

Serum Leptin Levels During the Dry Period and Various Lactation Stages in Holstein Dairy Cows

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Abstract:In this study we investigated the effects of dry period and various stages of lactation on serum leptin, glucose, total cholesterol and β hydroxybutyrate levels in Holstein dairy cows. Fourthly, four years old Holstein dairy cows in their second lactation were used as material. Ten of them were in dry period and the rest were in different stages of lactation periods (10 cows for each stages). Serum concentrations of leptin, glucose, total cholesterol and β hydroxybutyrate were compared between the dry period, and first, second and third stages of lactation period. Correlation between leptin and other parameters were analyzed. While no significant differences were observed in β hydroxybutyrate levels, significant differences were determined for leptin, glucose and total cholesterol levels between dry period and first, second, and third stages of lactation period ($p<0.05$, $p<0.001$, and $p<0.001$, respectively). Statistically significant positive correlations were observed between leptin and glucose concentrations. ($r = 0,401$, $p<0.01$).

Key Words : Leptin, Dairy Cows, Biochemical Parameters, Lactation Period.

Kuru Dönem ve Laktasyonun Çeşitli Evrelerindeki Holstein Irkı Süt İneklerinde Serum Leptin Düzeyleri

Özet;Bu çalışmada Holstein ırkı süt ineklerinde kuru dönem ve laktasyonun çeşitli evrelerinin serum leptin, glukoz, total kolesterol ve β hidroksi bütirat düzeyleri üzerine etkisi araştırıldı. Materyal olarak 40 adet, ikinci laktasyon döneminde olan, 4 yaşında holstein ırkı süt ineği kullanıldı. İneklerin 10 tanesi kuru dönemde, geri kalanlar ise laktasyon periyodunun çeşitli evrelerindeydiler (her evrede 10 inek). Kuru dönem ve laktasyonun I., II., ve III. evreleri arasında serum leptin, glukoz, total kolesterol ve β hidroksi bütirat konsantrasyonları karşılaştırıldı. Leptin ve diğer parametreler arasındaki korelasyonlar analiz edildi. Kuru dönem ve laktasyon periyodunun I., II., ve III. evreleri arasında β hidroksi bütirat düzeylerinde önemli bir farklılık gözlenmezken, leptin, glukoz ve total kolesterol düzeylerinde önemli farklılıklar ortaya koyuldu ($p<0.05$, $p<0.001$, and $p<0.001$, sırasıyla). Leptin ve glukoz konsantrasyonları arasında istatistik olarak pozitif bir korelasyon olduğu ortaya koyuldu ($r = 0,401$, $p<0.01$).

Anahtar Kelimeler: Leptin, Süt İneği, Biyokimyasal Parametreler, Laktasyon Periyodu

Introduction

Lactation markedly increases nutrient requirements in both rodents and ruminants. High yielding dairy cows are typically in a state of negative energy balance postpartum because

energy required for milk production and maintenance of body tissue functions exceeds energy ingested¹¹. Hence, metabolic and endocrine changes in early lactation allow enhanced mobilization of depot fat and skeletal muscle breakdown and favor partitioning of absorbed nutrients to the mammary gland in order to pro-

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vide sufficient substrates for milk synthesis. Some hormones have an important role in metabolic adaptation to lactation¹⁴. Leptin, a hormone secreted by adipocytes, plays an important role in the regulating feed intake in both ruminants and monogastrics, and influencing carbohydrate and lipid metabolism in monogastric species⁸. As leptin affects both fat deposition and Luteinizing Hormone (LH) concentrations, it may possibly play an important role in the regulation of metabolism during the lactation period in dairy cows. During early lactation, fat stores are first used for lactation maintenance, and growth associated with reproductive processes receives lower priority^{5,13}. In a study aimed to evaluate metabolic and endocrine adaptations to energy intake in multiparous Holstein cows, it was suggested that leptin is one of several factors involved in the regulation of energy metabolism and may be important for overall homeostatic and homeorhetic control of metabolism^{6,14}.

In this study, we aimed to investigate the effects of dry period and various stages of lactation on serum leptin, glucose, total cholesterol and β hydroxybutyrate levels in Holstein cows.

Material and Methods

In this study, Fourthly Holstein dairy cows of four years old in their second lactation from Bursa region of Turkey were used. Ten of them were in dry period and the rest were in different periods of lactation. Between the days 48-51, 147-161. and 180. and over of lactation period were accepted as first, second and third stage, respectively. There were 10 cows in each lactation stages. The amount of concentrated feed given to animals was calculated by the computer controlled feeding system in which the food requirement of each animal was taken in to account. Forage was given to the animals in the farm as ad libitum in the morning and evening. The amount of feed consumed by animals in their first, second, third and dry periods of lactation was 18, 22, 23 and 14 kg/day, respectively and, fifty percent of total feed contained concentrated feed, approximately. As forage, straw, corn silage, brewer's grain and as concentrated feed, a mixture containing wheat, barley, full fat soybean, cotton seed meal, sun flowers meal, corn and scurf was given to the animals.

Blood samples were collected in to evacuated tubes (Venoject, serum separator tubes) between 8:00 a.m. and 10:00 a.m. from v. subcutanea abdominis of each cow in all periods.

After clotting at room temperature, serum was separated by centrifugation and transferred to plastic tubes. Serum was stored at -20°C until analyses.

Serum glucose, total cholesterol and β hydroxybutyrate levels were measured using commercial kits (Biolabo, Glucose GOD-PAP, Cat. No 87109; Teco Diagnostics Total cholesterol, Cat. No. C509-150; Sigma, β .hydroxybutyrate 310- UV). Serum leptin level was measured using a double-antibody RIA kit containing guinea pig multispecies leptin antibody, human [^{125}I] leptin, and as standard, human leptin (Linco, Multispecies Leptin RIA Kit, Cat. No. XL-85K) following the manufacturer's instructions.

SPSS 10.0 programme was used for statistics. Results were expressed as mean (\bar{x}) and standard deviation (S.D.). Differences between groups were assessed by two-tailed Kruskal-Wallis test. The Pearson's correlation test was used to analyze correlations between leptin and other parameters such as glucose, β hydroxybutyrate and total cholesterol levels. Differences were considered to be significant at $P < 0.05$.

Results and Discussion

The objective of this study was to examine the effects of dry period and various stages of lactation on serum leptin, glucose, total cholesterol and β hydroxybutyrate levels in Holstein cows. We focused on the changes occurring in leptin plasma concentrations in high-yielding dairy cows throughout dry period and various stages of lactation. In the present study, serum leptin concentrations were significantly higher in the dry period than in the first stage of lactation ($p < 0.05$) (Table 1). As recently hypothesized (11), the fall in leptin plasma levels at parturition is possibly a signal of the negative energy balance; the animal activates a global energy-saving condition, thus mobilizing energy reserves¹⁷. It is also likely that leptin influences, through tissue-specific mechanisms, the sensitivity to insulin and hence glucose uptake by the cells, in order to direct nutrients towards organs or tissues that are metabolically more active (i.e. the mammary gland)^{4,6,12}. In this study glucose levels were found higher in the dry period than in the first, second and third stages of lactation, ($p < 0.001$) (Table 1). Different results were obtained in various studies assessing the relationship between leptin and glucose concentration. Subcutaneous administration of leptin was re-

ported to decrease plasma glucose concentration in rats¹⁵. Block et al⁵ reported that leptin concentrations tend to correlate positively with plasma glucose levels in dairy cattle. Consistent with this, we also found positive correlation between leptin and glucose concentrations ($r = 0,401$, $p < 0,01$).

Table 1. The serum leptin, glucose, total cholesterol and β hydroxybutyrate concentrations of dairy cows in dry period and various stages of lactation (n=10)

Tablo 1. Kuru dönem ve laktasyonun çeşitli evrelerindeki süt ineklerinde serum leptin, glukoz, total kolesterol ve β hidroksi bütirat konsantrasyonları (n=10)

	Dry period	Lactation periods		
	$\bar{X} \pm S.D.$	Stage 1 $\bar{X} \pm S.D.$	Stage 2 $\bar{X} \pm S.D.$	Stage 3 $\bar{X} \pm S.D.$
Leptin(ng/ml)	5.68 \pm 1.19 ^{a*}	4.36 \pm 1.07 ^b	4.65 \pm 1.24 ^{ab}	5.03 \pm 0.85 ^{ab}
Glucose(mmol/l)	3.80 \pm 0.53 ^{a**}	2.76 \pm 0.31 ^b	2.84 \pm 0.16 ^b	2.98 \pm 0.23 ^b
Total cholesterol (mmol/l)	2.90 \pm 0.41 ^{a**}	5.62 \pm 1.36 ^b	5.90 \pm 1.04 ^b	5.70 \pm 1.55 ^b
β hidroksi bütirat (mmol/l)	0.50 \pm 0.06 ^a	0.55 \pm 0.04 ^a	0.52 \pm 0.08 ^a	0.51 \pm 0.03 ^a

^{a,b} Means within a row with the different letters are significantly different

* $p < 0,05$, ** $p < 0,001$, \bar{X} =Mean, S.D=Standard deviation

In contrast to leptin and glucose, total cholesterol levels were lower in the dry period than in the first, second and third stages of lactation ($p < 0,001$) (Table 1). The decrease in leptin plasma concentration at calving may also be correlated with the intensive lipolysis that characterizes this period and thus with the reduction in fat tissue. During this period, the high GH concentrations and the low insulin levels stimulate a marked mobilization from adipose tissues, as confirmed by the increase in cholesterol levels; a negative correlation between leptin and total cholesterol has been already reported in humans^{1,10}. In this study we found no correlation between leptin and total cholesterol concentrations.

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period. Subclinical ketosis is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs of ketosis. Subclinical ketosis is a common disease in high production dairy cows and is caused by a negative energy balance which can affect milk production⁹ and reproduction^{2,3,16}. It has been shown that clinical and subclinical ketosis both result in increased concentrations of ketone

bodies in tissues and milk of the cows. Subclinical ketosis is detected by elevated levels of ketone bodies in body fluids. Ketone bodies include β hydroxybutyrate, acetoacetate, and acetone. β hydroxybutyrate is synthesized from absorbed butyrate in the ruminant epithelium and by the ketogenesis in hepatocytes through conversion of long-chain fatty acids during fat mobilization. Both acetoacetate and β hydroxybutyrate are freely distributed and transported in the blood and seem interconvertible in various tissues⁷. In the present study serum β hydroxybutyrate levels were not statistically different between dry period and various stages of lactation period (Table 1). In the present study, there were no correlation between leptin and β hydroxybutyrate concentrations.

In conclusion, in the present study we determined differences of leptin, glucose and total cholesterol levels of the cows between dry period and various stages of lactation periods. Moreover we observed a strong correlation between leptin and glucose concentrations. We believe that this study provides evidence that the decrease in leptin plasma concentrations at first, second, third stages of lactation periods could be related to the animals' energy deficit and to the extent of mobilization of adipose tissue.

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