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Effects of peripheral administration of ghrelin antagonist [D-Lys^3]-GHRP-6 on growth performance and blood biochemical indices in broiler chickens

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Abstract. In the present study, possible effects of peripheral administration of ghrelin antagonist [D-Lys^3]-GHRP-6 on chicken performance, thyroid hormones level and serum biochemical parameters were investigated. Broiler chicks divided into five experimental groups were reared up to day 42. On day 21, a treatment was assigned to the five groups: group 1 (control), chickens without any administration of peptide or solution; group 2 (G50), chickens with intraperitoneal (IP) injection of 50 ng per 100 g body weight (BW) of D-Lys^3 peptide on day 21; group 3 (G100), chickens with IP injection of 100 ng per 100 g BW of D-Lys^3 peptide on day 21; group 4 (G150), chickens with IP injection of 150 ng per 100 g BW of D-Lys^3 peptide on day 21; and group 5 (G200), chickens with IP injection of 200 ng per 100 g BW of D-Lys^3 peptide on day 21. On days 21 (post-injection) and 42 (post-rearing), blood samples were obtained from the animals for laboratory analyses. Experimental groups administered the GHS-R antagonist showed less feed intake – i.e., administration of greater doses led to less feed intake ($P < 0.01$). Daily weight gains within groups G150 and G200 decreased ($P < 0.01$) in comparison with the control. The feed conversion ratio (FCR) did not differ among the groups. There was a significant difference between control and experimental groups for glucose, total cholesterol and phosphorus levels ($P < 0.01$) in post-injection samples. In post-injection and post-rearing blood samples, the thyroid hormone (T_3 and T_4) in 6 h increased in treated groups in comparison with the control ($P < 0.01$). The infusion of ghrelin antagonist [D-Lys^3]-GHRP-6 reduces feed intake and body weight. With regard to increase in T_4 level, it can be inferred that [D-Lys^3]-GHRP-6 may increase metabolic rate, lipolysis and weight loss, which is similar to results obtained in mammalian species.

1 Introduction

Although endocrine regulation of growth in mammals is known and determined, its impact on poultry is far from clear (Kaiya et al., 2008; Kim, 2010). Therefore, the study of regulation of growth hormone in poultry is considered to be a research gap. Hence, in recent years, studies on endocrine regulation of growth in poultry have focused on growth hormone (GH)-releasing peptides such as ghrelin (Kaiya et al., 2007, 2008; Lotfi et al., 2013). The GHS-$R_1\alpha$ transcripts have been identified in peripheral organs of chicken. Whereas ghrelin antagonist ([D-Lys^3]-GHRP-6) has been studied in mammalian species, its antagonism has not been studied in poultry species. GH-releasing peptides have two similar functions: (1) a growth stimulatory effect and (2) regulation of appetite and food intake (Kojima et al., 1999; Kaiya et al., 2008, 2013). In mammals, ghrelin is the main regulatory peptide in appetite regulation (Kojima et al., 1999). However, although numerous studies have been conducted on appetite and the impact of novel regulatory peptides on appetite in birds, mechanisms of hypothalamic regulation of appetite and food intake in poultry remain open research questions which should be addressed and investigated by researchers (Kaiya et al., 2009; Fang et al., 2014; Liu et al., 2014). Interestingly, different administration/injection methods (central or peripheral) of ghrelin might bring about different results

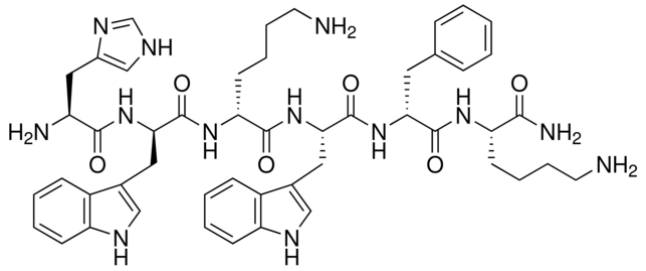

Figure 1. Chemical structure of [D-Lys^3]-GHRP-6.

(Kaiya et al., 2013). In an in ovo study (Lotfi et al., 2013), the pre-hatching infusion of exogenous ghrelin caused a decline in post-hatching feed intake.

Growth hormone secretagogue receptor (GHS-R) is a G protein-coupled receptor which binds ghrelin; indeed, it plays a considerable role in energy homeostasis, metabolism and regulation of body weight (Smith et al., 2001). Similar to the findings obtained about mammals, GHS-R1a transcripts have been identified in brain regions and peripheral organs of chickens (Geelissen et al., 2003; Saito et al., 2005; Yamamoto et al., 2008; Kaiya et al., 2014). The genes of different isoforms of GHS-R, i.e., GHS-R1a and GHS-Rb, can be found in all tissues of chickens (Kaiya et al., 2014).

Ghrelin receptors can bind with GHS-R antagonists; these receptors easily block them. By manipulating related peptides, GHS-R antagonists can be produced (Fig. 1). A minor alteration in GHRP-6a (a GH-releasing peptide) by replacing D-lysine with alanine can change GHS specification of GHRP-6 and produce a new biomolecule with an antagonistic effect on GHS-R (Smith et al., 1993). A synthetic peptidic ghrelin antagonist [D-Lys^3]-GHRP-6 (His-DTrp-D-Lys-Trp-D-Phe-Lys-NH_2), as shown in Fig. 1, is extensively used in vivo and in vitro as a preferred ghrelin receptor (Smith et al., 1993; Patel et al., 2012).

Ghrelin is documented as a strong GH stimulatory agent (Kojima et al., 1999). Almost all considerable regulatory effects of ghrelin have recently been identified (Kojima et al., 1999; Kaiya et al., 2003). If ghrelin is assumed to be an endocrine peptide which has numerous endocrine effects, blocking its receptors may change endocrine and metabolic body profile. As a case in point, Aghdam Shahryar and Lotfi (2014) conducted a simple study on peripheral infusion of ghrelin antagonist [D-Lys^3]-GHRP-6 in rats. They found that infusion of exogenous GHS-R antagonist may increase serum glucose and cortisol concentration; however, this effect was not completely different from the ghrelin effect. More recent studies did not find a significant impact of the infusion of ghrelin antagonist [D-Lys^3]-GHRP-6 on food intake and appetite in a rat model (Khazali and Mahmoudi, 2013; Gomez and Ryabinin, 2014). Costantini et al. (2011) compared the impact of a novel ghrelin receptor antagonist in dogs and mice; their findings were different from earlier studies. That is, they noted that infusion of GSK1614343 (a novel GHS-R antagonist) produced an unexpected increase in food intake and body weight. In another study, Khazali and Mahmoudi (2009) investigated thyroid hormones and found that GHSR-Iα antagonists can reduce body weight through an increase in T_3 and T_4 levels.

In the present study, possible impacts of ghrelin antagonists [D-Lys^3]-GHRP-6 on chicken performance (feed intake, weight gain, and feed conversion ration), thyroid hormones (T_3 and T_4) and some serum biochemical indices were investigated under commercial rearing conditions. Indeed, since there are differences in the impact of ghrelin between chicken and mammals in terms of appetite or feed intake (Kaiya et al., 2007, 2008, 2013), this study investigating how the ghrelin receptor antagonist might affect feed intake in chickens is of high significance.

Table 1. Ingredients and nutrient specifications of experimental diets (g kg^{-1}).

Item ingredient, %	Grower (21–42 days)
Corn (cp = 8.5 %)	545
Soybean meal (cp = 44 %)	390
Soybean oil	28
DCP	11.8
Oyster meal	14.7
Vitamin – mineral premix*	5
DL-Methionine	2.1
Lysine	0.9
Salt	2.5
Compositions (calculated)	
ME (kcal kg^{-1})	2950
Crude protein	210
Ca	8.6
P available	4.25
Met + Cys	8.9
Lysine	11.9

* Vitamin and mineral premix provided per kilogram of diet: vitamin A, 12 000 IU; cholecalciferol, 1500 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.

2 Materials and methods

In the present study, 300 one-day-old broiler chicks were randomly divided into five experimental groups (treatment). The treatments included four replicates, with 15 male chicks within each replicate. A completely randomized design was utilized in this study. Diets included two formulas (Table 1) according to NRC (1994) and were same for all groups during the whole rearing period. One group was considered to be the control group, while the four other groups were the experimental groups which were subjected to intraperi-

Table 2. Effects of peripheral GHS-R antagonist on performance in broiler chicks.

Treatment	Control	GHS-R-A (50)	GHS-R-A (100)	GHS-R-A (150)	GHS-R-A (200)	*P* value	SEM
Feed intake, g day^{-1} (FI)							
FI (21–28 days)	119.7^{a}	115.5^{b}	111.1^{c}	107.6^{c}	101.4^{d}	0.001	0.868
FI (28–35 days)	148.3^{a}	137.8^{b}	130.5^{c}	130.4^{c}	126.5^{d}	0.001	0.786
FI (35–42 days)	161.6^{a}	154.3^{b}	155.4^{b}	149.1^{c}	145.3^{d}	0.001	0.650
Total FI (21–42 days)	3007.2^{a}	2853.2^{b}	2779.0^{c}	2709.7^{d}	2612.4^{e}	0.001	22.20
Weight gain, g day^{-1} (wg)							
WG (21–28 days)	75.2^{a}	71.3^{a,b}	68.4^{b,c}	64.2^{c}	67.8^{b,c}	0.002	0.975
WG (28–35 days)	74.3^{a}	74.6^{a}	70.4^{a,b}	65.5^{b,c}	61.8^{c}	0.001	1.114
WG (35–42 days)	78.2^{a}	75.5^{a}	76.3^{a}	68.7^{b}	62.4^{c}	0.001	1.031
Total WG (21–42 days)	1593.9^{a}	1549.8^{a,b}	1505.7^{b}	1388.8^{c}	1365.0^{c}	0.001	14.722
Feed conversion rate (FCR)							
FCR (21–28 days)	1.60^{b}	1.62^{a,b}	1.62^{a,b}	1.67^{a}	1.50^{c}	0.001	0.015
FCR (28–35 days)	1.99^{a}	1.85^{b}	1.85^{b}	1.99^{a}	2.05^{a}	0.0019	0.012
FCR (35–42 days)	2.06^{b}	2.04^{b}	2.04^{b}	2.17^{a,b}	2.33^{a}	0.0013	0.038
FCR (21–42 days)	1.88	1.94	1.84	1.95	1.94	0.4509	0.017

Different letters (a–e) show significant difference between means. FCR = feed intake (g) / gain weight (g).

toneal (IP) injection of ghrelin antagonist [D-Lys^3]-GHRP-6 (Sigma Aldrich, USA). The experimental groups of the study were characterized and arranged as follows:

- Group 1 (control): chickens without any administration/injection of peptide or solution
- Group 2 (G50): chickens with IP injection of 50 ng per 100 g body weight D-Lys^3 peptide on day 21.
- Group 3 (G100): chickens with IP injection of 100 ng per 100 g body weight D-Lys^3 peptide on day 21.
- Group 4 (G150): chickens with IP injection of 150 ng per 100 g body weight D-Lys^3 peptide on day 21.
- Group 5 (G200): chickens with IP injection of 200 ng per 100 g body weight D-Lys^3 peptide on day 21.

2.1 Injection procedure

Lyophilized peptide ghrelin antagonist [D-Lys^3]-GHRP-6 was purchased from Sigma Aldrich (G4535, USA). The peptide-containing powder was dissolved in distilled water according to the manufacturer's instructions. Using 30G needles, 0.5 mL of the solution was injected per chicken on day 20 of the rearing period with single IP injection. The procedure was carried out in a sterile environment and in accordance with animal ethics. The mean body weight of chicks on day 21 (injection day) was 650 ± 30 g.

All chickens were then transferred to the rearing farm, and at the end of day 21 (6 h after injection), blood samples were taken from all the groups so as to examine and analyze the initial impact of the injected solution (short-term effect). The injection procedure and blood sampling was completed within 3 h.

The rearing period continued up to day 42 under conditions similar to those in commercial broiler farms. At the end of the rearing period, blood samples were taken for determination of long-term effects of infused peptide.

2.2 Data collection and analysis

The feed intake, BWG (body weight gain) and FCR (feed conversion ratio) were observed and evaluated for 3 weeks, from day 21 to day 42 in the experimental rearing period. After measuring the weight of chickens on day 42 (end of the rearing period), the second blood samples were analyzed to determine serum thyroid hormones (T_3 and T_4) and the following biochemical indices: glucose, total cholesterol, HDL cholesterol (HDL-C), triglyceride, total protein, albumin, globulin, calcium (Ca) and phosphorus (P). The measurements were examined by means of auto-analyzer biochemical analyses with Elisa kits of Pars Azmoon Co. (biochemical kits, Pars Azmoon Co., Tehran, Iran) for checking of biochemical parameters. Also, Roche testing kits (12017709122, Roche Ltd., Basel, Switzerland) were used for thyroid hormones.

Statistical analysis of the data was conducted using the GLM procedure in SAS (Statistical Analysis Software; SAS Inst. Inc., Cary, NC, 2000). The probability of significant differences among the experimental and control groups were statistically analyzed by ANOVA (analysis of variance) and the Tukey test. The probability value was set at $P < 0.05$ for

Table 3. Effects of peripheral GHS-R antagonist on biochemical parameters in broiler chicks (6 h after injection).

Treatment	Glucose	Cholesterol	Triglyceride	HDL	Calcium	Phosphorus	Total protein	Albumin	Globulin
			mg dL^{-1}					g dL^{-1}	
Control (0 ng / 100 g BW)	224.0^{a}	140.0^{a}	79.0	7.72^{b,c}	6.30	2.97^{b}	4.13^{b,c}	1.33^{b,c}	2.80^{a,b,c}
GHS-R antagonist (50 ng / 100 g BW)	219.7^{a,b}	141.0^{a}	70.0	74.0^{b,c}	5.80	3.87^{a}	3.80^{d}	1.27^{c}	2.53^{c}
GHS-R antagonist (100 ng / 100 g BW)	197.3^{c}	135.3^{a,b}	72.0	63.3^{c}	6.30	4.30^{a}	4.03^{c,d}	1.43^{a,b}	2.60^{b,c}
GHS-R antagonist (150 ng / 100 g BW)	215.0^{a,b,c}	133.3^{a,b}	77.3	80.0^{a,b}	6.03	4.20^{a}	4.36^{a,b}	1.50^{a}	2.87^{a,b}
GHS-R antagonist (200 ng / 100 g BW)	203.0^{c}	130.3^{b}	68.3	86.3^{a}	6.87	4.50^{a}	4.50^{a}	1.53^{a}	2.97^{a}
P value	0.0047	0.0097	0.3316	0.0012	0.1207	0.0004	0.0001	0.0004	0.0033
SEM	4.120	1.803	4.055	2.633	0.257	0.160	0.063	0.030	0.063

Different letters (a–d) show significant difference between means.
$n = 6$ per experimental group.

checking the statistical significance of difference among the independent treatments. The statistical model is given below:

$$Y_{ij} = \mu + \mathrm{T}_i + E_{ij},$$

where Y_{ij} are all dependent variables, μ is the overall mean, T_i is the effect of ghrelin levels ($i = 1, 2, 3$) and E_{ij} is the random effect of residual.

3 Results

3.1 Growth performance

The performance of broiler chickens with respect to feed intake, WG, and FCR is given in Table 2. The results obtained in this study indicate that the peripheral administration of GHS-R antagonist led to significant differences in feed intake from days 21 to 42 of the rearing period ($P < 0.01$). When compared with control group ($P < 0.01$), the experimental groups showed significantly less feed intake. The impact of GHS-R antagonist showed a linear relationship with feed intake; that is, increased GHS-R antagonist administration resulted in decreased feed intake (Table 2). It was noted that daily weight gain (42-day-old chickens) in groups G150 and G200 decreased significantly in comparison with the control group ($P < 0.01$).

Regarding FCR, feed intake and WG variations among the experimental groups did not have a significant impact on FCR. In other words, the experimental groups were not significantly different from each other in terms of FCR, as shown in Table 2.

3.2 Serum biochemical indices

3.2.1 Post-injection (6 h after injection of GHS-R antagonist)

Post-injection results of serum biochemical indices obtained from the first blood samples (6 h after GHS-R antagonist injection) are given in Table 3. Significant difference was detected between the control group and the experimental groups with respect to glucose, total cholesterol and phosphorus levels. Nevertheless, no significant difference was observed among the groups with regard to triglyceride and calcium levels following the treatment (Table 3).

When compared with the control group ($P < 0.05$), serum glucose and P concentrations significantly decreased within groups G100 and G200. Moreover, total cholesterol (TC) decreased within groups G100, G150 and G200, which was not the case in the control group ($P < 0.01$). Regarding HDL-C, the administration of different dosages of GHR antagonist resulted in moderate or non-significant impacts on HDL-C concentration. In contrast, HDL-C significantly increased within the G200 group ($P < 0.01$).

3.2.2 Post-rearing (on slaughtering)

Serum biochemical indices obtained from second blood samples (post-rearing) are given in Table 4. As shown in this table, there was a significant difference between control and experimental groups with regard to glucose, TC and HDL-C. However, as shown in Table 3, significant differences were not observed among groups with respect to triglyceride, Ca and P concentrations. The G200 group, which had the greatest dosage, had the greatest concentration of glucose in comparison with the control, G50 and G100 groups ($P < 0.01$). Similar to the data obtained from the first blood sample, the administration of different dosages of GHR antagonist resulted in a moderate or no effect on HDL-C concentration. Hence, the differences were significant only among the experimental groups (G50 and G150), but compared with the control group ($P < 0.05$), the difference was insignificant. Similarly, total cholesterol decreased in the G50, G100, G150, and G200 groups in comparison with control ($P < 0.01$).

3.3 Thyroid hormones (T_3 and T_4)

Thyroid hormone (T_3 and T_4) increased within G100, G150 and G200 but was not the case within the control and G50 groups (post-injection; $P < 0.01$). As shown in Table 5, the administration of 100–200 ng of GHR antagonists caused significant elevations in T_3 and T_4 concentrations in chickens immediately after the injection.

Table 4. Effects of peripheral GHS-R antagonist on biochemical parameters in broiler chicks (42 days).

Treatment	Glucose	Cholesterol	Triglyceride	HDL	Calcium	Phosphorus	Total protein	Albumin	Globulin
			mg dL^{-1}					g dL^{-1}	
Control (0 ng / 100 g BW)	210.0^{b,c}	157.3^{a}	71.7^{a}	75.3^{a,b}	8.37^{a,b}	4.80	5.33^{a}	1.73	3.60^{a}
GHS-R antagonist (50 ng / 100 g BW)	197.0^{c}	148.3^{b}	65.3^{a,b}	69.0^{b}	7.43^{b}	5.77	5.43^{b}	1.53	2.90^{b}
GHS-R antagonist (100 ng / 100 g BW)	201.3^{c}	143.0^{b}	63.7^{a,b}	83.7^{a,b}	8.13^{a,b}	5.10	5.53^{a}	1.77	3.77^{a}
GHS-R antagonist (150 ng / 100 g BW)	226.0^{a,b}	141.7^{b}	62.3^{a,b}	88.7^{a}	8.80^{a,b}	5.67	5.00^{a,b}	1.70	3.30^{a,b}
GHS-R antagonist (200 ng / 100 g BW)	241.7^{a}	133.0^{c}	61.0^{b}	76.0^{a,b}	9.13^{a}	5.80	5.43^{a}	2.13	3.30^{a,b}
P value	0.001	0.001	0.0335	0.426	0.0521	0.29	0.0044	0.1357	0.0097
SEM	4.371	1.843	2.071	4.000	0.352	0.408	0.162	0.146	0.135

Different letters (a–c) show significant difference between means.
$n = 6$ per experimental group.

Table 5. Effects of peripheral GHS-R antagonist on thyroid hormones in broiler chicks (ng mL^{-1}).

Treatment	21 days (6 h after injection)		42 days	
	T_3	T_4	T_3	T_4
Control (0 ng / 100 g BW)	1.327^{b}	5.767^{b}	2.34^{b}	6.67^{c}
GHS-R antagonist (50 ng / 100 g BW)	1.426^{b}	5.800^{b}	2.42^{b}	6.87^{c}
GHS-R antagonist (100 ng / 100 g BW)	2.017^{a}	7.200^{a}	2.51^{a,b}	6.87^{c}
GHS-R antagonist (150 ng / 100 g BW)	2.017^{a}	7.267^{a}	2.89^{a,b}	9.53^{a}
GHS-R antagonist (200 ng / 100 g BW)	2.033^{a}	7.567^{a}	2.98^{a}	8.03^{b}
P value	0.001	0.001	0.010	0.001
SEM	0.078	0.126	0.117	0.140

Different letters (a–c) show significant difference between means.
$n = 6$ per experimental group.

Measures of thyroid hormone (T_3 and T_4) related to post-rearing blood samples (Table 5) increased within G50, G100, G150 and G200 groups (all of the treated groups) in comparison with the control group. The administration of 200 ng of GHR antagonist led to significant elevations in T_3 concentration in chickens. Regarding T_4, significant increases were observed within G150 and G200 groups in comparison with the control group and the experimental groups treated with lower dosages ($P < 0.01$).

4 Discussion

As previously mentioned, studies conducted on the ghrelin system in chickens provide evidence indicating that endogenous chicken ghrelin is a "hunger signal" which is similar to mammalian ghrelin (Kaiya et al., 2007, 2013). However, the results obtained from earlier studies argued that the administration of exogenous ghrelin can inhibit feed intake (Geelissen et al., 2006; Buyse et al., 2009; Ocłón and Pietras, 2011; Lotfi et al., 2013). The impact of GHS-R antagonist on chicken feed intake, which is regarded as a research gap, was addressed by Asakawa et al. (2003), who found that the acute (high level) and central (intracerebroventricular) infusion of ghrelin receptor antagonist reduces food intake and body weight gain in laboratory mice. They maintained that changes in glycemic control attributed to infusion of GHS-R antagonist might cause less food intake and weight gain (Asakawa et al., 2003). In another study, Lin et al. (2011) mentioned that ablation of ghrelin receptor may cause less food intake and reduce adiposity in mice. In a study on growing pigs (Vizcarra et al., 2007), after active immunization against ghrelin, reduced feed intake (mild anorexia) and body weight gain were observed. However, a study on turkeys (avian) had completely different findings when compared with those observed in pigs (mammalian).

In the present study, as shown in Table 2, feed intake decreased in chickens after the peripheral administration of GHS-R antagonist with regard to dosage. Hence, groups treated with greater dosages showed the lowest feed intake compared to other groups ($P < 0.01$). Similarly, body weight gain within the experimental groups decreased after the peripheral administration of different dosages of GHS-R antagonist ($P < 0.01$).

The results obtained in this study are in line with those of Asakawa et al. (2003) and Puzio et al. (2011), who reported decreased feed intake and body weight in rodents after the administration of ghrelin receptor antagonist. Furthermore, the results reported in this study, as shown in Table 2, are in agreement with those of Lin et al. (2011) who reported less food intake in mice with ablation of ghrelin receptor. Nevertheless, the results observed in this study with respect to decreased feed intake in chickens, given in Table 2, contradict those of Vizcarra et al. (2012), who studied active immunization of ghrelin in turkeys. Such different research results may be attributed to the differences in the mechanisms of appetite/feed intake regulation (that is, the mechanism of ghrelin immunization differs with GHS-R antagonism) in poultry species (Kaiya et al., 2007, 2013) and also different birds strains (Saneyasu et al., 2011). The findings reported in this study on feed intake also confirm those of Kaiya et al. (2013),

who argued that endogenous ghrelin is a hunger signal in chickens.

Indeed, ghrelin is deemed to be a catabolic hormone which can stimulate hypothalamic pituitary–adrenal (HPA) axis in chickens (Kaiya et al., 2002; Ocłón and Pietras 2011); hence, it can stimulate appetite/feed intake via lipolysis and fat utilization (Kaiya et al., 2002, 2013). It seems that the blocking ghrelin receptor in the present study eliminated or reduced the effect of ghrelin on the HPA axis as it did not stimulate increased appetite in birds. In other words, the hunger signal (ghrelin signal) was weakened due to the infusion of GHS-R antagonist.

Asakawa et al. (2003) argued that continuous injection of [D-Lys^3]-GHRP-6 (200 ng, every 12 h for 6 days) in rats resulted in considerable decreases in serum glucose level and moderate decrease in total cholesterol. Lin et al. (2011) maintained that the ablation of ghrelin receptor led to a hypoglycemic effect in old rats.

The present study indicated that birds had significantly smaller glucose level after 6 h of GHS-R antagonist injection (Table 3). Also, total serum cholesterol and P decreased after the peripheral administration of GHS-R antagonist (Tables 3 and 4). With regard to post-injection glucose and cholesterol levels, which are illustrated in Table 3, it can be pointed out that the findings reported in this study are in agreement with those of Asakawa et al. (2003) and Lin et al. (2011). In metabolic terms, when the ghrelin signal (hunger signal) of appetite stops due to the injection of GHS-R antagonist, serum glucose and lipid reductions are assumed to be logical and natural. These findings in relation to the declines in feed intake (Table 2) and serum lipids (Tables 2 and 3) are also acknowledged by Zigman et al. (2005), who argued that ghrelin signaling is required for the development of diet-induced obesity.

A temporary reduction in serum P level in chickens (post-injection; Table 3) has been observed; nevertheless, the value of this parameter returns to normal levels over time (Table 4). It can be maintained that peripheral ghrelin level is related to mineral concentration in the bone (Misra et al., 2005). However, no considerable change in serum P level was detected after ghrelin administration in chickens (Aghdam Shahryar and Lotfi, 2013). In the present study, the peripheral administration of GHS-R antagonist did not have any significant impact on serum P level in the long term (post-rearing) (Table 4). Thus, it seems that, from a physiological perspective, the ghrelin system does not have a direct effect on serum P concentration.

In the present study, significant increases in thyroid hormones were observed within groups treated with GHS-R antagonist (Table 5). Khazali and Mahmoudi (2009) found that the injection of another GHS-R antagonist (analog of substance P) in rats increased mean plasma TSH, T_3 and T_4 concentrations based on dosage but was different in the control group (normal level), which is in agreement with Khazali and Mahmoudi (2009), who argued that T_3 and T_4 decreased after the central infusion of ghrelin. Aghdam Shahryar and Lotfi (2014) reported that the peripheral administration of [D-Lys^3]-GHRP-6 did not result in any considerable increases in T_3 or T_4 in rats. In other words, GHS-R antagonist seems to have more pronounced impact on thyroid hormones in chickens. It can be stated that an increase in T_4 is predictable when body weight of treated chickens decreases, which is indicated in Table 5. The possible mechanism for increased thyroid hormones can be attributed to the decrease in "hunger signal" and increase in the basal metabolism which can make up for the energy from feed. Lin et al. (2011) argued that the growth hormone secretagogue receptor antagonists can be considered as a new means of combating weight gain by shifting the energy balance from obesogenesis to thermogenesis.

The conclusion to be made in this study is that the peripheral administration of peptide ghrelin antagonist [D-Lys^3]-GHRP-6 significantly decreased feed intake and body weight in chickens. With regard to increase in peripheral T_4 level, it can be maintained that the antagonism of ghrelin can enhance the metabolic rate and stimulate lipolysis and weight losses, which is similar to the results observed in mammalian species. As a direction for further research, comparative studies can provide valuable data on ghrelin antagonism in other poultry species.

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