



PERIPARTURIENT PERIOD IN TERMS OF BODY CONDITION SCORE AND SELECTED PARAMETERS OF HORMONAL PROFILES

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ABSTRACT

The majority of all diseases in dairy cows occur during the period from three weeks before parturition to three weeks after parturition, in the periparturient or transitional period. The objective of this study was to evaluate the dynamics of selected parameters of: the hormonal profiles, the body condition score (BCS) and their interrelationships. The study was carried out on 15 dairy cows of the Slovak Pied Cattle, from three weeks before to nine weeks after parturition, which were divided into six groups. The concentrations of leptin during *ante partum* increased from $23.08 \pm 10.58 \text{ ng.ml}^{-1}$ to $26.80 \pm 11.47 \text{ ng.ml}^{-1}$, then gradually decreased ($P > 0.05$), and conversely, the concentrations of ghrelin before parturition were found to be decreasing and during the postpartal period, the concentrations increased, with the highest value of $35.94 \pm 16.85 \text{ pg.ml}^{-1}$. In the case of insulin, we found the opposite tendency of ghrelin. We observed significantly higher values of BCS in dry cows than in cows after parturition ($P < 0.001$). Comparing the BCS and the parameter of the hormonal profiles, we found

both positive and negative correlations: leptin and ghrelin ($r = -0.235$, $P < 0.05$), and BCS and insulin ($r = 0.232$, $P < 0.05$), and BCS and leptin ($r = 0.360$, $P < 0.001$). The interrelationships between the hormones and the body condition score, provided evidence that the variations in concentrations of leptin, ghrelin and insulin were related to variations in the BCS.

Key words: body condition score; dairy cow; ghrelin; insulin; leptin; periparturient period

INTRODUCTION

The periparturient period is characterized by a sudden increase in energy requirements imposed by the onset of lactation and by a decrease in voluntary dry matter intake (DMI) which results in a negative energy balance (NEB) [18]. Dairy cows undergo tremendous metabolic and physiological adaptations around the time of parturition in order to support lactation. The dry period, in particular, the transitional period, is characterized by dramatic

changes in the endocrine status [11]. These changes prepare the cow for lactogenesis and parturition. Essentially all dairy cows experience a period of: insulin resistance, reduced feed intake, negative energy balance, hypocalcemia, reduced immune function, and bacterial contamination of the uterus soon before, or within the weeks after calving. Physiological and pathological changes associated with NEB are important factors related to the development of: ketosis, displaced abomasum, and retained placenta [7]. These changes may also impact the immune system which can lead to the occurrence of infectious diseases such as mastitis and metritis.

During the last three weeks of pregnancy, nutrient demands by the fetal calf and placenta reach their maximum, yet DMI may decrease by 10 to 30 % compared with the intake during the early dry period. This, in itself, may not be a cause for alarm, as decreased food or feed intake around parturition is a common finding in many mammalian species [9]. Cows lose body condition during early lactation due to NEB. As a result of NEB, cows have their lowest BCS at approximately one to two months *post partum* [28]. Dairy cows, like other mammals, undergo a normal cycle of body energy storage and mobilization, with an increased body fat storage during mid-gestation and increased body fat mobilization during early lactation [9]. Several aspects of dietary management and body condition may affect DMI and increase or decrease the susceptibility to periparturient health problems. Overfeeding energy during the dry period is a prominent risk factor. Management or environmental circumstances that force cows away from their optimal body condition may result in increased risk for health problems [15]. It is well known that overconditioned cows are at a greater risk for the development of metabolic problems [26], and have poorer DMI after calving and readily break down their excessive stores of body fat [24], and they lose more muscle fiber area after parturition than thin cows, suggesting a greater mobilization of body protein as well as fat. Obesity leads to increased susceptibility to the complex of metabolic disorders and infectious diseases known as the “fat cow syndrome”. Overconditioning results in the impairment of the immune system [16] and also results in greater indices of oxidative stress [3].

A number of metabolic hormone concentrations also change during this critical period. Leptin may have a role in the activation of lipid oxidation and may protect non-adipose tissue from excessive fat accumulation. Leptin is

involved in the central and/or peripheral regulation of: body homeostasis, energy (intake, storage and expenditure), fertility, and immune functions [6]. The reduction of plasma leptin to the NEB is caused by the initiation of copious milk secretion. Temporal changes of plasma leptin and energy balance near calving are parallel, and plasma leptin and NEB nadirs seem to coincide. Undernutrition, or even short-term restriction of access to food, results in a significant reduction in leptin concentrations in ruminants [1]. Leptin acts in opposing fashion to ghrelin by signaling satiation.

Ghrelin has a role in signaling the deposition of fat tissue by increasing food intake and reducing fat utilization [5]. This causes ghrelin to be expressed at lower concentrations during states of positive energy balance (EB) and increased during NEB [21]. It has been shown that ghrelin may play a role in regulating the EB.

Changes in insulin play an important role in the metabolic adaptation of cattle to changes in weight and body condition [17]. Insulin is also a putative mediator of the nutritional status. However, the process of adaptation to the NEB in dairy cows usually is accompanied with a decrease of blood insulin [31]. The genetic selection for milk production has been associated with a decline in circulating insulin levels in dairy cows in early lactation. In addition, adipose and muscle become insulin resistant in late gestation and develop an increased sensitivity to lipolytic agents. Holtenius [13] reported that cows fed a higher energy allowance during the dry period had a greater degree of insulin resistance before and after calving, which allowed for greater non-esterified fatty acids (NEFA) concentrations.

The aim of this investigation was to determine the plasma concentrations of selected parameters of hormonal profiles: leptin, ghrelin and insulin; their changes in relation to the period of *ante partum* and *post partum*; evaluation of BCS and assessment of their interrelationships.

MATERIALS AND METHODS

The monitored parameters of the hormonal profiles: leptin, ghrelin, insulin and body condition score, were evaluated in dairy cows ($n=15$) of the Slovak Pied Cattle breed (aged three—five years). Blood samples were taken from three weeks before parturition until nine weeks after parturition by direct puncture of *v. jugularis*. The dairy

cows were classified into six different groups based on the calving date — according to certain phases of *ante partum* (*a.p.*) and *post partum* (*p.p.*):

- Group 1 – dairy cows 3 weeks before parturition (3 wk *a.p.*) (n = 15)
- Group 2 – dairy cows 1 week before parturition (1 wk *a.p.*) (n = 15)
- Group 3 – dairy cows 1 week after parturition (1 wk *p.p.*) (n = 15)
- Group 4 – dairy cows 3 weeks after parturition (3 wk *p.p.*) (n = 15)
- Group 5 – dairy cows 6 weeks after parturition (6 wk *p.p.*) (n = 15)
- Group 6 – dairy cows 9 weeks after parturition (9 wk *p.p.*) (n = 15)

All of the variables were analyzed in blood serum. The blood samples were collected three hours after feeding. The mean production age was 2.5 lactations. The milk yield during the previous lactation was 6668.5 kg of milk. The animals were fed a total mixed ration (TMR) twice daily and had free access to drinking water. The nutrient composition of the TMR varied with the stage of pregnancy and lactation. The concentrations of leptin (ng/ml) and ghrelin (pg.ml⁻¹) were determined by RIA kits from Millipore (St. Charles, Missouri, USA). The insulin (IU.ml⁻¹) was determined by ELISA using commercial assays (Cusabio, China). The body condition score was determined using a 5-point scale and backfat thickness (BFT) measurements were obtained using a 3.5 MHz linear transducer. The body condition score and the BFT were assessed according to Staufenbiel [27]. The evaluation of the results were performed by the assessment of the mean values (x) and the standard deviations (S.D.) in each monitored period. The significance of differences in the average values in relation to several monitored periods were evaluated by the one way analysis of variance (ANOVA). The significance of differences in the average values between the different time of blood taking were evaluated by the Tukey's Multiple Comparisons Test. The Pearson's correlation coefficients were calculated to describe the relationships between the variables; the relationships were evaluated by a linear regression analysis, including significance of the correlation at the same time. The statistical analyses were done with the GraphPad Prism 3.0 software.

RESULTS

The components of *pre partum* and *post partum* diets (kg.head⁻¹day⁻¹) are shown in Table 1. The concentrations of the metabolic hormones leptin, ghrelin, and insulin in the blood serum during *ante partum* and *post partum* are presented in Table 2. The values of the BCS are shown in Table 3. Correlation between the concentration of leptin and ghrelin is shown in Figure 1; correlation between the concentration of leptin and BCS is shown in Figure 2 and correlation between the concentration of insulin and BCS is shown in Figure 3.

The concentrations of leptin during *a.p.* increased, then gradually decreased without any statistical significance. In the case of ghrelin, we found an opposite tendency; the concentrations before parturition decreased and in the early postpartal period the concentrations increased; the highest value was recorded six weeks after parturition. The concentrations of insulin in the time before calving were significantly higher than the values recorded after calving

Table 1. Components of prepartum and postpartum diets
[kg.head⁻¹day⁻¹]

	3 and 1 wk <i>a.p.</i>	1wk <i>p.p.</i>	3 wk <i>p.p.</i>	6 wk <i>p.p.</i>	9 wk <i>p.p.</i>
Meadow hay	5.5	1.5	1.5	1.5	1.5
R24	0.3	0.25	0.3	0.25	0.25
Haylage	4	4	6	6	6
Lucerne silage	13	24	22	22	22
Green fodder		25	25	25	25
Soybean meal		0.8	0.8		
Rape meal		2.5	2.5	2.5	2.5
Wheat meal		3	4	2.5	
Limestone		0.2	0.2	0.2	0.2
Flaxseed meal			0.5	1	1
Maize meal				1	
Triticale					3.5

R-24 – mineral supplement
(10.4% Ca, 9% P, 11% Na, 4% Mg, 7000 mg Cu, 3000 mg inorganic Mn, 6000 mg inorganic Zn, 40 mg Se, 100 mg I, 20 mg Co, 1 000 000 IU vitamin A, 100 000 IU vitamin D3, 2000 IU vitamin E); wk – weeks before and after parturition; *a.p.* — ante partum; *p.p.* — post partum

Table 2. Concentration of parameters of hormonal profile

Parameter		3 wk a.p.	1 wk a.p.	1 wk p.p.	3 wk p.p.	6 wk p.p.	9 wk p.p.	P
Leptin [ng.ml ⁻¹]	x	23.08	26.80	24.98	19.59	19.12	18.91	ns
	SD	10.58	11.47	8.92	6.51	5.03	7.90	
Ghrelin [pg.ml ⁻¹]	x	29.25	26.57	30.54	32.73	35.94	35.63	ns
	SD	4.82	5.35	5.40	15.70	16.85	18.02	
Insulin [IU.ml ⁻¹]	x	580.8	625.5 ^{ao}	483.3	437.1	375.3 ^a	388.7 ^a	< 0.01
	SD	66.30	174.9	289.0	222.7	169.9	172.5	

Results are presented as mean $x \pm$ SD. The same indices in lines represent significance of differences in the mean values between the groups:
^{α,β} — $P < 0.05$; ^a — $P < 0.01$; P — significance of the differences of the results during monitored time; ns — not significant

Table 3. Mean values of the body condition score

BCS		3 wk a.p.	1 wk a.p.	1 wk p.p.	3 wk p.p.	6 wk p.p.	9 wk p.p.	P
	x	4.42 ^{ABC}	4.25 ^{aDE}	3.90 ^{αβ}	3.48 ^{Aa}	3.35 ^{BDα}	3.25 ^{CEβ}	< 0.001
	SD	0.75	0.58	0.45	0.51	0.40	0.30	

Results are presented as mean $x \pm$ SD. The same indices in lines represent significance of differences in the mean values between the weeks:
^{α,β} — $P < 0.05$; ^a — $P < 0.01$; ^{A, B, C, D, E} — $P < 0.001$

($P < 0.01$). The mean values of BCS during three weeks and one week before parturition were significantly higher than at three weeks after parturition ($P < 0.001$). By assessment of the correlation between the leptin and ghrelin, we recorded a significant negative correlation ($r = -0.235$, $P < 0.05$). The leptin and BCS exhibited a statistical significant positive correlation ($r = 0.360$, $P < 0.001$) and the insulin and BCS displayed a positive correlation which was statistically significant ($r = 0.232$, $P < 0.05$).

DISCUSSION

The period of transition between late pregnancy and early lactation presents a huge metabolic challenge to the high-yielding dairy cow and the biochemical profiles are important in evaluating the health status of animals during this transition [2]. The health status of dairy cows, especially during the transition period is a limiting factor of production and reproduction. The production diseases of

dairy cows are caused by: a level of production inconsistent with nutrient intake, provision of an inadequate diet, an unsuitable environment, an inappropriate breeding policy, or various combinations of these factors. They have a higher incidence during the transitional period [20].

The onset of lactation is generally characterised by a NEB, due to a drastic increase in energy requirements for milk yield and a simultaneous depression in DMI. The energy status of dairy cows is evaluated by energy intake and output [25] and expressed by the BCS [22]. Several studies have shown that the over-conditioned of dry cows have a greater depression of feed intake during the peripartal period and deeper NEB than cows with a lower BCS.

The transition from pregnancy to lactation in dairy cows is associated with a reduction

in the plasma concentration of leptin. We found non-significantly lower concentrations of leptin during the postpartal period, compared with concentrations during the prepartal period. The changes in the plasma concentration of leptin, could also be an important adaptation,

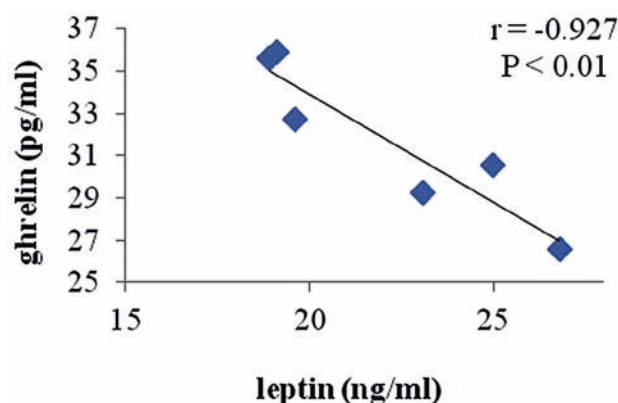


Fig. 1. Correlation between the concentrations of ghrelin and leptin *ante partum* and *post partum*
r — Pearson's correlation coefficient

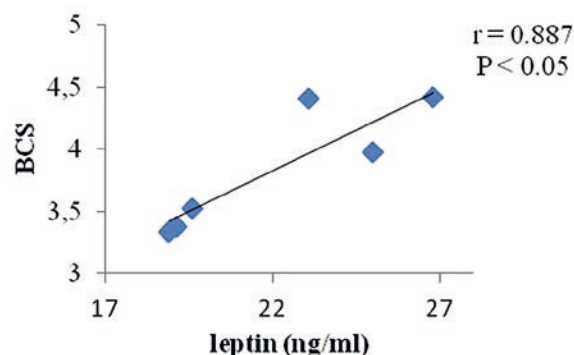


Fig. 2. Correlation between the concentrations of plasma leptin and body condition score *ante partum* and *post partum*

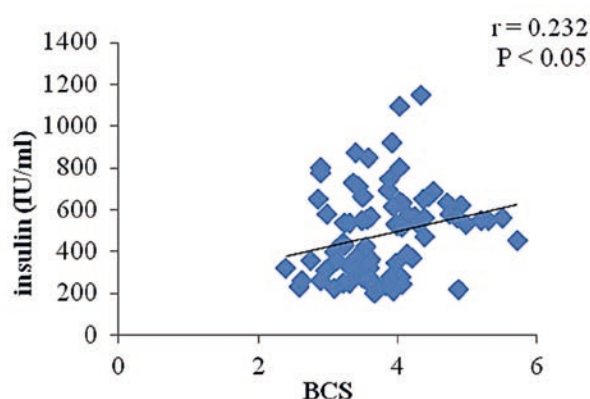


Fig. 3. Correlation between the concentrations of insulin and body condition score *ante partum* and *post partum*

particularly given the role of white adipose tissue (WAT) in support of early lactation in dairy cattle. It is likely to promote centrally mediated adaptations required during periods of energy deficits. The reduced synthesis of leptin in WAT is largely responsible for the lower concentration of plasma leptin in early lactating dairy cows. This reduction could benefit early lactating dairy cows by promoting a faster increase in feed intake and by diverting energy from non-vital functions such as reproduction [4].

Circulating ghrelin concentrations increase during fasting or NEB in dairy cows, and exogenous administration of ghrelin stimulates feed intake in rats and cattle [5, 32, 33]. We did not detect a significant difference in plasma ghrelin concentrations during the monitored period. The highest concentrations of ghrelin were found six weeks p.p. The highest value of ghrelin immediately after calving has been

associated with changes in the feed intake and the initiation of lactogenesis.

Our data indicated that insulin blood concentration decreased from the dry period towards early lactation. The insulin concentration was significantly different in dried cows compared to dairy cows after parturition. In contrast, Illek et al. [14] observed a gradual increase of insulin concentrations during the progressing lactation. A decrease in insulin blood concentration at calving is a metabolic adaptation to cope with the energy demands of lactation [28, 30], as low insulin concentrations favour gluconeogenesis and lipolysis [12].

If valid, the interpretation that leptin negatively regulates ghrelin could imply that the weight reducing effects of leptin are mediated, not only via direct central actions, but also via the peripheral inhibition of ghrelin. In this study, leptin concentrations decreased during the postpartal period, while the concentrations of ghrelin increased.

The data observed in our study confirmed the relationship between leptin and BCS. Our results are in agreement with previous studies showing that plasma leptin was positively correlated with BCS in cows during lactation [8]. Reist et al. [23] also observed a positive relationship between BCS and plasma leptin, whereas Holtenius et al. [13] did not. These results confirm that plasma leptin in ruminants is related to body fat, as previously observed in humans and rodents [19]. We detected a positive relationship between plasma leptin and BCS. This is in agreement with a previous study [29]. However, in the study of León et al. [17], as heifers achieved a higher BCS, the rate of increase in insulin differed.

CONCLUSIONS

In conclusion, our results suggest that selected parameters of hormonal profiles changed throughout the time of *ante partum* and *post partum*, which suggests that they have a physiological role in the dairy cow's energy metabolism. These data provide evidence that the variations in the concentrations of leptin, ghrelin and insulin are related to variations in the BCS. The negative correlation between leptin and ghrelin contributes to the argument that leptin negatively regulates ghrelin. The high rates of the BCS loss in the early postpartum period are associated with a severe NEB alteration in the hormonal profiles. In the period we recorded significant correlations between leptin and ghrelin, leptin and body condition score, as well as between insulin and BCS.

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