Associations between leptin, body condition score, and energy metabolites in Holstein primiparous and multiparous cows from 2 to 8 weeks postpartum

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SUMMARY

The present study aimed to investigate the relationship between serum leptin and body condition score (BCS) during the postpartum period in Holstein cows. We also explored the potential associations with other metabolic traits and fertility parameters. Primiparous and multiparous cows were subjected to leptin analysis on calving day. Starting at the second week postpartum, cows underwent seven weeks of weekly monitoring of serum concentrations of NEFA, BHB, leptin, cholesterol, AST, and bilirubin. During study, BCS changes were recorded, and the genital tract was assessed by ultrasonography and some fertility parameters were evaluated. During the study period, primiparous cows showed a lower mean BCS (P<0.05). Leptin concentrations between calving and 8 weeks postpartum were higher in multiparous cows than primiparous cows (P<0.001). Between postpartum weeks 2 and 8, primiparous cows showed higher concentrations of NEFA, BHB, and AST and lower cholesterol concentrations. Multiparous cows showed greatest largest follicle diameters and ovulated earlier. Primiparous cows showed higher intervals of calving-to-first insemination and calving-to-conception, more insemination indices for the conception. Improved cow metabolic and reproductive performance was associated with lower concentrations of NEFA, BHB, and AST; higher concentrations of leptin and cholesterol; and greater BCS change during the early postpartum period. Delayed ovarian activity in primiparous cows might be caused by lower postpartum concentrations of leptin and cholesterol.

Keywords: Postpartum period, Leptin, Fertility

Introduction

The energy requirements of dairy cows increase with fetal development and colostrum production during the prepartum period, and with lactation in the postpartum period [25,46]. This can result in a period of negative energy balance (NEB) when rations do not meet the energy demand for a high-yielding dairy cow. Although cows feed with a high-energy diet, reduced dry matter intake causes to failure in meeting enough energy demand. This NEB reaches a peak at two or three weeks after calving, and may continue for 60 days postpartum due to peak lactation. To meet the cow’s energy requirement during this time, body reserves are mobilized [47]. Body condition score (BCS) and changes of non-esterified fatty acids (NEFA), beta hydroxybutyrate (BHB), bilirubin, and aspartate aminotransferase (AST) are practical parameters to determine the nutrition, energy status and liver function of dairy cows [11,36].

Several hormones-including leptin, insulin-like growth factor-1 (IGF-1) and insulin also play major roles in maintaining the metabolic balance of dairy cows [23,46]. Leptin regulates food intake in mammals, and impacts the inhibition of lipogenesis and stimulation of lipolysis in energy metabolism. Leptin concentrations reflect fat reserves and satiety, and decreases in plasma leptin near parturition are observed in association with NEB. Furthermore, in both people and animals, reduced reproductive capacity is related to leptin deficiency [5,24,46]. Early in the lactation period,
lower leptin concentrations in cows reflect delayed ovarian activity. Leptin receptors exist in bovine granulosa cells, corpus luteum and endometrium [43,46]. Leptin is important in stimulating the release of GnRH, LH, and FSH and in inhibiting ovarian steroids [22,49]. Leptin also reportedly inhibits production of pregnenolone, progesterone, and 20 alpha-hydroxy-4-pregnen-3-one, via decreasing the expression of adrenodoxin [4].

In primiparous cows, the energy demands of growth, load of the first lactation, and calving combine to create stress for the animals. The negative effect of energy requirements in the prepartum and postpartum periods on reproductive performance are more harmful to primiparous cows compared to multiparous cows [2,47]. It must also be considered that heifers have different metabolic and reproductive characteristics compared to mature cows during the lactation period [21,33]. To date, the effects of parity on the BCS, leptin concentrations, and concentrations of some metabolites on reproductive performance remain controversial.

The present study was designed to compare the concentrations of leptin and BCS, and to determine the potential relationship between serum leptin and body fat reserves in Holstein primiparous and multiparous cows during the early postpartum period. This investigation also focused on how the leptin and BCS alterations reflect the subsequent fertility and other metabolic traits in primiparous compared with multiparous cows within pp 8 weeks.

**Materials and methods**

**STUDY DESIGN AND HERD DESCRIPTION**

The present study was conducted at a commercial dairy farm in the Southern Marmara region of Turkey where cows are housed at an in or outdoor paddock with a free stall system. We selected Holstein primiparous (n = 80) and multiparous cows (between 2 to 4 parturitions, n = 80) that were calving between November and February to minimize seasonal effects. All cows were housed in the same building under identical environmental and nutritional conditions. Throughout the experimental period, the cows were fed a mixed ad libitum ration providing 1.57–1.70 Mcal/kg dry matter, with a composition designed to meet the requirements for living and growing performance and lactation. Their total mixed rations consisted of grass, corn silage and commercial concentrate which were given twice a day, with mineral supplements according to their requirements.

Cow’s having common diseases, mastitis, puerperal metritis and lameness were excluded in the study. All clinical examinations were performed two hours after morning milking. Body condition scores were recorded by the same observer using the 1-5 scale according to Ferguson et al. [10] on the day of calving. Animals were then scored weekly until the eighth week postpartum.

**SPECIMEN COLLECTION AND BIOCHEMICAL ANALYSES**

For monitoring all parameters, blood specimens were collected from the coccygeal vein into vacutainer tubes without anticoagulant at 2, 3, 4, 5, 6, 7, and 8 weeks after calving. For leptin analysis, blood specimens were also collected on calving day. After collection, blood specimens for serum were stored at 4°C for 3 hr and then specimens were centrifuged at 3000 rpm for 15 min. Serum was decanted and stored at -20°C until biochemical analysis. Serum leptin concentrations were determined using a leptin ELISA kit (Cusabio, CSB-E06771b-Bovine) as per the manufacturer’s instructions. Leptin assay was validated by serial dilutions of a specimen and curve response found to be compatible with the stand curve generated using kit standards. In each serum specimen, the concentrations of AST, cholesterol, total bilirubin, nonesterified fatty acids and beta hydroxybutyric acid were measured on an automatic chemistry analyzer using commercial kits (Erba Mannheim test kits for AST, cholesterol, and total bilirubin, Wako NEFA-HR (2) test kit, Wako Autokit-3HB test kit) according to the manufacturer’s instructions. All the analytes except NEFA and BHB were calibrated using ERBA XL Multical (XSYS0034) and calibration verified using two level control serum (ERBA Norm-BLT00080 and ERBA Path-BLT00080). NEFA and BHB reagents were calibrated using their respective standards supplied by the manufacturer and calibration was verified with two level control serum (Randox Quality Control-HS2611) obtained from Randox Company.

**GYNECOLOGIC EXAMINATIONS AND EVALUATION OF REPRODUCTIVE PERFORMANCE**

Starting at the second week after calving, the genital tract was examined [32] weekly using an Aloka SSD-500 ultrasonography unit equipped with a 7.5 MHz transrectal transducer. The diameters of the largest follicles on examination days and the presence of corpora lutea were recorded as indicators of prior ovulation rate. Animals with a corpus luteum on one ovary were considered cyclic animals. The herd fertility was assessed based on the calving-to-first insemination interval, insemination index, and the calving-to-conception interval.

**STATISTICAL ANALYSES**

For between-groups comparisons of data collected from calving to the eighth week of lactation, we performed repeated measures of the analysis of variance using commercially available software (SPSS 14.1). First, the Kolmogorov-Smirnov test and Leven’s test were performed to determine the normality of distribution and homogeneity of investigated parameters. All parameters in our study were distributed normally. Data were expressed as mean ± SEM. Additionally, differences for each week of primiparous and multiparous cows were evaluated by t-test. Pearson’s correlation was used to analyze the association between all studied parameters.
The calving-to-first insemination interval and calving-to-conception interval were evaluated with a t-test. Results are shown as coefficient of correlation and P value. The values P<0.05 were considered statistically significant.

**Results**

**BODY CONDITION SCORE PROFILES**

In primiparous cows, BCS changed over time (P<0.001, Fig. 1), showing significant differences between all timepoints except 4 and 6 weeks postpartum. However, similar BCS changes was observed in multiparous cows (P<0.001, Fig. 1) with significant differences between all timepoints except 4 and 5 weeks postpartum. During the study period, the mean BCS was significantly lower in primiparous compared to multiparous cows (P<0.05).

**METABOLIC PARAMETERS**

The leptin concentration changed with time (P<0.05) in both groups and at all timepoints, excluding 6 and 7 weeks postpartum (Fig. 2). Additionally, the leptin concentrations significantly differed between primiparous and multiparous cows during the study period (P<0.001), with multiparous cows showing higher leptin concentrations. Although primiparous cows showed a slight increase in leptin concentration at postpartum week 6, it remained lower than in multiparous cows (P<0.001).

The serum concentrations of cholesterol, NEFA, BHB, bilirubin, and AST changed with time throughout the study period (Fig. 3). In both study groups, plasma NEFA concentrations changed over the time (P<0.001). Throughout the study, NEFA concentrations were markedly higher in primiparous cows compared to multiparous (P<0.05). BHB concentrations also changed over time in both groups (P<0.001). The BHB concentrations were significantly higher in primiparous than multiparous cows throughout the study (P<0.05).

Additionally, bilirubin concentrations changed over time in both groups (P<0.001). However, compared to primiparous, multiparous cows had lower bilirubin concentrations at postpartum 2, 3, 4th weeks (P<0.05), and then subsequently had higher bilirubin concentrations (P<0.05). In both study groups, AST concentrations was changed over the time (P<0.001). Primiparous cows had higher AST concentrations at during the study period, but this differences was significant until only 5th weeks (P<0.05).

The cholesterol concentrations also changed over time in both groups (P<0.001). The multiparous cows had higher cholesterol concentrations than primiparous during the experimental period (P<0.01).

In primiparous cows, significantly negative correlations were observed between BCS and NEFA in the second (r=-0.78; P<0.05) and sixth (r=-0.67; P<0.05) postpartum weeks. At the same times, the BCS were negatively correlated to concentrations of BHB (r=-0.69; P<0.05). Concentrations of NEFA was negatively correlated with concentrations of cholesterol (r=-0.71; P<0.05) and positively correlated with concentrations of AST (r=0.76; P<0.05) in the second postpartum week. NEFA concentrations were correlated to concentrations of T. bilirubin in the second (r=0.62) and third (r=0.63) postpartum weeks (P<0.001 for all).

In primiparous cows, throughout the study period—with the exception of the sixth postpartum week—NEFA concentrations were positively correlated with BHB concentrations in the second (r=0.74), third (r=0.69), fourth (r=0.56), and fifth (r=0.59) postpartum weeks (P<0.01 for all). NEFA concentrations were correlated to concentrations of T. bilirubin in the second (r=0.62) and third (r=0.63).
Significantly positive correlations were observed between BHB and leptin concentration in the second postpartum week ($r = 0.70, P < 0.001$ for all). NEFA was positively correlated with AST ($P < 0.001$ for all) during the second ($r = 0.71$, third ($r = 0.83$) weeks of postpartum. Finally, primiparous cows showed a significant negative correlation between cholesterol and AST concentrations in the second week of postpartum ($r = -0.68, P < 0.05$).

**ULTRASONOGRAPHIC FINDINGS AND FERTILITY PARAMETERS**

After calving, the diameter of the largest follicles changed in multiparous cows but not in primiparous cows (Fig. 4). After postpartum 3 weeks, primiparous cows showed an increased diameter of the largest follicle, while multiparous cows were showed a decrease in the largest follicle diameters ($P < 0.05$). All cows experienced at least one ovulation by 6 weeks postpartum, and ovarian cyclicity began in the first 6-week period. Multiparous cows ovulated earlier than primiparous cows ($P < 0.05$). The calving-to-first insemination interval and relatively prolonged interval from calving to conception was higher in primiparous cows than in multiparous cows and required more insemination counts to achieve conception compared to multiparous ($P < 0.05$, Fig. 5).
Discussion

In recent years, genetic studies have achieved advancements leading to improved milk production but also dramatic decreases in fertility [9,30]. This paradox may be related to the metabolic profile and energy requirements during the growing process and the pregnancy and lactation period, which impacts the subsequent fertility of dairy herds [3,14].

BCS is a simpler and more practical method of evaluating body energy and fat reserves in dairy cows [35]. However, previous results regarding BCS in primiparous and multiparous cows are controversial. Meikle et al. [33] and Kara et al. [20] reported that primiparous cows showed significantly lower BCS than multiparous cows during the postpartum period. However, other authors have not found any significantly differences in BCS between primiparous and multiparous cows [38,42,47]. In our present study, compared to multiparous cows, the primiparous cows showed a significantly lower mean BCS at parturition through postpartum week 8. Primiparous cows have lower adipose tissue reserves and require additional energy for continued growth. Therefore, it could be suggested that primiparous and multiparous cows have physiologically different energy requirements and energy utilization. The physiological differences between primiparous and mature cows might also impact the antepartum and subsequent body condition scores.

Circulating leptin concentrations are related to adipose tissue amount, and are associated with BCS alterations [5,7,44]. In contrast, other authors have found that no relationship between leptin concentrations and BCS after parturition [16]. Also, some studies were conducted to evaluate the relationship between leptin and parity but published data are inconsistent. Some authors reported no differences in leptin concentrations between multiparous
and primiparous cows [41,47], whereas others described that leptin was either lower [40] or higher [33] in multiparous cows than primiparous cows, respectively. In contrast to previous studies [16,40,47], our present data showed significantly higher BCS during the 8 weeks after calving in multiparous cows than primiparous. Similar to previous studies, also a significant correlation between leptin and BCS was determined at postpartum period in both study groups. Based on significant correlation between leptin and BCS in early postpartum period, multiparous cows with higher BCS during early lactation period compared to primiparous cows in the same period had higher leptin concentration during 2 months of lactation. It is reported that reduced reproductive capacity in animals is related to leptin deficiency [5,33,46]. Some authors have also described the relationship between leptin concentration and resumption of cyclic ovarian activity during postpartum period [17,18,28,33]. Lower leptin concentration in cows reflects the delayed ovarian activity in early lactation [43,46]. Butler [6] have described that cows with better reproductive performance had higher IGF-1 and leptin concentration. Liefers et al. [28] have also reported that higher leptin concentration were associated with shorter intervals to first observed estrus. Meikle et al. [33] reported that cows with good condition at parturition had also a higher leptin concentration during early lactation and thus these cows had a better reproductive activity. Results of the study presented here are in agreement with previous studies showing that the calving-to-estrus interval, the calving-to-conception interval, and the insemination index (rate) were higher in heifers that exhibited low concentrations of leptin. These results suggested that during postpartum periods of NEB, leptin concentrations may be used as indicators of energy balance and fertility prognosis in practice. Additionally, the different production system, energy intake and range of BCS, could be effect the leptin levels among primaparous and multiparous cows.

Prior results regarding NEFA and BHB concentrations in primiparous and multiparous cows are controversial. Wathes et al. [47] reported that multiparous cows had higher plasma BHB and lower NEFA concentrations than primiparous cows. In contrast, other authors have found that primiparous cows have higher circulating NEFA [33,42] and BHB concentrations [33] than multiparous cows during the early postpartum period. In our present study, compared to multiparous cows, primiparous cows had higher plasma NEFA and BHB concentrations, which were similar to those reported by Tanaka et al. [42] and Meikle et al. [33]. The discrepancies in the literature may be due to study animals having lower antepartum BCS concentrations and a higher decrement of BCS during the study period in primiparous cows. The present study results showed high NEFA concentrations in cows of both study groups, although primiparous cows had higher NEFA and BHB values around the early lactation period. Our findings may suggest that primiparous cows are adversely affected by the NEB, resulting in loss of BCS and mobilized fat tissue, which was finally reflected in higher NEFA and BHB concentrations.

AST is one indicator enzyme for hepatic disease determination [13,39]. Until the 5th weeks, AST concentrations were significantly higher in primiparous cows than multiparous cows. These higher AST concentrations could be associated with the higher fat mobilizations and negative energy balance in primiparous cows due to the higher metabolic load associated with the first lactation. Additionally, we detected a significant positive correlation between AST and NEFA concentrations around the second and third postpartum weeks, which is a time of high energy requirement. Alterations in hepatic function may affect the cow’s metabolism, health, and reproduction.

Similar to AST, bilirubin is associated with liver damage and energy balance [45]. In our study, compared to multiparous cows, primiparous had higher bilirubin concentrations throughout 2, 3 and 4 weeks. Total bilirubin concentrations were not significantly different between groups since fifth weeks. Also there was no correlations between bilirubin and the other parameters in multiparous cows and except 2 and 3 weeks in primiparous. This may indicate that there were no crucial hepatic damage in either study groups, but still primiparous cows were more sensitive to this condition than multiparous.-

Cholesterol concentrations are affected by parity. Higher cholesterol concentrations are related to ovarian activity, and cholesterol may be a precursor of energy balance during the early lactation period [7]. Similar to the findings of Kappel et al. [19] here we observed that plasma cholesterol concentrations increased in parallel in primiparous and multiparous cows. Ling et al. [29] suggested that a lower cholesterol concentration is associated with a shorter interval from calving to first luteal response. Kim and Suh [22] reported that decreases of cholesterol concentration during the first months of lactation reflect body reserve utilization, and are associated with a delayed time to first insemination postpartum. We found that primiparous cows had lower cholesterol and greater losses of BCS during the study period. Additionally, longer intervals of calving to detection of first luteal activity and of calving to first insemination were observed in primiparous cows. These results suggest that these parameters were affected by parity.

Previous studies have reported that BCS at calving and during the postpartum period is associated with the cow’s health, fertility and milk yield [1]. Low BCS and high loss of BCS are associated with increased incidences of anestrus and anovulatory cycles, and with reduced conception rate [10]. Additionally, higher NEFA concentration as a result of lipomobilization is associated with nutritional anestrus [12,31]. Ospina et al. [34] reported that elevated prepartum and postpartum NEFA concentrations have detrimental effects on conception. Leroy et al. [26,27] further suggested direct toxic effects of BHB and NEFA on ova maturation. The results of our present study were in accordance with these proposals. We found that the calving-to-estrus interval, the calving-to-conception interval, and the insemination index
(rate) were higher in heifers that exhibited high levels of BCS loss, NEFA and BHB.

Heifers are generally calved around 24 months of age to maximize the economic gain of commercial farms [15]. However, this practice results in decreased fertility rates after parturition due to the continuing high growth of heifers after this age [47]. Accordingly, different management strategies should be utilized for primiparous and multiparous dairy cows to improve their metabolic health and fertility [36,37].

**Conclusions**

The presently obtained data suggest that maintaining optimal BCS during the early lactation period led to higher leptin and cholesterol concentrations, and lower NEFA and BHB concentrations. This, in turn, may result in decreased calving-to-first insemination and calving-to-conception intervals, thus improving fertility expectations from calving-to-first insemination and calving-to-conception intervals. BHB concentrations. This, in turn, may result in decreased fertility rates after parturition as compared to multiparous cows. Routine monitoring of BCS and leptin concentrations should be utilized for primiparous and multiparous dairy cows. Primiparous cows. Routine monitoring of BCS and leptin concentrations appears to be useful for nutritional and reproductive management of primiparous cows, which have a higher tendency towards an unbalanced metabolic profile as compared to multiparous cows.

**References**


FERTILITY: ASSOCIATIONS BETWEEN LEPTIN AND BODY CONDITION SCORE


