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The Physiological Effect of Peripheral Ghrelin on the Plasma Levels of Serotonin and Insulin in Sheep

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Abstract: The gut hormone ghrelin plays an important physiological role in modulating GH secretion, insulin secretion and glucose metabolism. To test the hypothesis that long term effect of peripheral ghrelin on serotonin and insulin levels in ruminant species, ten male lambs were 2 months old with an average body weight of 26 kg and 2 groups according to ghrelin treatment in group I, animals were fed *ad libitum* in the group II, animals were fed *ad libitum* and intravenously injected with the ghrelin. In the laboratory, researchers have shown that ghrelin administration was significantly decreased plasma serotonin, insulin and glucose concentrations in the long term. Although, the results obtained by ghrelin treatment in the long term are not enough clear in ruminant species and further research should validate the obtained results before applying this information on relationships between ghrelin, insulin and serotonin.

Key words: Ghrelin, serotonin, insulin, lambs, Turkey

INTRODUCTION

Energy intake and expenditure are under a fine control exerted by several neurotransmitters, neuropeptides and hormones, among which complex interactions exist (Kalra *et al*., 1999; Mercer and Speakman, 2001). Studies of spontaneous feed intake patterns and associated changes in blood metabolites and hormones have provided important background data for the investigation of the factors controlling feed intake in ruminants (Baile, 1975; Chase *et al*., 1976).

Serotonin inhibits food intake and stimulates energy expenditure (Le Feuvre *et al*., 1991). Peripheral serotonin is known to be associated with glucose metabolism mainly because of its regulation of the secretion of insulin in pancreatic β cells. Hypothalamic as well as peripheral administration of serotonergic agonists affects feeding patterns by producing a significant decrease in the size and duration of individual meals in association with a reduced rate of eating (Blundell, 1984). Since, the latency to meal onset and the frequency of meals taken are not affected, it is proposed that endogenous 5-HT may influence primarily the termination rather than the initiation of eating (Leibowitz *et al*., 1998). The evidence predominantly indicates that the major effect of hypothalamic 5-HT is on eating behavior and particularly meal size and this effect in turn has impact on body weight. The serotonergic system is also responsive to insulin which is released by the ingestion of carbohydrate. Insulin administration enhances 5-HT turnover and release *in vitro* (Dunbar *et al*., 1995; Vahabzadeh *et al*., 1995). These studies suggest that serotonin may play important roles with regard to glucose and lipid metabolism.

Ghrelin, a recently discovered gastric hormone has been implicated in the control of food intake and energy homeostasis. Changes in blood ghrelin levels are associated with feeding behavior in rats (Date *et al*., 2002) and goat (Hayashida *et al*., 2001). A large preprandial rise and a postprandial fall in plasma ghrelin levels were observed in man (Cummings *et al*., 2001) and ruminants (Sugino *et al*., 2002). In addition to a role in the regulation of food intake and body weight regulation, ghrelin has been proposed to play a direct role in glucose homeostasis. A number of early reports have demonstrated ghrelin expression in pancreatic islets (Date *et al*., 2002; Wierup *et al*., 2002). Ghrelin's role in regulation of insulin secretion and action remains controversial, however with some studies showing an ability of ghrelin to increase insulin secretion (Dezaki *et al*., 2004; Irako *et al*., 2006; Adeghate and Ponery, 2002; Lee *et al*., 2002). Recent study of a cohort study has shown that low plasma ghrelin is associated with elevated fasting insulin levels, insulin resistance and type 2 diabetes (Poykko *et al*., 2003). In this study,

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researchers have investigated the physiological effect of peripheral ghrelin on the plasma levels of serotonin and insulin in scheduled fed sheep.

MATERIALS AND METHODS

This study was conducted and validated at the Animal Welfare and Animal Welfare and Application Center of Faculty of Veterinary Medicine in Bursa (Protocol No.: 26.07.2004/020/333). Sixteen male Awassi lambs were tested for homogeneity with respect to weight and age. The animals were 2 months old with an average body weight of 26 kg and each lamb within each group was housed individually 100×150×120 cm pen inside a closed shed.

Experimental animals and treatments: This study was conducted and validated at the Animal Welfare and Animal Welfare and Application Center of Faculty of Veterinary Medicine in Bursa (Protocol No. 26.07.2004/020/333). Ten male lambs were tested for homogeneity with respect to weight and age. The animals were 2 months old with an average body weight of 26 kg and each lamb within each group was housed individually 100×150×120 cm pen inside a closed shed. The lambs were randomly assigned to the following 2 groups with 5 animals per group according to ghrelin treatment in group I, animals were fed *ad libitum* in the group II, animals were fed *ad libitum* and intravenously injected with the ghrelin (1 μg kg^{-1}, Ghrelin rat, 24160 Anaspec) twice a week.

The daily food allowance was adjusted to the metabolic energy in per day to maintain an average body weight of 43 kg. The animals were given alfaalfa hay as roughage. Water was available *ad libitum*. The dry matter content of the dietary samples was determined by drying at 105°C for 12 h and the crude protein content was determined by the Kjeldahl method (AOAC, 1990). Ash was determined by combustion at 550°C for 6 h. The Neutral Detergent Fibre (NDF) contents were determined using the methods described by Van Soest.

Determination of insulin and serotonin levels in plasma: Blood samples for ghrelin measurements were obtained by puncturing the jugular vein of lambs weighning of 43 kg. All samples were collected in vacutainer tubes containing EDTA at 30 min before feeding (08:30) and at 60 min after feeding (10:00). Researchers have collected the blood samples at 15 days intervals until day 45. Whole blood was centrifuged at 2,200 g and 4°C for 10 min and plasma was collected and stored in microtubes containing EDTA, at -20°C until analysis.

Consentrations of insulin in plasma were determined by ELISA (Sheep Insulin, Mercodia Elisa). Plasma serotonin concentrations were determined by 5 Hydroxytryptamine/Serotonin (5HT/ST) ELISA kit (sheep serotonin, mybiosource).

Biochemical parameter measurment: Glucose was measured by the glucose oxidase enzymatic method (BIOLABO, Glucose GOD-PAP, Cat. No. 87109), spectrophotometrically (Schimadzu UV-1601).

Statistical analysis: The statistical package for the Social Sciences, Version 13.0 (SPSS, Chicago, IL, USA) was used for data analysis. Values are expressed as arithmetic Mean±Standard Error of Mean (SEM). Within-group effects and group interactions with time were analyzed using an ANOVA for repeated measures. When violations in parametric assumptions were found within the data set, within-group effects and between-group interactions with time were analyzed using a univariate ANOVA. Differences in carcass traits between different groups were compared using the Kruskal-Wallis test. Significance was determined with Tukey's Honestly Significant Differences (HSD) test with a cut-off of $p<0.05$.

RESULTS AND DISCUSSION

Changes in plasma insulin and serotonin levels in lambs subjected to the two different group are presented in Table 1 and 2. Plasma insulin levels decreased at 3rd period (30 day) in all groups. Furthermore, a decrease in plasma insulin levels at 3rd period was significantly <1st period (0 day) ($p<0.01$), 2nd period (15 days) ($p<0.05$) and 4th period (45 days) ($p<0.01$). However, there was no significant changes among the groups.

Data showed no significant difference ($p>0.05$) for plasma serotonin concentrations among periods during

Table 1: Plasma insulin concentration during periods (measured at 15 days intervals) in male lambs subjected to the 2 groups (μg L^{-1})

		Feding regimen groups	
Periods	n	*ad libitum*	*ad libitum*+Ghrelin
1st	5	0.27±0.06	0.25±0.06
2nd	5	0.23±0.06	0.28±0.07
3rd	5	0.11±0.03	0.08±0.02
4th	5	0.35±0.10	0.31±0.05

Table 2: Plasma serotonin concentration during periods (measured at 15 day intervals) in male lambs subjected to the 2 groups (ng mL^{-1})

		Feding regimen groups	
Periods (days)	n	*ad libitum*	*ad libitum*+Ghrelin
1st (0)	5	140.62±16.05	83.22±7.15
2nd (15)	5	132.19±8.600	91.20±9.40
3rd (30)	5	111.56±15.07	93.78±6.59
4th (45)	5	128.25±11.87	77.74±6.81

±values represents the $\bar{X}$±SEM

the experiment. However showed a significant difference ($p<0.001$) among groups, especially serotonin concentrations of lambs injected intravenous ghrelin had lower values than lambs fed *ad libitum* group.

Glucose concentrations showed significantly differences among periods; there was change significantly ($p<0.05$) between 3rd period with 1st, 2nd and 4th periods but there was no significant change in groups (Table 3).

The action of insulin in the brain is modulated by several hormones and neurotransmitters of particular interest among those factors is the neurotransmitter serotonin (5-HT) that controls food intake and energy homeostasis (Leibowitz and Alexander, 1998; Wade *et al*., 2008) through the same type of neurons as insulin (Xu *et al*., 2010; Zhou *et al*., 2007). Insulin is a critical regulator of energy metabolism and evidence suggests a close relationship between circulating ghrelin levels and insulin secretion. Blood ghrelin and insulin concentration fluctuate reciprocally before and after feeding (Cummings *et al*., 2001).

Several of early reports have demonstrated ghrelin expression in pancreatic islets (Date *et al*., 2002; Wierup *et al*., 2002), as well as shown the ability of ghrelin to regulate insulin secretion and promote β-cell proliferation and survival (Irako *et al*., 2006; Granata *et al*., 2007). In this study, ghrelin treatment group showed no significant differences compare to lambs fed *ad libitum*. Studies about the effects of ghrelin on insulin secretion have shown both stimulatory (Adeghate and Ponery, 2002; Lee *et al*., 2002) and inhibitory effects (Broglio *et al*., 2001; Egido *et al*., 2002). Researchers did not observe any effect of ghrelin on insulin secretion in lambs. Circulating insulin levels are decreased at 3rd period ghrelin administration during the experiment. This finding suggests that ghrelin might have some role in the insulin secretion during long term in lambs.

Plasma serotonin changes have been shown in male lambs during the periods of the experimet (Table 2). Serotonin levels were significant changes among groups. Ghrelin treatment group exhibited significant decrease in plasma serotonin levels as compared to *ad libitum* group. Ghersi *et al*. (2011) showed that ghrelin administered into the hippocampus or in the superfusion medium, decreased the serotonin release from hippocampal slices. Brunetti *et al*. (2002), who demonstrated that Ghr inhibits 5-HT release from rat hypothalamic synaptosomes.

Table 3: Plasma glucose concentration during periods (measured at 15 days intervals) in male lambs subjected to the 2 groups (mg dL^{-1})

		Feding regimen groups	
Periods (days)	n	*ad libitum*	*ad libitum*+Ghrelin
1st (0)	5	91.85±2.400	94.81±0.15
2nd (15)	5	96.52±5.030	100.00±4.95
3rd (30)	5	93.33±16.63	95.33±8.06
4th (45)	5	107.20±7.410	123.20±8.33

Considering the earlier obtained findings and the present results, it is reasonable to think that the decrease in the plasma serotonin levels in ghrelin treatment group. At the present study, plasma glucose concentrations showed high values in ghrelin treatment group, although there was not significantly differences. However, glucose levels exhibited significantly a decrease of concentrations at the 3rd period as same plasma insulin concentrations. Generally, long-term ghrelin treatment induced an increase in plasmatic values of glucose whereas plasmatic insulin levels, unlike short-term effects did not change or enhanced after ghrelin treatment (Sangiao-Alvarellos and Cordido, 2010) but researchers observed that with ghrelin treatment a decrease plasma values of glucose during long term experiment. Long-term effects of exogenous ghrelin on glucose and insulin levels are not conclusive. Although, the results obtained by ghrelin treatment in the long term are not enough clear.

CONCLUSION

At the present study, indicates that regulation of ghrelin secretion is complex and that its secretion is stimulated by seemingly contradictory signals. These results do not support a role of ghrelin alone as a signal mediating the effects of nutrition on insulin secretion in sheep. The findings also, when considered with the earlier reports from other laboratories indicate that ghrelin is an important stomach hormone that may be mediated insulin secretion during long term in ruminant species.

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