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Hormonal profile and body condition scoring in dairy cows during *pre partum* and *post partum* periods

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Abstract

The aim of this study was to evaluate the dynamics of selected indicators of energy, hormonal profile, body condition score (BCS) and their relationships in dairy cows of the Slovak Pied Cattle from 3 weeks before parturition to 9 weeks after. Significant differences were found in the mean values of non-esterified fatty acids (NEFA) ($P < 0.001$) and β-hydroxybutyrate (BHB) ($P < 0.05$). According to BCS results, the dry cows were overweight (4.42 ± 0.75 points). After calving the cows lost weight significantly, as the BCS was 3.25 ± 0.30 points at 9 weeks *post partum* ($P < 0.001$). The highest concentration of leptin was recorded before calving (26.80 ± 11.47 ng/ml). The concentrations of insulin and ghrelin did not change significantly (an increase in insulin concentrations from 580.8 ± 66.30 IU/ml to 625.50 ± 174.90 IU/ml and a decrease in ghrelin concentrations from 29.25 ± 4.82 pg/ml to 26.57 ± 5.35 pg/ml were found comparing 3 weeks to 1 week before parturition, respectively). Relationships between the hormones showed positive correlation between insulin and leptin ($r = 0.220$, $P < 0.05$), BCS and leptin ($r = 0.360$, $P < 0.001$), BCS and insulin ($r = 0.232$, $P < 0.05$) and negative correlation between leptin and ghrelin ($r = -0.235$, $P < 0.05$), BCS and ghrelin ($r = -0.257$, $P < 0.05$). These data provide evidence that the variations in the concentrations of leptin, ghrelin, and insulin are related to variations in the BCS. Negative correlation between leptin and ghrelin contributes to the argument that leptin negatively regulates ghrelin.

Body condition score, leptin, ghrelin, insulin, cows, periparturient period

The majority of all diseases occur during the period from three weeks before parturition to three weeks after parturition, i.e. in the periparturient or transition period (Bauman 2000). The transition period is associated with a peak incidence of production disease, and the effects of these diseases on dairy cows' health and productivity extend far into the subsequent lactation. These diseases include the fatty liver syndrome, ketosis, alkalosis, oxidative stress, laminitis, mastitis, milk fever, retained placenta, metritis, infertility, etc. Their effects on the production of dairy cows are different, e.g., Illek et al. (1994) found a close relationship between blood values and milk constituents, and a reduction in milk proteins during metabolic alkalosis. The onset of lactation is generally characterised by a negative energy balance (NEB), due to a drastic increase in energy requirements for milk yield and a simultaneous depression in dry mater intake (DMI). The energy status in dairy cows is evaluated by energy intake and output (Rukkwamsuk et al. 1999) and expressed by the body condition score (BCS) (Pavlata et al. 2008). Energy deficiency during lactation causes fat mobilization from the body deposits, increased concentration of non-esterified fatty acids (NEFA) in the blood and increased milk fat synthesis in the mammary gland (Pechová and Pavlata 2005). Metabolic profile indicators are of great importance for early identification of energy metabolism disturbances in cows. Most reliable indicators of the cow's energy status are β-hydroxybutyrate (BHB) and glucose concentrations during the dry period. Concentrations of these indices are in high correlation with blood NEFA

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concentrations and BCS during the peripartal period (Stengärde et al. 2008; Prodanović et al. 2010). Cows lose in 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*. Several studies have shown that over-conditioned dry cows have a greater depression of feed intake during the peripartal period and deeper NEB than cows with a lower BCS. A number of metabolic hormone concentrations also change over this critical period. Genetic selection for milk production has been associated with a decline in circulating insulin concentrations in dairy cows (Taylor et al. 2003) and insulin concentrations tend to fall in early lactation. In addition, adipose and muscle become insulin resistant in late gestation, but develop an increased sensitivity to lipolytic agents. Changes in insulin play an important role in the metabolic adaptation of cattle to changes in weight and body condition (León et al. 2004).

Changes in the plasma concentration of leptin could also be an important adaptation, particularly given the role of white adipose tissue (WAT) in support of early lactation in dairy cattle. Leptin is involved in the central and/or peripheral regulation of body homeostasis, energy intake, storage and expenditure, fertility and immune functions (Chilliard et al. 2005). Undernutrition or even short-term restriction of access to food results in a significant reduction in leptin concentrations in ruminants (Amstalden et al. 2000). It has been shown that ghrelin may play a role in regulating energy balance (EB). Ghrelin has a role in signalling the deposition of fat tissue by increasing food intake and reducing fat utilization (Bradford and Allen 2008). Leptin acts in opposing fashion to ghrelin by signalling satiation. This causes ghrelin to be expressed at lower concentrations during states of positive EB and increased during NEB (Nogueiras et al. 2008). The aim of this study was to determine the plasma concentrations of hormonal profile indicators: leptin, insulin, ghrelin and selected variables of the energy profile: NEFA, glucose and BHB, their changes in relation to the peripartal period; evaluation of BCS which was changed due to NEB and assessment of their relationships.

Materials and Methods

Selected indicators of the hormonal profile - leptin, ghrelin, insulin; energy profile – NEFA, glucose, BHB, and body condition score were evaluated in dairy cows (aged 3–5 years) of the Slovak Pied Cattle breed from April to August 2013. Dairy cows (n = 15) were classified into different groups based on the calving date (according to certain phases of *ante partum* (a.p.) and *post partum* (p.p.). The animals were divided individually into 6 groups:

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)

The mean production age was 2.5 lactations. The milk yield during the previous lactation was 6 668.5 kg of milk during a 305-day lactation. The animals were fed a total mixed ration (TMR) twice daily, nutrient composition of the TMR varied with the stage of pregnancy and lactation (Table 1). The dairy cows had free access to drinking water. The experiment was carried out in accordance with the established standards for animal care and use on a farm near Košice. The protocol was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice, Slovak Republic (14/2014). All the evaluated variables were analysed in blood serum. The blood samples were collected 3 h after feeding by direct puncture of v. jugularis. The concentrations of NEFA (Randox, UK) was assessed by spectrophotometric method - Specord 210 Plus (Analytik Jena, Germany). The concentrations of BHB and glucose were determined using commercial diagnostic kits (Randox, UK) on automatic biochemical analyser Alizé (Lisabio, France). Insulin (IU/ml) was determined by ELISA using commercial assays (Cusabio, China) according to the manufacturer's instructions. The concentrations of leptin (ng/ml) and ghrelin (pg/ml) were determined by RIA kits from Millipore (St. Charles, Missouri, USA) according to the manufacturer's instructions. 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 (BCS) and BFT were assessed according to Staufenbiel (1997). The examination site was located in the sacral region between the caudal one-quarter and one-fifth connection line going from the dorsal part of the tuber ischia (pins) to the tuber coxae (hooks). This site corresponds to the area between the end of the crista sacralis and the end of the os sacrum (i.e., beginning of the first coccygeal vertebra). Results for BCS are presented as mean BCS and

Table 1. Components of *prepartum* and *postpartum* diets (kg/head/day).

	3-1 wk a.p.	1wk p.p.	3 wk p.p.	6 wk p.p.	9 wk p.p.
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, 1000 000 IU vitamin A, 100 000 IU vitamin D3, 2 000 IU vitamin E)

as digression from physiological values at examined periods. Animals were scored for body condition on the day of their blood collection. Evaluation of the obtained results was performed by the assessment of mean values (x) and standard deviations (S.D.) in each monitored group of dairy cows. Significance of differences in the mean values in relation to the several monitored periods was evaluated by one way analysis of variance (ANOVA). Significance of differences in the mean values between groups was evaluated by Tukey's multiple comparisons test. Pearson's correlation coefficients were calculated to describe relationships between the monitored variables, relationships were evaluated by linear regression analysis, including significance of the correlation at the same time. Statistical analyses were done with the GraphPad Prism 3.0 software.

Results

Concentrations of metabolic hormones (leptin, ghrelin, and insuline) in blood serum during prepartal and postpartal periods are presented in Figs 1, 2 and 3. Body condition scoring results are shown in Table 2. Concentrations of selected metabolites of the energy

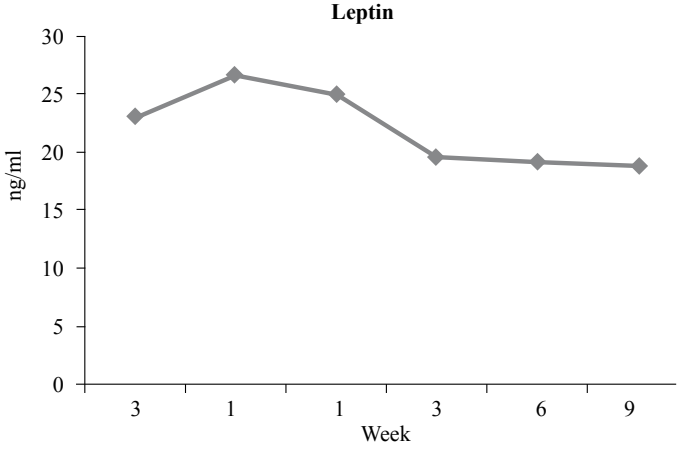

Fig. 1. Mean concentrations of leptin in the monitored groups of dairy cows during prepartal and postpartal periods

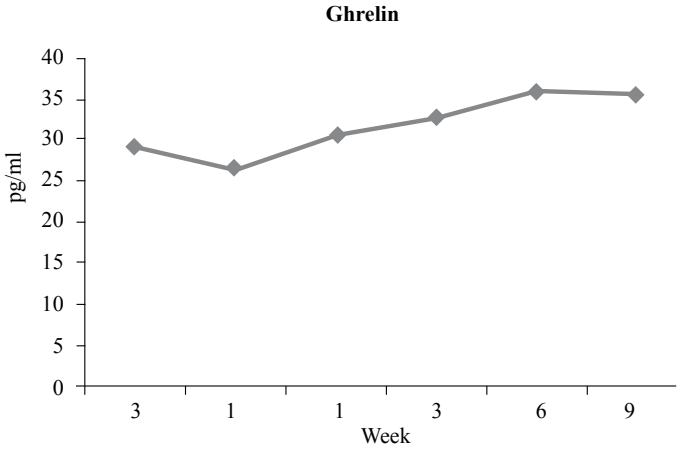

Fig. 2. Mean concentrations of ghrelin in the monitored groups of dairy cows during prepartal and postpartal periods

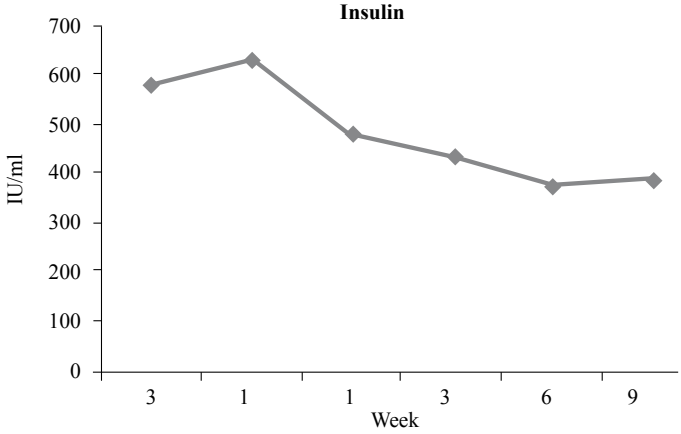

Fig. 3. Mean concentrations of insulin in the monitored groups of dairy cows during prepartal and postpartal periods

profile that were achieved after analyses of blood samples are shown in Table 3. Correlation between the concentration of plasma leptin and BCS is shown in Fig. 4; correlation between the concentration of ghrelin and leptin is shown in Fig. 5; correlation between the concentration of insulin and leptin is shown in Fig. 6; correlation between the concentration of ghrelin and BCS is shown in Fig. 7; and correlation between the concentration of insulin and BCS is shown in Fig. 8.

The concentrations of leptin during the a.p. increased from 23.08 ± 10.58 ng/ml (Group 1) to 26.80 ± 11.47 ng/ml (Group 2) then gradually decreased ($P > 0.05$). Ghrelin concentrations before parturition were found to be decreasing, in the early postpartal period the concentrations increased and the highest value (35.94 ± 16.85 pg/ml) was recorded 6 weeks after parturition. In the case of insulin we found an opposed tendency, the

Table 2. Mean values of the body condition score at different stages.

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}	$3.90^{\alpha\beta}$	3.48^{Aa}	$3.35^{BD\alpha}$	$3.25^{CE\beta}$	< 0.001
SD	0.75	0.58	0.45	0.51	0.40	0.30	

Results are presented as mean ± SD (n = 15). The same indices in lines represent significance of differences in the mean values between the groups: $P < 0.05$ – α, β; $P < 0.01$ – a; $P < 0.001$ – A, B, C, D, E
P – significance of the differences of the results during monitored time
wk – weeks before and after parturition
a.p.– *ante partum*
p.p. – *post partum*

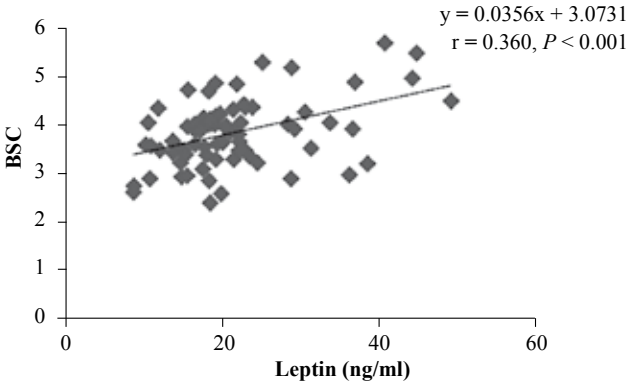

Fig. 4. Correlation between the concentrations of plasma leptin and BCS *ante partum* and *post partum* (n = 90)

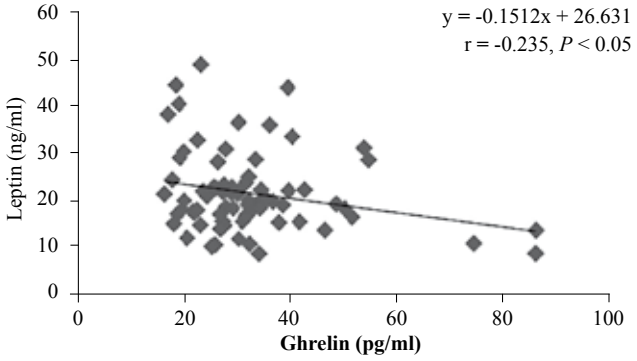

Fig. 5. Correlation analysis between the concentrations of ghrelin and leptin *ante partum* and *post partum* (n = 90)

concentrations in the time before calving were higher (from 580.8 ± 66.3 to 625.5 ± 174.9 IU/ml) than the values recorded after calving (from 483.3 ± 289.0 to 388.7 ± 172.5 IU/ml). The differences in average values of insulin concentrations during a.p. and p.p. periods in the monitored groups were not significant.

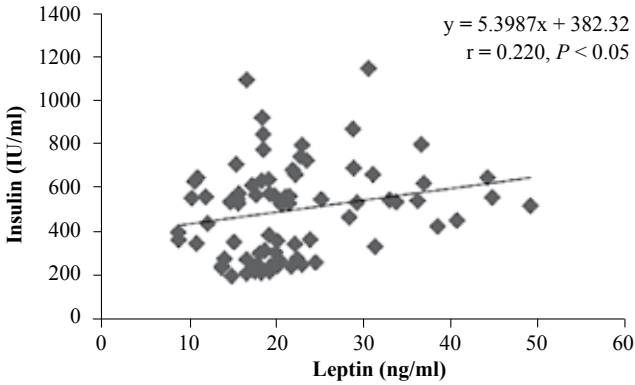

Fig. 6. Correlation analysis between the concentrations of insulin and leptin *ante partum* and *post partum* (n = 90)

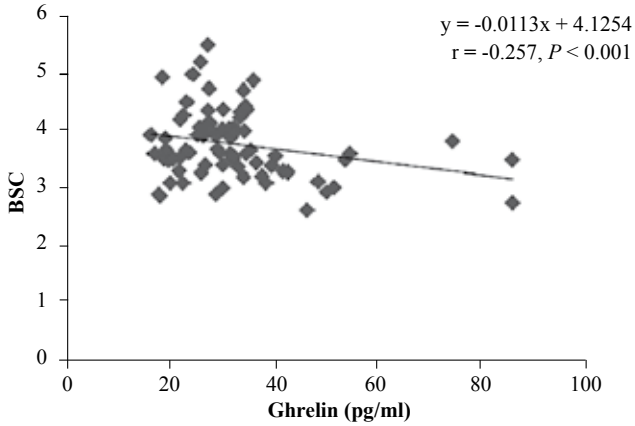

Fig. 7. Correlation analysis between the concentrations of ghrelin and BCS *ante partum* and *post partum* (n = 90)

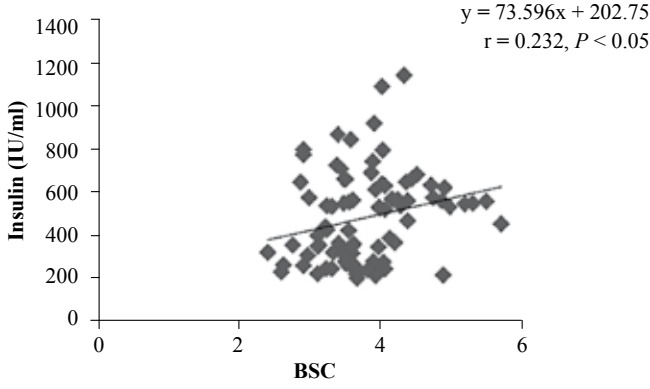

Fig. 8. Correlation analysis between the concentrations of insulin and BCS *ante partum* and *post partum* (n = 90)

Table 3. Concentrations of selected metabolites of the energy profile in dairy cows during the prepartal and postpartal periods.

Indicator		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*
Glucose	x	3.97	3.83	3.40	3.70	3.90	3.88	ns
(mmol/l)	SD	0.19		1.13	0.40	0.44	0.38	0.27
NEFA	x	0.44^{AB}	$1.29^{A\alpha C}$	1.69^{BDEF}	0.93^{D}	$0.73^{\alpha E}$	0.51^{CF}	< 0.001
(mmol/l)	SD	0.190.78	0.55	0.39	0.27	0.23		
BHB	x	0.40	0.69	0.72	0.83	0.54	0.39	< 0.05
(mmol/l)	SD	0.12	0.33	0.29	0.89	0.28	0.17	

Results are presented as mean ± standard deviation (n = 15). The same indices in lines mean significance of differences in the mean values between the groups: $P< 0.05$ – α; $P< 0.001$ – A, B, C, D, E, F
P – significance of the differences of the results during monitored time
wk – weeks before and after parturition
NEFA - non-esterified fatty acids
BHB - β-hydroxybutyrate
a.p.- *ante partum*
p.p. – *post partum*
ns – not significant

Table 2 shows that the mean BCS 3 wk *a.p.* was above the reference values. During the postparturient period we found the BCS gradually decreasing from 3.90 ± 0.45 to 3.25 ± 0.30 points with significance. Mean BCSs in dairy cows 3 and 1 wk a.p. were significantly higher than at 3 weeks p.p. ($P < 0.001$) and the BCS in cows 3 weeks a.p. was significantly higher than in cows 9 weeks p.p. ($P < 0.001$ and $P < 0.01$).

Glucose concentrations remained within the physiological range; cows after calving (1 week p.p.) exhibited the lowest concentration of glucose. In NEFA concentrations (Table 3) significantly lower values were observed in the cows 3 wk a.p. and 6, 9 wk p.p. than those determined in the cows 1 wk a.p. and 1 wk p.p. ($P < 0.001$ and $P < 0.05$). The concentrations of BHB from 3 wk a.p. to 3 wk p.p. increased from 0.40 ± 0.12 to 0.83 ± 0.89 mmol/l and then gradually decreased.

In our study (Fig. 4) leptin and BCS were in significant positive correlation ($r = 0.360$, $P < 0.001$); also insulin and BCS (Fig. 8) were in significant positive correlation ($r = 0.232$, $P < 0.05$). The BCS and ghrelin were negatively correlated ($r = -0.257$, $P < 0.05$) (Fig. 7). By evaluation of correlation between leptin and ghrelin we found negative correlation ($r = -0.235$, $P < 0.05$) (Fig. 5). By assessment of correlation (Fig. 6) between insulin and leptin we recorded their significant positive correlation ($r = 0.220$, $P < 0.05$).

Discussion

The transition from pregnancy to lactation in dairy cows is associated with a reduction in the plasma concentration of leptin. It is likely to promote centrally mediated adaptations required in periods of energy deficit. We observed non-significantly lower concentrations of leptin during the *post partum* than during the *ante partum*. 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 (Block et al. 2001). Circulating ghrelin concentrations increase during fasting or NEB in dairy cows, and exogenous administration of ghrelin stimulates feed intake in rats and cattle (Wren et al. 2000; Wertz-Lutz et al. 2006; Bradford and Allen 2008). Although we did

not detect a significant difference in plasma ghrelin concentrations over time in our study, we did observe a trend for the lowest concentrations in the dairy cows 1 week a.p. and the highest concentrations in the cows 6 weeks p.p. The highest value of ghrelin in the dairy cows immediately after calving is associated to changes in feed intake and the initiation of lactogenesis. Changes in insulin play an important role in the metabolic adaptation of cattle to changes in the weight and body condition (Leon et al. 2004). Our data indicate that insulin concentration was non-significantly different in dried cows compared to *post partum* dairy cows. It seems that the blood insulin concentration varied widely from the 1st to the 3rd week *post partum*. Similarly, gradual increase of this index was observed during the progressing lactation (Illek et al. 2009). Our data also indicate that insulin blood concentration decreases from the dry period (1 week a.p.) towards early lactation (1 week p.p.) A decrease in insulin blood concentration at calving is a metabolic adaptation to cope with the energy demands of lactation (Taylor et al. 2003; Wathes et al. 2007), as low insulin concentrations favour gluconeogenesis and lipolysis (Herdt 2000). Insulin is also a putative mediator of the nutritional status. However, the process of adaptation to the NEB in dairy cows is usually accompanied with a decrease of blood insulin (Jorritsma et al. 2003; Hammon et al. 2009; Wathes et al. 2011).

Cows with high BCS before calving had greater risks of metabolic problems because of excessive mobilization of body reserves resulting in metabolic disorders with devastating effects on health, production and reproduction of these animals. High rates of BCS loss in the early postpartal period are associated with severe NEB, alterations in blood metabolites and hormone profiles (Wathes et al. 2007). Cows with optimal BCS at calving had higher insulin concentrations in the postpartal period and adapted better than cows with a high BCS at calving (over-conditioned) to increased energy requirements after parturition. According to BCS results dry cows in this study were overweight (4.42 ± 0.75 points) because the physiological range of BCS for Slovak Pied Cattle is 3.25 to 3.75 (dry period), 3.25 to 3.75 (puerperium) and 2.50 to 3.0 (the first 100 days of lactation) (Strapák et al. 2004). Although there were no visible signs of health disorders, selected indicators of the energy profile and body condition scoring obtained in this work showed that the cows suffered from NEB during the *post partum* period. The decrease in glucose concentrations in puerperal dairy cows previously reported in different studies (Veenhuizen et al. 1991; Drackley et al. 2001; Dann et al. 2005), may be related to the sudden activity of the mammary gland and increased lactose synthesis. Furthermore, the NEB associated with lipomobilization and increased fat accumulation in hepatocytes may induce a considerable reduction in the liver gluconeogenesis, also contributing to reduction of glycaemia. A *pre partum* rise in NEFA suggested that the cows were already in NEB at this time and were mobilizing lipids as an energy source (Duffield 2000). According to Bernabucci et al. (2005) increased circulating NEFA concentrations are directly associated with large amounts of adipose stores during a time of energy deficiency. The increase in NEFA is generally of short duration (< 5 weeks), but our results showed that the NEFA peak remained high longer. Decreased concentrations of NEFA in dairy cows 9 weeks after calving indicate that NEB is gradually corrected by reduction of lipomobilization as a direct indicator of NEB. Serum BHB concentration is another indicator of energy metabolism disruptions which is more sensitive than glycaemia and which fluctuates in parallel to lipomobilization (Veenhuizen et al. 1991). Increasing concentrations of ketones are thought to suppress the feed intake. Cows with high values of BHB have lower reproductive capacity, significant loss in body condition, produce a small amount of milk and suffer from extreme metabolic changes (Kessel et al. 2008). The increased value of BHB indicates the possibility of fatty liver, which can affect the process of metabolic adaptation (Šamanc et al. 2011).

Our data confirm the strong link between leptin and BCS. Ehrhardt et al. (2000) estimated that BCS in late pregnant cows explained 37% of the variation in plasma leptin.

Reist et al. (2003) also observed a positive relationship between BCS and plasma leptin, whereas Holtenius et al. (2003) did not. Our results are in agreement with previous studies showing that plasma leptin was positively correlated with BCS in cows during lactation (Ehrhardt et al. 2000). These results, in addition to those obtained recently in adult sheep (Blache et al. 2000; Delavaud et al. 2000), confirm that plasma leptin in ruminants is related to body fat, as previously observed in humans and rodents (Maffei et al. 1995). Ghrelin concentrations were negatively correlated with changes in BCS, indicating that as BCS decreased, plasma ghrelin concentrations increased. Leptin negatively regulates ghrelin, and the increases of ghrelin induced by weight loss arise because of diminished inhibitory input from leptin. If valid, this interpretation could imply that the weight-reducing effects of leptin are mediated not only via direct central actions, but also via peripheral inhibition of ghrelin. The hypothesis that leptin negatively regulates ghrelin also predicts that conditions of excessive leptin signalling should be associated with low ghrelin concentrations and vice versa. In this study, leptin concentrations decreased after calving, meanwhile ghrelin concentrations increased. The significant correlations between plasma concentrations of leptin and insulin could represent co-regulation by EB, and perhaps a role for these factors in mediating the effect of EB on leptin synthesis. We observed significant positive correlation between leptin and insulin. Insulin upregulates leptin expression *in vivo* and *in vitro* in bovine WAT explants. These effects of insulin are dependent on adequate uptake of glucose, suggesting that cellular energy availability is the primary factor regulating leptin synthesis (Wellhoener et al. 2000). Our results are in agreement with previous studies showing that plasma insulin was positively correlated with BCS (Vizcarra et al. 1998). However, in the study of León et al. (2004), the rate of increase in insulin differed as heifers achieved a higher BCS.

In conclusion, our results demonstrate that variables of hormonal profile such as leptin, ghrelin and insulin, and the indicators of the energy profile such as NEFA, BHB and glucose changed throughout the time around calving, which suggests that they have a physiological role in the dairy cow's energy metabolism. Reduction in plasma leptin during the postpartal period could benefit early lactating dairy cows by promoting a faster increase in the feed intake and by diverting energy from non-vital functions such as reproduction. The presented results also showed positive or negative relationships between indicators of hormonal profile and body condition.

Acknowledgements

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