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Effects of Ghrelin on LH and T Hormones Secretion in Mature and Immature Zell Rams Fed by Two Different Levels of their Daily Food Requirements

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This study was established for determining the effects of ghrelin in the control of LH (luteinizing hormone) and T (testestrone) hormones secretion in Zell Rams fed by two different levels of their daily food requirements. Ten mature and ten immature rams were selected randomly and divided into two groups (n=5). Animals in 4 groups were fed either 100% or a 50% energy content diaet, for two weeks. Then all rams received jugular vein injection of 5 μg ghrelin/KgBW. Blood samples were withdrawn by jugular venipuncture at 30-minute intervals, 3 h before and 3 h after ghrelin injection. For determining the concentration of LH and T hormones, plasma was isolated and subjected to radioimmunoassay. The results showed that ghrelin injection in both mature and immature rams who were subjected to 50% energy content diaet, significantly reduced T hormone level. Furthermore ghrelin injection in both groups of mature rams significantly reduced LH hormone level. It seems that ghrelin have inhibitory effects on reproduction of Zell Rams by reducing sexual hormones level.

Key words: Ghrelin, Ram, Reproduction, LH and T hormones.

Ghrelin is a 28-amino acid peptide that was found in the stomach and is an endogenous ligand for GHS-R (Kojima *et al*., 1999). It is synthesized predominantly in the stomach but has also been identified in a variety of other organs, such as bowels, kidney, thyroid, lung, lymphatic tissue, placenta, hypothalamus, and pituitary (Kluge *et al*., 2007). Ghrelin is the natural ligand of the GH secretagogue receptor (GHS-R) and stimulates feeding behavior and GH levels in rodents and humans. Accordingly, ghrelin stimulates GH secretion in humans and rodents (Wren *et al*., 2000, Javed *et al*., 2006). Ghrelin is secreted by a highly specialized population of cells in stomach epithelium. Secretion is tightly regulated in response to circadian rhythms, food ingestion, stress, and other factors. Plasma ghrelin concentrations rise dramatically before meals and decline immediately after eating commences (Nass *et al*., 2008). Its secreted into the blood stream by the endocrine stomach mucosal cells named "X/A like" in rat (Rindi *et al*., 2002, Perdona *et al*., 2011) and P/D1 cells in humans (Sakata *et al*., 2002). Once released, ghrelin reaches the pituitary, where it

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binds to its receptor and stimulates the secretion of growth hormone. Ghrelin receptors are also located in several other central nervous system sites, including many known to be involved with body weight determination (Zigman *et al*., 2006). Administration of excess ghrelin leads to increased food intake in rodents and humans. Therefore, ghrelin has been considered to be an important factor in regulating food intake (Kojima *et al*., 2006, Cummings *et al*., 2006). Ghrelin has been implicated in the regulation of other endocrine functions, with evidence of effects in the rat, human, and monkeys on the secretion of prolactin (PRL), adrenocorticotrophic hormone, cortisol (Tassone *et al*., 2003, Broglio *et al*., 2004, Javed *et al*., 2006), and LH (Fernandez *et al*., 2004, Vulliemoz *et al* 2004). Whereas leptin (Ahima and Osei, 2004), insulin (Plum *et al*, 2005), and other peripheral factors act as satiety factors (Park and Bloom, 2005). Although ghrelin is not essential for food intake in mice, it is required for certain food reward behaviors that occur in the setting of chronic calorie restriction (Perello *et al*., 2010). Ghrelin is also essential in mice to prevent hypoglycemia and death when the animals are subjected to severe calorie restriction. The latter function of ghrelin became apparent in studies of genetically engineered mice that lack the gene encoding ghrelin O-acyltransferase (GOAT), the enzyme that attaches octanoate to ghrelin, a reaction that is essential for the biological activity of the hormone (Zhao *et al*., 2010). Administration of ghrelin decreases LH and T in human and animal models (Barreiro and Sempere 2004, Kluge *et al*. 2007, Garcia *et al*. 2007). There are few studies about the effect of ghrelin on reproduction activity axis in different strains of ruminants under different level of energy. So, the goal of this study was to determine the effect of ghrelin on LH and T secretions in the mature and immature Zell rams fed by two different levels of their daily food requirements.

MATERIALS AND METHODS

Ten mature (Average Weight= 57±2.3 kg) and ten immature (Average Weight= 32±1.4 kg) rams were randomly selected from Rasht Research Center for Agriculture and Natural Resources. Animals in four groups (n=5) were fed respectively either by 50% or 100% of their daily food requirement for two weeks. After two weeks, rams received 5 µg ghrelin/kg body weight via their jugular vein. Total energy and chemical compositions of feedstuffs, namely, crude fiber, crude protein, fresh and dry matter, NDF, ADF, ether ex-tract, ash, calcium, and phosphorous, were analyzed in the Rasht Research Center for Agricul-ture and Natural Resources. Animals were maintained on the diets for two weeks. Diets were formulated based on maintenance energy requirements and according to standard tables (Ensminger and Parker, 1986). Blood samples were collected by jugular vein at 30-min intervals (Mcmanus *et al*., 2000), 3 h be-fore and 3 h following galanin injection. The samples were then centrifuged for 15 minutes and the plasma was stored at -20°C until assayed for LH and T levels. Blood samples that were collected before the injec-tion of ghrelin in the four treatments served as the con-trols for each group. Plasma concentrations of LH and T were determined by double-antibody radioimmunoassay (RIA) with stan-dard LH and T ovine antigens (Tabeshyarnoor Co., Iran). ANOVA was used to assess any statistical differences in mean LH and T levels in animals in response to ghrelin. All statistical analyses were performed using SPSS (version 13). The results are shown as means ± SE

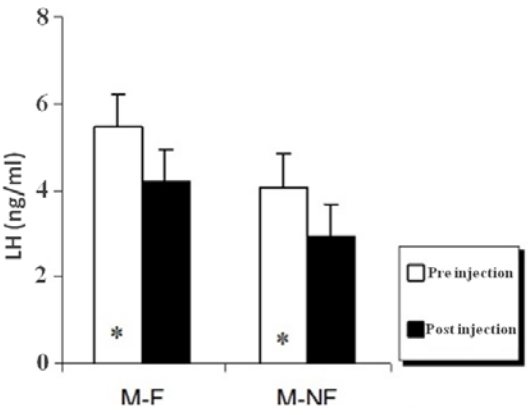

Fig. 1. LH Concentration in mature Rams fed by 100% (M-F) and 50% (M-NF) of their daily food requirement, before and after the Administration of 5 µg/kg BW Ghrelin.
* $p < 0.05$
Data is presented as the mean ± SE.

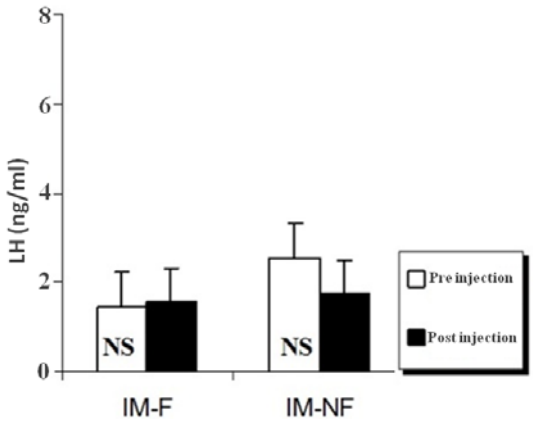

Fig. 2. LH Concentration in immature Rams fed by 100% (IM-F) and 50% (IM-NF) of their daily food requirement, before and after the Administration of 5 µg/kg BW Ghrelin.
* $p < 0.05$
Data is presented as the mean ± SE.

and the level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

LH

Mean LH plasma levels were significantly (*P*_0.05) decreased in both groups of mature rams fed by 100% (M-F) or 50% (M-NF) of their daily food requirement, after the Administration of Ghrelin (Figure 1). Since mean LH plasma levels were not affected significantly in immature rams (Figure 2).

T

The ANOVA analysis also revealed that the mean T plasma levels were significantly (*P*_0.05) decreased in both groups of mature and immature rams fed by 50% (M-NF) of their daily food requirement, after the Administration of Ghrelin (Figure 3). Since its concentration were not affected significantly in mature and immature rams fed by 100% (M-F) of their daily food requirement (Figure 4).

The data of the present study indicate that ghrelin decreases LH and T Concentration especially in mature Zell rams. Similar results were reported on human (Unniappan, 2009), rodents (Javed *et al*., 2006), monkey (Vulliémoz *et al*., 2004) and rats (Fernandez *et al*., 2004). There is only one study in humans, which indicates that Ghrelin has no effect on LH (Takaya *et al*., 2000). In rat gonadotropes, two distinct subsets of secretory granules have been identified (small dense granules rich in LH and large lucent granules rich in FSH) (Farifteh *et al*., 2008). Ghrelin was found to affect the hypothalamic-pituitary-gonadal axis at all levels. However, only for the hypothalamic level has evidence been provided from both in vitro and in vivo experiments (Tenasempere, 2005). It is possible that ghrelin differentially modulates the intracellular actions of LHRH (Fernandez *et al*., 2004), a finding previously described for peptides involved in the control of LHRH action, such as endothelins (Kauyicska *et al*., 1991), galanin (Aboutalebi *et al*., 2011) and NPY (Evans, 1999). Ghrelin significantly decreased hypothalamic LHRH in vitro (Fernandez *et al*., 2006). In addition, a decrease of the LH pulse frequency in vivo, as found in humans (Ogata *et al*.,2009) and animals (Furuta *et al*., 2001, Vulliemoz *et al*., 2004), indicates an inhibition of the hypothalamic LHRH pulse generator. This inhibitory effect was suggested to be mediated by hypothalamic neuropeptide Y and agouti-related peptide (Vulliemoz *et al*., 2004) because both peptides have inhibited pulsatile LH release (Shahab *et al*., 2003), and their synthesis has been decreased by ghrelin (Goto *et al*., 2006). Interestingly, not only acylated ghrelin but also

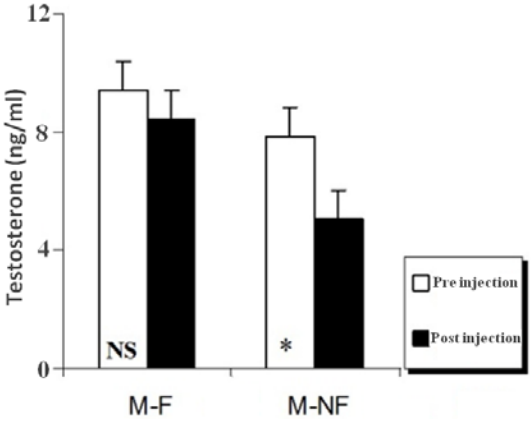

Fig. 3. T Concentration in mature Rams fed by 100% (M-F) and 50% (M-NF) of their daily food requirement, before and after the Administration of 5 µg/kg BW Ghrelin.
* $p < 0.05$
Data is presented as the mean ± SE.

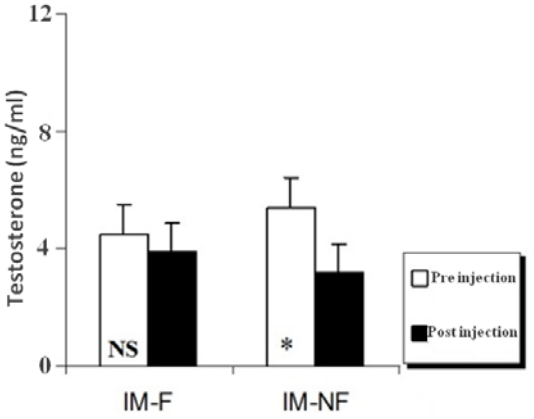

Fig. 4. T Concentration in immature Rams fed by 100% (IM-F) and 50% (IM-NF) of their daily food requirement, before and after the Administration of 5 µg/kg BW Ghrelin.
* $p < 0.05$
Data is presented as the mean ± SE.

the unacylated isoform of ghrelin, which has been considered widely inert (VanderLely *et al*., 2004), decreased LH secretion in rats to a similar extent as the acylated isoform. This finding indicates that central effects of ghrelin on the hypothalamic-pituitary-gonadal axis are not, or at least not only, mediated through the GHS-R that the unacylated form does not bind to (Martini *et al*., 2006).

To the best of our knowledge, this is the first study in which the effects of ghrelin on LH and T secretion in rams fed by two different energy levels have been investigated. The results of this study suggest that the effect of ghrelin on LH and T secretion in rams is independent from the meta-bolic state and energy balance of the animal. Most prob-ably, the decrease of energy in the diet can not change the sensitivity of hormones secretion toward ghrelin. In conclusion, ghrelin is a peptide hormone mainly secreted from the stomach into the circulation, but it can be synthesized by other tissues such as reproductive tissues suggesting local actions (autocrine and/or paracrine). Ghrelin participates in the regulation of different aspects of the reproductive functions of rams. Ghrelin through its extensive biological functions including energy metabolism by promoting fat deposition and food intake could be a key signal between energy status and control of fertility. However, further studies are required to gain insights into the understanding of the fine mechanisms of ghrelin action.

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