

## **Relations Between Leptin and Testosterone and Glucose Concentrations, Fattening Performance and Reproductive Development in Growing Bulls**

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### **ABSTRACT**

This study were performed in 7 Brown Swiss and 7 Holstein bulls. The leptin concentrations at 12 months of age and testosterone concentrations at 9 months of age were statistically different between Brown Swiss and Holstein bulls ( $P < 0.05$ ). In hip heights, a statistical difference at the level of  $P < 0.01$  and  $P < 0.05$  was observed at the 7, 11 and 12 months of age values and at the 8, 9 and 10 months of age values, respectively, between the two breeds of bulls. Furthermore, live weight gains and feed conversion values at 8 months of age were also significantly different ( $P < 0.05$  and  $P < 0.01$ , respectively). Statistically negative correlations ( $P < 0.05$ ) were observed between leptin concentrations and hip heights and scrotal circumferences of Brown Swiss bulls. The correlation was observed only between the leptin and testosterone concentrations in Holstein bulls ( $P < 0.05$ ). In conclusion, the data obtained from Holstein bulls implies that leptin might be involved in growth and reproductive development, no association, however, was obtained between leptin and feeding performance.

**Key Words:** Leptin, fattening performance, reproductive development, bull.

### **INTRODUCTION**

Leptin acts through both central and peripheral mechanisms to affect feeding behavior, lipid and glucose metabolism, thermogenesis, reproductive and endocrine functions, and cardiovascular and immune functions (Veniant 2003). Although a nimity of information exist regarding the physiological role of leptin in rodents and humans, the regulation and action of leptin in domestic animals is less certain (Wauters 2000). Recent reports have provided evidence that the leptin gene is functionally relevant in cattle and sheep, and may contribute to an array of important reproductive events, including puberty (Williams 2002). Fertility in mammals requires adequate nutrition and reserves of metabolic fuel. The effect of nutritional status on reproduction is postulated to reflect the action of metabolic signals that are recognized by the brain and serve as indices of metabolic state, however, the identity of these metabolic factors has remained elusive (Barash 1996).

While a great deal of research focusing on leptin have been performed in humans and rodents, little is known in ruminant species. However, in recent years, there has been interest in leptin and elucidation of its function in other species and, particularly, in ruminants. In these studies, the emphasis has been mainly placed on relation of leptin to puberty, feeding regimens and carcass traits, etc. (Williams 2002, Minton 1998, Tokuda 2000).

The objective of this study is to evaluate relationships between serum leptin, and testosterone and glucose concentrations, fattening performance and reproductive development in growing Brown Swiss and Holstein bulls.

### **MATERIALS AND METHODS**

The study was conducted at the Animal Welfare and Animal Production Research and Application Center in Uludag University, Faculty of Veterinary Medicine. Fourteen Brown Swiss and Holstein bulls ( $n = 7$  bulls per breed) at 7 months of age were used in the study. The bulls were fed on a concentrate ration mixture (crude protein = 15.38 %, crude fat = 2.57 %, crude cellulose = 7.17 %) consisting of corn, wheat, sun flowers meal, dicalcium phosphate, limestone, salt, vitamin premix. As roughage, straw (crude protein = 4.88 %, crude fat = 1.51 %, crude cellulose = 43.0 %) was given to animals.

From 7 to 12 months, the amounts of ration consumed were weighed, live weights were recorded, hip height and scrotal circumference were measured monthly. On the day of measuring, blood samples were collected by jugular venipuncture (Venoject, serum separator tubes). After clotting at room temperature, serum was separated by centrifugation and transferred to plastic tubes. Serum was stored at  $-20^{\circ}\text{C}$  until analyses. Serum glucose concentration was measured by glucose/oxidase method using commercial kit (Biolabo, Glucose GOD-PAP, Cat.No 87109). Serum leptin concentration was measured using a double-antibody RIA kit containing guinea pig multispecies leptin antibody, human [ $^{125}\text{I}$ ] leptin, and as standard,

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human leptin (Linco, Multispecies Leptin RIA Kit, Cat#XL-85K) following the manufacturer's instructions. The quantification was carried out in 100µl of serum. Serum testosterone level was also measured using a RIA (DPC Gambyt CR, England 95-3/1097) commercial kit (Testosterone RIA DSL-4000 Active®).

**Table 1.** Composition of Concentrate feed

| Ingredient              | %    |
|-------------------------|------|
| Corn grain              | 50.0 |
| Sunflower meal          | 16.5 |
| Soybean meal            | 13.2 |
| Limestone               | 1.2  |
| Salt (NaCl)             | 0.5  |
| Vitamin-Mineral Premix* | 0.1  |

\* Supplied per kilogram lambs of premix (Kavimix VM, Kartal Kimya A.S., Gebze, Turkey) : Vitamin A 12000000 IU, Vitamin D<sub>3</sub> 3000000 IU, Vitamin E 30 g, Mn 50 g, Fe 50 g, Zn g, Cu 10 g, I 0.8g, Co 0.1 g, Se 0.15 g, Antioxidant 10 g.

**Table 2.** Chemical composition of feed ingredients

| Chemical Composition | Concentrate Feed (%) | Alfalfa Hay (%) |
|----------------------|----------------------|-----------------|
| Dry Matter           | 88.30                | 90.30           |
| Ash                  | 4.67                 | 9.93            |
| NDF*                 | 15.09                | 39.40           |
| Crude Protein        | 15.80                | 15.5            |
| Ether Ekstract       | 2.56                 | 2.26            |
| Calcium              | 0.59                 | 1.33            |
| Phosphorus           | 0.41                 | 0.25            |

\* NDF: Neutral Detergent Fiber

SPSS 10.0 programme was used for calculations. Results are expressed as mean (X) and standard deviation (S.D.). Mann-Whitney U test was used to assess the differences between Brown Swiss and Holstein bulls. The Pearson's correlation test was used to analyze correlations between leptin and other parameters testosterone and glucose levels, live weight gain, feed conversion, hip height, and scrotal circumference.

## RESULTS and DISCUSSION

In this study, leptin concentrations in Brown Swiss and Holstein bulls (average 1.84-3.93 ng/ml) (Table 3) were found consistent with previous studies in which the same method was used to measure serum leptin concentration (Chelikani 2004; Delavaud 2002). It was reported that, in humans and cattle, the circulating leptin levels vary greatly among individuals; however, it is not clear whether this is also the case in sheep and other species (Tokuda 2000). In this study, leptin concentrations were significantly different between two breeds only at 12 month of age ( $P < 0.05$ ) (Table 3). However, though statistically seemed insignificant, Holstein bulls have always exhibited higher leptin concentrations than Brown Swiss bulls.

Breed type and sex are major factors that affect fatty acid composition of lipids of carcass dissectible or intramuscular depot fats (Zembayashi 1995). Bellman et al (2004) reported that leptin concentration was higher in Holstein breed, which is secretion type of cattle, than was in Charolais breed, which is accretion type of cattle. The accretion type of cattle are apt in accreting feed as meat, while the secretion type of cattle secrete metabolised feed as milk. They suggested that the amount of subcutaneous adipose tissue is directly correlated with leptin. Brown Swiss breed is considered both accretion and secretion type cattle (Arpacik 1995). However, this breed of cattle tend to be more accretion type and their ability to gain carcass weight is more than that of Holstein cattle. Holstein cattle, on the other hand, are considered secretion type and tend to accumulate more fat in their bodies. This might partially explain why Holstein bulls exhibited higher leptin concentrations in this study.

**Table 3.** The means and differences between various parameters of Brown Swiss and Holstein bulls throughout the study (n=7, for each breed)

|                                   | Months of Age |            |           |          |           |           |
|-----------------------------------|---------------|------------|-----------|----------|-----------|-----------|
|                                   | 7.            | 8.         | 9.        | 10.      | 11.       | 12.       |
|                                   | X±S.D.        | X±S.D.     | X±S.D.    | X±S.D.   | X±S.D.    | X±S.D.    |
| <b>Leptin (ng/ml)</b>             |               |            |           |          |           |           |
| Brown Swiss                       | 3.13±0.7      | 3.52±0.9   | 3.09±1.1  | 2.75±0.6 | 2.81±0.5  | 1.84±0.9  |
| Holstein                          | 3.22±1.1      | 3.59±1.5   | 3.39±1.5  | 3.55±1.7 | 3.78±2.2  | 3.93±2.0* |
| <b>Testosterone (ng/ml)</b>       |               |            |           |          |           |           |
| Brown Swiss                       | 2.99±2.9      | 3.39±5.8   | 3.01±2.4  | 4.94±4.5 | 7.04±5.0  | 7.73±1.9  |
| Holstein                          | 3.00±2.7      | 2.87±2.7   | 8.31±3.5* | 9.40±2.8 | 8.28±4.9  | 6.44±4.0  |
| <b>Glucose (mmol/l)</b>           |               |            |           |          |           |           |
| Brown Swiss                       | 4.78±0.8      | 4.58±1.3   | 3.87±0.4  | 3.49±0.4 | 3.69±0.6  | 3.97±0.3  |
| Holstein                          | 5.65±1.4      | 4.47±0.4   | 3.93±0.3  | 3.80±0.5 | 3.48±0.7  | 4.00±0.7  |
| <b>Body weight (kg)</b>           |               |            |           |          |           |           |
| Brown Swiss                       | 216±38.5      | 238±38.3   | 275±38.0  | 314±35.9 | 346±45.0  | 385±39.4  |
| Holstein                          | 235±22.7      | 262±25.9   | 290±24.6  | 326±30.1 | 371±32.2  | 420±39.0  |
| <b>Live weight gain (kg)</b>      |               |            |           |          |           |           |
| Brown Swiss                       | 22±6.4        | 36±9.2*    | 39±7.8    | 32±16.7  | 40±10.1   | 40±12.2   |
| Holstein                          | 27±6.9        | 27±4.8     | 36±8.9    | 45±12.9  | 48±16.7   | 35±13.6   |
| <b>Feed conversion</b>            |               |            |           |          |           |           |
| Brown Swiss                       | 7.85±1.8      | 5.47±1.1** | 5.80±1.1  | 8.49±3.4 | 7.42±2.4  | 7.96±2.9  |
| Holstein                          | 6.41±2.1      | 7.90±1.4   | 6.89±1.7  | 6.03±1.7 | 6.32±2.2  | 9.27±3.0  |
| <b>Hip height (cm)</b>            |               |            |           |          |           |           |
| Brown Swiss                       | 103±4.0       | 107±5.0    | 110±3.1   | 113±3.2  | 118±4.1   | 120±3.1   |
| Holstein                          | 110±3.4**     | 113±2.7*   | 117±3.9*  | 120±4.2* | 123±2.3** | 127±3.7** |
| <b>Scrotal circumference (cm)</b> |               |            |           |          |           |           |
| Brown Swiss                       | 23±3.4        | 25±2.5     | 27±2.1    | 29±1.9   | 31±2.0    | 32±1.8    |
| Holstein                          | 23±2.2        | 24±2.1     | 27±1.6    | 29±2.0   | 31±1.7    | 33±1.8    |

\* : The difference between means of two breed within a column is significant at P<0.05

\*\* : The difference between means of two breed within a column is significant at P<0.01

X: Mean, S.D.: Standard Deviation

Different results were obtained in various studies evaluating the relationship between leptin and glucose. Subcutaneous administration of leptin was reported to decrease plasma glucose concentration in rats (Sivitz 1997). Block et al (2003) reported that leptin concentrations tend to correlate positively with plasma glucose levels in dairy cattle. In this study, no differences were found between glucose concentrations of two breeds, and nor was there any correlation between leptin and glucose concentrations.

The testosterone concentrations at 9 month of age were statistically different (P < 0.05) between Brown Swiss and Holstein bulls (3.01 and 8.31 ng/ml, respectively) (Table 3). The extent of the testosterone

secretion increases as puberty advances (Hafez 1993). The difference between testosterone concentrations in the breeds used may be associated with onset of puberty.

Nutrition is a key determinant of reproductive potential in cattle and other mammals. Therefore, responses of the reproductive system to changes in nutrition and metabolic status influence reproductive and economic efficiency of food-producing species. It has been proposed that there are complex and complicated relationships between the control of the central reproductive axis and body mass index or adiposity. This relationship has been implicated in the timing of onset of puberty and maintenance of normal reproductive cycles in many species. Leptin has been proposed as the elusive factor linking metabolic status to reproduction (Williams 2002). Garcia et al (2003) reported that leptin increases as puberty approaches in beef heifers. In a study performed in Angus, Brangus and Brahman bulls, it was reported that there is a positive correlation between leptin and testosterone (Thomas 2002). In this study, correlation between leptin and testosterone concentrations was obtained only in Holstein bulls ( $r = 0.319$ ,  $P < 0.05$ ).

Positive correlations between scrotal circumference and growth traits and semen quality have been reported (Martinez-Velazquez 2003; Smith 1989). In a study performed in Angus, Brangus and Brahman bulls, it was shown that body weight, daily weight gain and scrotal circumference have correlated well with serum leptin levels (Thomas 2002). Leon et al (2004) reported that a positive relationship exists between leptin concentrations and body weight and weight gain in heifers.

Daily live weight gain and feed efficiency of Brown Swiss and Holstein cattle, most commonly raised breeds in Turkey, are similar to each other, with Brown Swiss cattle being slightly better in feed efficiency (Arpacik 1995). In this study, body weight and hip height increased from 7 to 12 months of age in both breeds, with those of Holstein bulls always being heavier and taller than those of Brown Swiss bulls. In hip heights, a statistical difference at level of  $P < 0.01$  and  $P < 0.05$  was observed between two breeds at the 7, 11 and 12 months of age values and at the 8, 9 and 10 months of age values, respectively. Live weight gain and feed conversion values in Brown Swiss at 8 months of age were superior than Holstein bulls ( $P < 0.05$  and  $P < 0.01$ , respectively) (Table 3). No differences were determined between scrotal circumferences of two breeds. Statistically negative correlations were observed between leptin concentrations and hip heights ( $r = -0.288$ ) and scrotal circumferences ( $r = -0.307$ ) of Brown Swiss bulls ( $P < 0.05$ ). This findings are not consistent with results of Thomas et al. This might be due to the decrease at the level of leptin of Brown Swiss bulls at 12 month of age. Though statistically insignificant, there seemed to be a positive relationship between leptin concentrations and hip heights ( $r = 0.108$ ) and scrotal circumference values ( $r = 0.184$ ) of Holstein bulls. There was no correlation between leptin and live weight, live weight gain and feed conversion in either breed of bulls.

In conclusion, the data obtained from Holstein bulls implies that leptin might be involved in growth and reproductive development. We did not observe a strong association between leptin and feeding performance. When compared, the reason for different values obtained in Brown Swiss bulls might be because this breed of cattle is more of an accretion type and can build more muscle tissue at early months of age than Holstein breed. We believe that, as a hormone proved to be crucial for the regulation and coordination of energy metabolism and reproductive development in humans and other mammals, elucidation of the effects and functions of leptin in ruminants, which are raised mostly for economic reasons, will be a major task in near future. We anticipate that data obtained in this study will serve as a reference in future studies.

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