80 mg; Zn, 60 mg; Fe, 40 mg; Cu, 5 mg; Se, 0.15 mg; Co, 0.1 mg; I, 0.4 mg

On day-21 of the experimental rearing period, one group was considered as the control while the two other groups served as the experimental groups and they were subjected to ghrelin Ip-Injection (Sigma Aldrich, USA).

The experimental groups were characterized as follows:

- Group 1 (control): Birds without any administration of peptide or solution.
- Group 2 (G50): Ip-injection of 50 ng/100g body weight ghrelin peptide on day-21.
- Group 3 (G100): Ip-injection of 100 ng/100g

body weight ghrelin peptide on day-21.

Injection procedure

Lyophilized ghrelin powder (rat) was purchased from Sigma Aldrich Company (G8903, USA). Similar peptide with chicken ghrelin was used in the present experiment, in accordance with Saito et al. (2002b). The powder was solved in distilled water according to the manufacturer's instructions. The infused solution (0.5 ml) was administered to each chicken. Injections were performed using 30g needle on day 21. The procedure was carried out in a sterile environment and in a special injection chamber, according to animal ethics (Reg. no.7884).

At the end of day 21 (12 h after injection), blood samples (9 samples/group) were collected from all groups for the examination and assessment of the initial effect of the injected solution. The rearing period continued up to day 42 under commercial rearing conditions, which were similar to those in the commercial broiler farms.

Data collection and analysis

Feed intake, BWG (body weight gain) and FCR (feed conversion rate) were recorded for three weeks, namely from day 21 up to day 42. On day 42 (end of the rearing period), after measuring the weight of chickens, additional blood samples were collected (from the vein of the wing). Blood samples were analyzed to determine the serum thyroid hormones (T₃ and T₄) and the following biochemical indices: glucose, total cholesterol (TC), triglyceride, total protein (TP). Measurements were performed by using a biochemical auto-analyzer with Elisa kits of Pars Azmoon Company (Biochemical kits, Pars Azmoon Co. Tehran, Iran) for checking biochemical parameters. Also, Roche testing kits (12017709122, Roche Ltd., Basel, Switzerland) and Chicken CORT ELISA Kit (MBS701668, MyBiosource, Inc., San Diego, CA) were used for thyroid hormones, and corticosterone, respectively.

Carcass traits

On the 42 day of the study, 5 birds from each replicate (pen) were randomly selected and slaughtered by decapitation. After removal of skin and feather, carcass, breast and thigh muscles, liver, and abdominal fat were weighed individually. Yields were expressed as the percentage of live BW.

Statistical analysis

Statistical analysis of the data was conducted using the GLM procedure by SAS Statistical Analysis Software (SAS Inst. Inc., Cary, NC, 2000). Significant differences among the experimental and control groups were detected by ANOVA (analysis of variance) and Tukey test. The probability value was set at $P < 0.05$ for checking the statistical significant differences among groups. The applied statistical model is the following:

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

Where,

Y_{ij} : all dependent variable

μ : overall mean

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

E_{ij} : the random effect of residual

RESULTS

Growth performance and carcass characteristics

The performance of broiler chickens with respect to feed intake, BWG, and FCR is presented in Table 2. The results obtained in this study indicate that the peripheral administration of exogenous ghrelin caused minor reductions (*ns*) in feed intake and in BWG (*ns*) during the rearing period. Hence, there was no significant change in FCR, due to ghrelin administration.

In other words, the administration of two different doses of ghrelin did not cause any effect on the performance of broiler chickens, as illustrated in Table 2.

In Table 3, no significant difference between the control and ghrelin-treated groups for live weight, carcass yield, and relative weight of breast muscle, abdominal fat, and liver (*ns*) was also observed and there was only a significant decrease in thigh percentage of carcass in the ghrelin-treated groups ($P < 0.01$).

Blood biochemical parameters

In Table 4 (short-term effect), the glucose level was increased in the ghrelin-treated groups (G50 and G100) compared with the control (162 and 151 mg/dl vs 117 mg/dl, respectively; $P < 0.01$) and a significant decrease for TC, and triglyceride in the ghrelin-treated groups (G50 and G100) compared with the control. Triglyceride levels were lower in G100 group. In long-term effect (table 5), the glucose level was at a greater level in G50 and G100 as compared with the control (182 and 200.66 mg/dl vs 133.60 mg/dl, respectively; $P < 0.01$), in accordance with the findings of short-term effects. A significant decline was observed for TC and triglyceride in the ghrelin-treated groups ($P < 0.05$). There was no significant change among groups for TP.

Blood hormonal parameters

In Table 6 (short-term effect), there was no significant differences among groups. A trend of lower T_3 and corticosterone and higher T_4 levels existed in ghrelin administered groups. In Table 7, (long-term effect), there was a significant increase for T_4 in the ghrelin-treated groups (G50 and G100), compared with the control (4.57 ng/ml vs 4.20 ng/ml; $P < 0.05$). There was no significant difference among groups for T_3 and corticosterone.

Table 2. Performance of male broiler chick subjected to IP-injection of ghrelin

Treatment	Injection dosage (ng/100g BW)	Growing period d 1-21			Finishing period d 21-42		
		Feed intake (g)	BWG ¹ (g)	FCR ²	Feed intake (g)	BWG (g)	FCR
Intact (control)	0	890.25	552.50	1.61	1471.10	775.30	1.82
G50	50	845.50	544.20	1.55	1389.40	768.10	1.81
G100	100	840.20	553.40	1.52	1361.30	770.30	1.76
<i>P</i> -value		0.4129	0.3351	0.0857	0.2341	0.1412	0.0905
SEM		21.29	15.14	0.04	20.16	12.20	0.03

¹ BWG: body weight gain.

² FCR: feed conversion ratio.

Table 3. Carcass traits ¹ of male broiler chick subjected to IP-injection of ghrelin (% of live weight)

Treatments	Injection dosage (ng/100g BW)	Live weight (g)	Carcass yield (%)	Breast muscle (%)	Thigh (%)	Abdominal fat (%)	Liver (%)
Intact (control)	0	1903	69.29	25.10	25.30 ^a	1.88	2.99
G50	50	1855	67.04	23.90	23.98 ^c	2.01	2.65
G100	100	1955	69.83	24.27	24.67 ^b	1.95	2.65
<i>P</i> -value		0.2156	0.4017	0.0942	0.004	0.5283	0.0578
SEM		35.22	1.43	0.323	0.11	0.07	0.09

^{a-c} Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

¹ Carcass traits are presented with percent of carcass weight.

Sample number per organ = 5

Table 4. Serum biochemical indices in male broiler chick subjected to IP-injection of ghrelin in day 21(12 h after ghrelin administration)

Treatment	Injection dosage (ng/100g BW)	Glucose (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)
(control)	0	117.00 ^b	191.00 ^a	78.30 ^a	5.03 ^a
G50	50	162.00 ^a	156.33 ^b	85.33 ^a	4.30 ^b
G100	100	151.00 ^a	157.31 ^b	61.00 ^b	4.40 ^b
<i>P</i> -value		0.005	0.0014	0.0022	0.0106
SEM		6.17	4.03	2.80	0.12

^{a-b} Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

Table 5. Serum biochemical indices in male broiler chick subjected to IP-injection of ghrelin in day 42

Treatment	Injection dosage (ng/100g BW)	Glucose (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)
(control)	0	133.60 ^c	192.31 ^a	79.62 ^a	5.10
G50	50	182.00 ^b	137.65 ^c	69.31 ^b	5.00
G100	100	200.66 ^a	168.33 ^b	69.66 ^b	4.80
<i>P</i> -value		0.001	0.02	0.0313	0.0893
SEM		3.73	4.06	2.08	0.14

^{a-c} Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

Table 6. Some of serum hormones indices in male broiler chick subjected to IP-injection of ghrelin in day 21(12 h after ghrelin administration)

Treatment	Injection dosage (ng/100g BW)	T3 (ng/mL)	T4 (ng/mL)	Corticosterone (ng/mL)
(control)	0	2.16	4.10	4.83
G50	50	1.73	4.63	3.96
G100	100	1.80	4.53	4.43
<i>P</i> -value		0.0544	0.0673	0.076
SEM		0.16	0.21	0.32

Table 7. Some of serum hormones indices in male broiler chick subjected to IP-injection of ghrelin in day 42.

Treatment	Injection dosage (ng/100g BW)	T3 (ng/mL)	T4 (ng/mL)	Corticosterone (ng/mL)
(control)	0	1.70	4.20 ^b	5.93
G50	50	1.53	4.55 ^a	4.80
G100	100	1.66	4.57 ^a	4.83
<i>P</i> -value		0.1517	0.0250	0.081
SEM		0.05	0.19	0.43

^{a-c} Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

DISCUSSION

As already pointed out, ghrelin is mainly considered as a “hunger signal” in chicken, similar to its function in mammalian systems (Kaiya et al., 2007, 2013). This function is referred to the endogenous ghrelin (Kaiya et al., 2002, 2007) and is possibly not connected with the peripherally administered exogenous ghrelin that was applied in the present study. The administration of exogenous ghrelin has different effects on appetite or feed intake in chickens (Stimulation/No effect/Inhibition) as reviewed by Kaiya et al., (2013). Tachibana et al., (2011) reported that feed intake is not affected by the administration of ghrelin in neonatal broiler chicks, whereas Buyse et al. (2009) found that peripherally injected ghrelin inhibited feed intake. In the present study (table 2), feed intake was not affected by ghrelin-administration, and there was no significant effect on the growth performance or feed conversion ratio. Therefore, the present findings are in accordance with the results of Tachibana et al. (2011), who stated that the administration of ghrelin did not affect feed intake. In a recent study conducted by Hühne et al. (2017) on laying hens and also in a study of our research team in geese (Aghdam Shahryar and Lotfi, 2015), peripheral ghrelin administration did not affect energy demands or feed intake at whole growing period or at least finishing rearing-period. It appears that in agreement with Kaiya et al., (2013), only central-/ or endogenous- ghrelin may have a significant effect on appetite (feed intake) regulation in birds, and not exogenous or peripherally-administered ghrelin, especially in finishing rearing-period of chickens. Conversely, exogenous -(or administered) ghrelin may play a potential role in appetite regulation, only in the growing period (before d 21). In the studies of Buyse et al. (2009) and Lotfi et al. (2013) in neonatal chickens, the infusion of exogenous ghrelin significantly affected subsequent feed intake and metabolism.

These findings indicate that the endogenous ghrelin system may be completely developed in a later period and is not affected by peripherally-administered excessive ghrelin. This hypothesis is in agreement with the findings of Hühne et al. (2017), who found that plasma ghrelin has no significant effects on energy status and performance of layer-type chickens.

As indicated, there were significant short-term increases for glucose, and decreases in TC, triglyceride, and TP levels in groups subjected to ghrelin-administration (table 4). On the other hand, TP level did not dif-

fer among experimental groups on day 42 (ns; table 5).

The infusion of ghrelin that is one of the key regulatory peptides in glucose metabolism (Vestergaard, et al., 2008; Delhanty and Van der lely., 2011; Deza-ki., 2013) may cause notable elevation in serum glucose levels.

Lotfi et al. (2013) showed a hyperglycemic effect of *in ovo*-administered ghrelin in newly-hatched chicks. It seems that the up-regulation of plasma glucose level due to ghrelin infusion is a physiological pathway to obtain energy for hemostasis in birds (Geelissen et al., 2006; Lotfi et al., 2013; Aghdam Shahryar et al., 2014). Therefore, the findings of the present study for glucose level in both short- and long-term samples is in agreement with the regulatory effect of ghrelin on glucose metabolism. On the other hand, reduction in the levels of lipidemic indices (TC and triglyceride) in the present study (table 4 and 5) revealed the lipolytic effect of ghrelin, which was reported in newly-hatched chicks by Buyse (2009). In this regard, molecular studies showed that the infusion of exogenous ghrelin may stimulate the mRNA expression of lipolytic genes in birds (Furuse et al., 2001; Geelissen et al., 2006; Buyse et al., 2009; Ocelon and Pietras., 2011; Kaiya et al., 2013). Ghrelin may trigger lipolysis to obtain energy and glucose for homeostasis in broiler chickens, as suggested by Briggs and Andrews (2011).

In accordance with the findings of the present study, T₄ as a main thyroid hormone and as a hormone which plays a key role in metabolism (Kim, 2010) increased significantly in the groups treated with ghrelin as a long-term result (table 7), whereas the thyroid hormones did not differ in the short-term samples (table 6). It has been reported that the administration of ghrelin might cause an increase in fT₄ and there was a direct effect of ghrelin on the activity of the thyroid gland (Benso et al. 2013). Our previous study on newly-hatched chickens showed that an *in ovo* administration of ghrelin caused an increase in T₄ (Aghdam Shahryar and Lotfi., 2013) and in another study on domesticated turkey (Aghdam Shahryar and Lotfi., 2017), the peripheral-administration of ghrelin caused a significant increase in the T₄ long-term levels. Also, similar results were reported in ruminants by Khazali (2005). A possible mechanism for this stimulatory effect on the level of T₄ could be correlated to the direct effect of ghrelin on the hypothalamus-pituitary-thyroid (HPT) axis (Kluge et al., 2010).

CONCLUSION

In conclusion, the peripheral administration of ghrelin in broiler chicken, during the commercial rearing period did not affect the overall growth performance, carcass quality and feed conversion ratio. However, it may increase the levels of serum glucose, and decrease the total cholesterol and triglyceride levels. In serum hormones, T_4 increases in the long-term (and not in the short-term). It seems that ghrelin affected and stimulated the hypothalamic - pituitary - thyroid axis and subsequently increased the levels of T_4 . This mechanism indicated that exogenous ghrelin might increase metabolic rate and regulate lipogenesis in birds, whereas it is insufficient to affect feed intake or the total growth performance. These findings indicate that the endogenous ghrelin system may have been developed in an earlier period and it is not affected by the peripheral-administration of excess-

sive ghrelin. Also, the possible effect of ghrelin on appetite/ feed intake in chicken, may only be considerable in the earlier period (growing period: d 0-21) as reported in the related literature. In a comparative term- it can be suggested that ghrelin has some similar and some various physiological effects in difference species of poultry, also been mentioned in the reviews by Honda et al., (2017) and Kaiya et al., (2013). Also, with attention to the temperate or weak metabolic effect of peripherally-administered exogenous ghrelin in mature chickens (finishing period), the administration of exogenous ghrelin cannot be useful during commercial rearing, in terms of performance. Further studies are warranted for the full elucidation of ghrelin activities.

CONFLICT OF INTEREST

None declared.

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