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Original article

Ghrelin and its correlation with leptin, energy related metabolites and thyroidal hormones in dairy cows in transitional period

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Abstract

The transition from late gestation to early lactation is a critical period in a dairy cow's life so that dairy cows undergo tremendous changes during this period.

The aim of this study was to determine blood levels of ghrelin, leptin, glucose, β -hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), triiodothyronine (T3) and thyroxine (T4) in dairy Holstein cows ($n = 20$) and their correlations during the transition period.

Blood samples were collected weekly from 3 wk antepartum to 6 wk postpartum from 20 high-yielding Holstein-Friesian cows. Ghrelin and leptin of plasma and glucose, BHB, NEFA, TG, T3, T4 of serum were then measured.

Early lactation cows showed significantly higher ($p < 0.05$) values of ghrelin, BHB and NEFA, and lower levels of leptin, TG, T3 and T4 ($p < 0.05$) compared to late dry cows. Serum concentrations of glucose did not differ significantly at any time ($P > 0.05$).

Plasma ghrelin concentrations showed positive correlations with the serum BHB and NEFA ($p < 0.01$), while plasma ghrelin had negative correlations ($p < 0.01$) with leptin, TG, T3 and T4. In addition, no significant correlation ($p > 0.05$) was found between ghrelin and glucose.

The results of the study showed that blood ghrelin, leptin, BHB and NEFA levels are sensitive indicators of the energy balance during the peri-partum period in dairy cows and glucose values may not be considered as a precise indicator of negative energy balance in dairy cows.

Key words: dairy cows, ghrelin, leptin, energy related metabolites, thyroid hormones, transition period

Introduction

The recognition of the importance of the period from late pregnancy until the adaptation phase of early lactation has led to the development of the concept of the transition period, which is commonly

defined as the period from 3 weeks before to 3 weeks after calving (Drackley 1999). Nutrient requirements of the fetus reach maximal levels three weeks prepartum, yet dry matter intake (DMI) decreases by 10-30% (Bell 1995). Within three weeks of the onset of lactation, milkyield, milk proteins, fat and lactose

increase rapidly and exceed feed intake (Bertoni et al. 2009). Moreover, the diet of most dairy cows changes sharply at calving from being mainly forage-based to concentrate-rich diets. Postpartum milk production and the requisite nutritional adaptations induce a physiological state of negative energy balance (NEB). The dramatic increase in energy requirements of high-producing dairy cows during this period requires homeorhetic control of metabolism to direct endogenous and dietary nutrients to the mammary gland for lactogenesis (Drackley 1999). The use of blood metabolites for herd-level health assessment during the period around calving (transition period) has also been an area of study for many years (Oetzel 2004). During the last 20 yr, there have been extensive research efforts to find strategies to improve the nutritional, metabolic and health status of periparturient dairy cows (Duffield et al. 1998, Melendez et al. 2002). Nevertheless, diseases and production stress are still prevalent, raising concern about animal productivity and welfare. Feed intake is a complex mechanism, regulated by several factors including hormones, metabolites, environmental factors, physical constraints, digestive and neuronal peptides (Ingvarsen et al. 2000). There is renewed interest in this topic, however, new research shows that blood metabolite concentrations around calving not only can predict disease, but also are associated with economically important herd parameters including milk yield and reproductive performance. Much of this work has focused on metabolites related to negative energy balance including nonesterified fatty acid (NEFA) and beta-hydroxybutyrate (BHB); however, biomarkers such as ghrelin and leptin concentrations around calving may also be useful for assessing transition cow health and performance. Ghrelin displays a wide spectrum of biological functions such as the regulation of appetite and food intake, gastrointestinal motility, gastric acid secretion, endocrine and exocrine pancreatic secretions, cell proliferation, glucose and lipid metabolism, and cardiovascular and immunologic processes (De Vriese et al. 2008, Soares et al. 2008). Leptin acts to regulate food intake, energy expenditure, homeostatic body weight, and consequently to influence fat deposition in both animals and humans (Yamada et al. 2003). Thyroid hormones modulate energy metabolism (Huszenicza et al. 2002), in which carbohydrates and lipids are the major constituents. In farm mammals the interrelation between the circulating levels of ghrelin, leptin and thyroid hormones has been poorly documented up to now. In the present study, the concentrations of ghrelin and its relationship with leptin, glucose, β -hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), triglycerides (TG), triiodothyronine (T3) and thyroxine (T4) were investigated.

Materials and Methods

The study was approved by the Animal Ethics Committee of the Islamic Azad University, Kazerun Branch. A total of 20 clinically healthy pregnant multiparous Holstein cows (2-6 lactation) weighing 600 ± 75 Kg were randomly selected from a dairy herd around Shiraz, Fars Province, Iran. A TMR diet was fed based on alfalfa, corn silage, and concentrate (mixture of barley, corn grain, soybean meal and bone meal).

Cows were housed in a free stall barn before calving and fed a TMR that was formulated for late pregnant, nonlactating cows (dry cow diet). After calving, cows were housed in a freestall barn and fed a lactating cow TMR. The TMR was formulated to meet or exceed NRC (2001) recommendations. Feed was provided twice daily; once at approximately 0800 h and a second time at 1600 h. After calving, cows were milked twice daily at 0400 and 1600 h. The experiment began 21 d before expected calving and continued until 42 d after calving.

Blood samples were collected from the jugular vein at the same time of day and the same day of the week, weekly from three weeks before until 6 weeks after calving in test tubes.

Collected blood was immediately emptied into two tubes, one containing potassium EDTA and plasma was prepared immediately and frozen at -20°C until analyzed, and other tube containing no anticoagulant where the blood was allowed to coagulate. Serum was then harvested following centrifugation, frozen and stored at -20°C , until analyzed.

Plasma ghrelin was determined using enzyme-linked immunosorbent assay (SPI Bio ghrelin EIA assay kits, Montigny, France) and the concentration of plasma leptin was measured using ELISA method (Kit available from BioVendor Laboratory Medicine Inc. Czech Republic).

Serum β -Hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) were determined using a β -hydroxybutyrate kit and a NEFA kit (Randox Laboratories Ltd., Ardmore, UK).

Serum glucose was measured by glucose oxidase method and serum triglycerides was measured by enzymatic procedure of McGowan et al. (1983).

Serum T3 and T4 were measured by radioimmunoassay (RIA) method (kits available from

Immunotech Company, Immunotech-Radiova, Prague, Czech Republic) in Namazi Research Center, Shiraz, Iran.

All values were expressed as mean and standard deviation (SD), and $p = 0.05$ was considered as statistically significant.

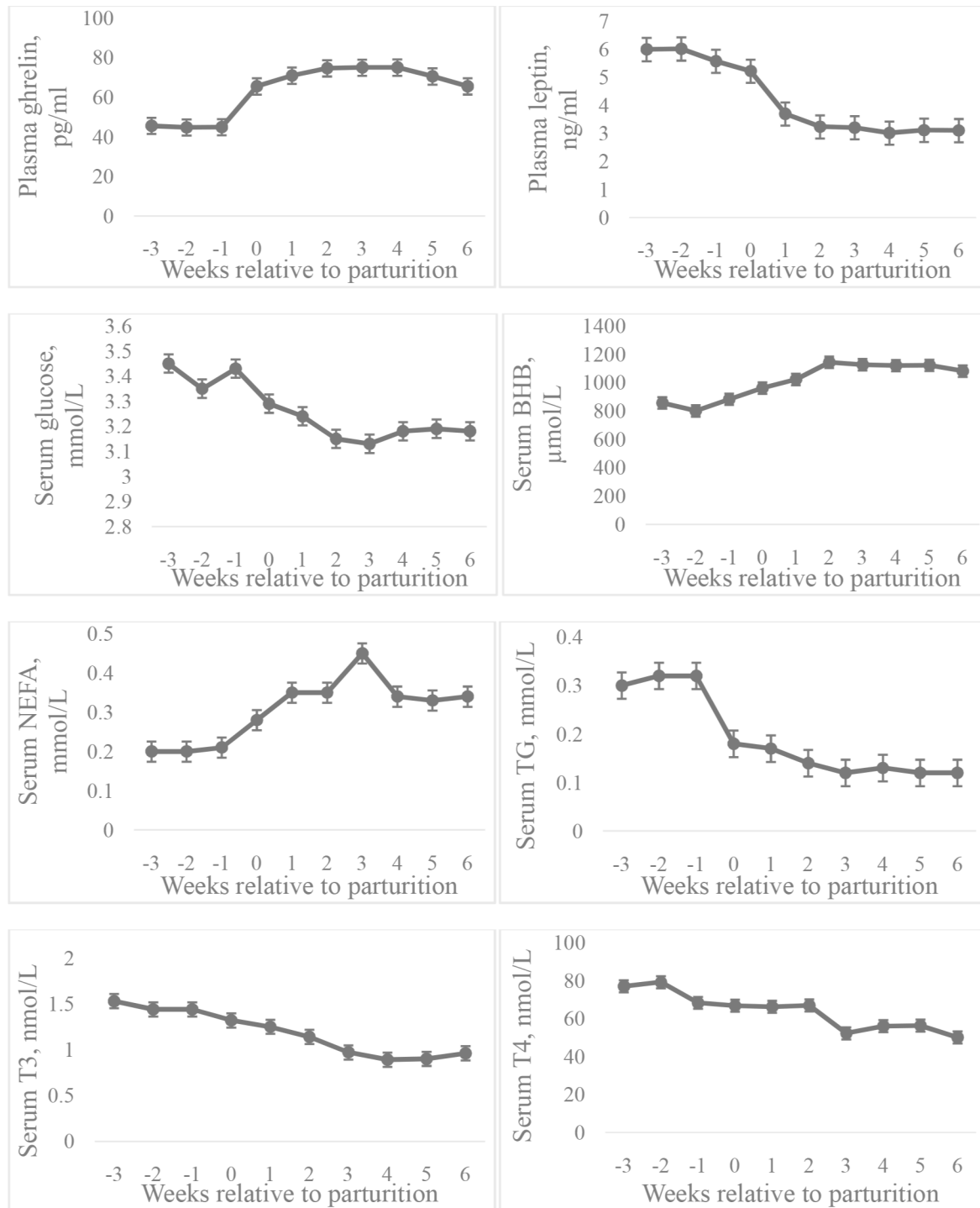


Fig. 1. Changes of the concentrations (mean \pm SEM) of plasma ghrelin and leptin and serum glucose, BHB, NEFA, TG, T3 and T4 in dairy cows during late gestation and early lactation. All of the variables were affected by transition period ($p \leq 0.05$) except glucose ($p > 0.05$).

Data obtained were statistically analyzed by ANOVA-procedure and Tukey's multiple range test for comparison among various weeks. The correlations between various parameters were assessed by Pearson's correlation test. The SPSS statistical software, version 22 was used for study.

Results

Mean (\pm SD) concentrations of ghrelin, leptin, glucose, BHB, NEFA, TG, T3 and T4 on different weeks in cows are presented in Table 1. The timely changes of the concentrations of plasma ghrelin and

Table 1. Concentrations of plasma ghrelin and leptin and serum energy related metabolites and thyroidal hormones in dairy cows in transition period (n=20).

Weeks relative to parturition	Ghrelin Pg/ml	Leptin ng/ml	Glucose mmol/L	BHB μ mol/L	NEFA mmol/L	TG mmol/L	T3 nmol/L	T4 nmol/L
-3	5.50 \pm 8.66 ^a	5.99 \pm 0.11 ^a	3.45 \pm 0.09 ^a	856.55 \pm 134.24 ^a	0.20 \pm 0.04 ^a	0.30 \pm 0.04 ^a	1.53 \pm 0.14 ^a	77.10 \pm 4.28 ^a
-2	44.70 \pm 7.32 ^a	6.01 \pm 0.09 ^a	3.35 \pm 0.55 ^a	800.60 \pm 107.13 ^a	0.20 \pm 0.03 ^a	0.32 \pm 0.04 ^a	1.44 \pm 0.20 ^a	79.24 \pm 1.40 ^a
-1	44.85 \pm 6.96 ^a	5.57 \pm 0.17 ^b	3.43 \pm 0.58 ^a	882.75 \pm 167.58 ^{ab}	0.21 \pm 0.04 ^a	0.32 \pm 0.04 ^a	1.44 \pm 0.26 ^a	68.36 \pm 1.54 ^b
0	65.40 \pm 5.30 ^b	5.22 \pm 0.06 ^c	3.29 \pm 0.08 ^a	961.35 \pm 52.54 ^{bc}	0.28 \pm 0.02 ^b	0.18 \pm 0.03 ^b	1.32 \pm 0.07 ^b	66.85 \pm 1.59 ^b
1	70.85 \pm 4.92 ^{cd}	3.69 \pm 0.09 ^d	3.24 \pm 0.65 ^a	1022 \pm 117.39 ^{cd}	0.35 \pm 0.04 ^c	0.17 \pm 0.05 ^b	1.25 \pm 0.08 ^b	66.29 \pm 7.71 ^b
2	74.55 \pm 6.84 ^{de}	3.23 \pm 0.15 ^e	3.15 \pm 0.53 ^a	1143 \pm 128.67 ^e	0.35 \pm 0.04 ^c	0.14 \pm 0.04 ^c	1.14 \pm 0.11 ^c	67.00 \pm 1.52 ^b
3	74.90 \pm 4.49 ^e	3.20 \pm 0.04 ^e	3.13 \pm 0.56 ^a	1126 \pm 117.17 ^e	0.45 \pm 0.11 ^d	0.12 \pm 0.03 ^{cd}	0.97 \pm 0.11 ^d	52.29 \pm 1.20 ^c
4	74.95 \pm 4.50 ^e	3.01 \pm 0.05 ^f	3.18 \pm 0.44 ^a	1120 \pm 177.31 ^e	0.34 \pm 0.03 ^c	0.13 \pm 0.03 ^{cd}	0.89 \pm 0.13 ^d	56.09 \pm 2.23 ^c
5	70.45 \pm 2.89 ^e	3.11 \pm 0.10 ^e	3.19 \pm 0.64 ^a	1122 \pm 216.19 ^e	0.33 \pm 0.03 ^c	0.12 \pm 0.02 ^d	0.90 \pm 0.12 ^d	56.41 \pm 1.60 ^c
6	65.45 \pm 6.97 ^b	3.10 \pm 0.09 ^e	3.18 \pm 0.56 ^a	1081 \pm 142.22 ^{de}	0.34 \pm 0.02 ^c	0.12 \pm 0.03 ^{cd}	0.96 \pm 0.05 ^d	50.14 \pm 1.39 ^c

BHB beta-hydroxybutyrate, NEFA nonesterified fatty acid, TG triglyceride, T3 triiodothyronine, T4 thyroxine
 Data are presented as means \pm SD.

Data with different superscripts in each column significantly differ, $p \leq 0.05$.

Table 2. Correlation coefficients between the measured parameters for total samples (n=20).

	Ghrelin	Leptin	Glucose	BHB	NEFA	TG	T3	T4
Ghrelin		-0.809**	-0.130	0.562**	0.666**	-0.756**	-0.609**	-0.411**
Leptin			0.188**	-0.634**	-0.735**	0.841**	0.795**	0.501**
Glucose				-0.121	-0.223**	0.185**	0.226**	0.077
BHB					0.529**	-0.565**	-0.497**	-0.157*
NEFA						-0.675**	-0.583**	-0.368**
TG							0.743**	0.439**
T3								0.391**
T4								1

BHB beta-hydroxybutyrate, NEFA nonesterified fatty acid, TG triglyceride, T3 triiodothyronine, T4 thyroxine

** Correlation is significant at the 0.01 level(2-tailed).

* Correlation is significant at the 0.05 level(2-tailed).

leptin and serum concentrations of glucose and BHB, NEFA and TG, as well as thyroidal hormones in serum recorded throughout the study, are shown in Figure 1. All of the variables were affected by transition period ($p \leq 0.05$) except glucose ($p > 0.05$). The plasma concentration of ghrelin did not differ during pregnancy ($p > 0.05$), but was higher during lactation ($p < 0.05$). The plasma concentration of leptin was lower during lactation than during pregnancy ($p < 0.05$). This reduction was initiated at the end of pregnancy ($p < 0.05$), and was sustained until the end of the study ($p < 0.05$). Serum concentrations of glucose did not differ significantly at any time (Table 1; $p > 0.05$). In the early lactation cows, serum concentrations of BHB and NEFA were significantly higher ($p < 0.05$) than in late dry cows and serum concentrations of TG, T3 and T4 were significantly lower

($p < 0.05$) than in late dry cows. Correlations between plasma ghrelin and other measured factors in transitional period are shown in Table 2. In this study, significantly positive correlations were determined between plasma ghrelin and serum concentration of BHB ($r = 0.562$; $p < 0.01$) and NEFA ($r = 0.666$; $p < 0.01$) as well as the negative ones between the plasma ghrelin and plasma concentration of leptin ($r = -0.809$; $p < 0.01$) and serum concentrations of triglyceride ($r = -0.756$; $p < 0.01$), T3 ($r = -0.609$; $p < 0.01$) and T4 ($r = -0.411$; $p < 0.01$) in the transitional period of dairy cows. In addition, no significant correlation ($r = -0.130$, $p > 0.05$) was found between ghrelin and glucose, indicating that glucose values may not be considered as a precise indicator of negative energy balance in dairy cows. Blood plasma leptin levels correlated significantly and negatively with BHB

($r = -0.634$, $p < 0.01$) and NEFA ($r = -0.735$, $p < 0.01$) concentrations. A significantly positive correlation was obtained in leptin and glucose ($r = 0.188$, $p < 0.01$), TG ($r = 0.841$, $P < 0.01$), T3 ($r = 0.795$, $p < 0.01$) and T4 ($r = 0.501$, $p < 0.01$) values. Serum glucose was significantly and positively correlated with TG ($r = 0.185$, $p < 0.01$) and T3 ($r = 0.226$, $p < 0.01$) and significantly and negatively correlated with NEFA ($r = -0.223$, $p < 0.01$). Serum BHB was significantly and negatively associated with TG ($r = -0.565$, $p < 0.01$), T3 ($r = -0.497$, $p < 0.01$) and T4 ($r = -0.157$, $p < 0.05$) and significantly positively with NEFA ($r = 0.529$, $p < 0.01$) concentrations in blood serum. NEFA concentrations were significantly and negatively correlated with TG ($r = -0.675$, $p < 0.01$), T3 ($r = -0.583$, $p < 0.01$) and T4 ($r = -0.368$, $p < 0.01$) concentrations in blood serum. TG concentrations showed significantly and positively correlation with T3 ($r = 0.743$, $p < 0.01$) and T4 ($r = 0.439$, $p < 0.01$). Additionally, a significantly positive correlation was evidenced between T3 and T4 ($r = 0.391$, $p < 0.01$).

Discussion

The transition from late gestation to early lactation is a critical period in a dairy cow's life because failure to successfully overcome the negative energy balance (NEB) caused by the sudden increase in energy demand attributed to lactation and lagging DMI (Drackley et al. 2001) can increase the risk of detrimental health and reproductive outcomes (Herd 2000). One of the major physiological changes occurring during the transition period of dairy cows is the depression of dry matter intake (DMI) as parturition approaches (Drackley et al. 2001). As a result, several metabolic disorders such as ketosis, fatty liver, hypocalcemia, and other diseases are commonly experienced by the cow during the transition period (Goff et al. 1997). Feed intake is a complex mechanism, regulated by several factors including hormones and metabolites (Ingvartsen et al. 2000). Ghrelin is a peptide hormone synthesized by the abomasal and ruminal tissues of cattle (Hayashida et al. 2001, Gentry et al. 2003). In the present study the transition from pregnancy to lactation in dairy cows is associated with an increase in the plasma concentration of ghrelin. Bradford et al. (2008) observed ghrelin surge prior to the conditioned meal only in early lactation cows. Itoh et al. (2005) reported greater plasma ghrelin concentration in early lactation compared to late lactation cows. Greater ghrelin secretion in early lactation is consistent with experimental models of energy deficiency. Fasting steers for 36 h increased mean plasma ghrelin concentration by more than five-fold (Wertz-Lutz et

al. 2006), and long-term energy restriction increased plasma ghrelin concentration in ovariectomized sheep (Kurose et al. 2005). Potential causes of increased ghrelin secretion during periods of energy deficiency include decreased plasma concentrations of insulin (Mohlig et al. 2002) and glucose (Briatore et al. 2003). Felix (2010) observed circulating ghrelin concentrations did not change with stage of lactation, not similar to our findings. In this study plasma leptin concentrations in early lactating cows were significantly lower ($p < 0.05$) than in late dry cows. Negative correlation ($r = -0.809$; $p < 0.01$) between the ghrelin and leptin concentrations in blood of cows during transition period was observed. During the periparturient period, high-yielding dairy cows experience major changes in energy metabolism (Bauman 2000). Despite the energy shortfall, partitioning of nutrients to the mammary gland is favored, and represents over 70% of available energy (ingested and endogenous). In ruminants, key adaptations of early lactation that have been identified in recent years include increased secretion of growth hormone (GH) and decreased responsiveness of skeletal muscle and white adipose tissue to insulin (Etherton et al. 1998). Changes in the plasma concentration of leptin, a protein hormone secreted almost exclusively by adipocytes (Ahima et al. 2000), could also be an important adaptation, particularly given the role of white adipose tissue in support of early lactation in dairy cattle. In ruminants and other animals, leptin is synthesized in proportion to the overall degree of adiposity (Ahima et al. 2000, Ehrhardt et al. 2000), and acts on the central nervous system (CNS) to reduce voluntary feed intake (Ahima et al. 2000, Schwartz et al. 2000). Our results indicate that the energy deficit of early lactation reduces leptin synthesis in white adipose tissue. Leptin, because of its role in the regulation of feed intake and energy disposition, could also participate in the co-ordination of metabolism during the transition from pregnancy to lactation. Therefore, reduced synthesis of leptin in white adipose tissue is largely responsible for the lower concentration of plasma leptin in early lactating dairy cows. The CNS, via sympathetic innervation of white adipose tissue, could also play an important role in reducing leptin synthesis in early lactation: β -adrenergic signals are potent inhibitors of leptin expression in adipocytes (Carulli et al. 1999), and ruminant white adipose tissue is particularly sensitive to their metabolic effects in early lactation (Bauman 2000). The peripartum decrease of the concentrations of leptin we found is in line with the observations of Block et al. (2001) and Chilliard et al. (2005). A fall in leptin acts through the hypothalamus to increase appetite, decrease energy expenditure, and modify neuroendocrine function in a direction that favors survival. In

this study serum BHB and NEFA concentrations in early lactating cows were significantly higher and TG was significantly lower ($P < 0.05$) than in close-up dry cows. NEFA's are useful for monitoring the energy status of dry cows during the last month of gestation – when rapid changes in energy balance status may not be detectable from changes in body condition score (Herdt 2000). High values of NEFA indicated a negative energy balance to occur in animals which are not prone to any disease (Radostits et al. 2007). Early lactation in dairy cows resulted in negative energy balance and high mobilization of lipids from bodily fat reserves (Reist et al. 2002). Increased amounts of NEFA removed by the liver along with carnitine palmitoyltransferase-1 activity regulate ketogenesis and thus, BHB production (Hegardt 1999). These results were in accordance with the results of other authors (Veenhuizen et al. 1991, Reist et al. 2002), clearly showing that a significant increase of NEFA concentrations in blood causes an increase of the content of lipids in liver cells and a decrease of TG concentrations in blood. Serum TG concentration was significantly lower ($p < 0.01$) in ketotic cows compared to healthy cows (Djoković et al. 2007). This suggests that TG accumulate in the liver cells of early lactation cows and cause their blood values to decrease. The present study showed that early-lactation cows had TG concentration less than late pregnancy cows. These results are in accordance with observations by Holtenius (1989) and Sevinc et al. (2003). The serum glucose concentration in early lactation cows and late dry cows was not different ($p > 0.05$). In cases of subclinical ketotic dairy cattle can become ketonaemic without the presence of significant hypoglycaemia (Groöhn et al. 1983), so it appears that glucose has a low sensitivity and cannot be a good criterion for diagnosis of subclinical ketosis and negative energy balance (Asl et al. 2011). Djocovic et al. (2007) reported in the group of postpartum healthy cows the glucose concentration in blood is significantly lower ($p < 0.05$) compared to the groups of late pregnant cows, contrary to our observations. In this study serum T3 and T4 concentrations in early lactating cows were significantly lower ($p < 0.05$) than in late pregnancy cows. These results are in agreement with those of other authors (Kasagić et al. 2011, Dejojovic et al. 2014), suggesting that under conditions of marked NEB characterized by increased mobilization of NEFA from body reserves in postpartum cows, blood levels of thyroid hormones are significantly decreased, particularly in ketotic cows, i.e. in animals diagnosed with hepatic lipidosis. This finding has been confirmed in this study by the significantly negative correlation between the blood levels of T3 and NEFA, and be-

tween levels of T3 and BHB. The hormonal activity of the thyroid gland has an important role in the transitional period for determining cell metabolism intensity, metabolism of lipids and carbohydrates and the lactation course itself by its thyroid hormones (Nikolić et al. 1997). A positive correlation was established between thyroid hormones in blood and energy balance (Reist et al. 2002). Under the conditions of negative energy balance and high lipid mobilisation, the concentrations of thyroid hormones were reduced in the blood of dairy cows in the transitional period, with a markedly declined triiodothyronine in blood shortly before and after calving (Reist et al. 2002, Pezzy et al. 2003). Romo et al. (1997) reported that in consequence of liver steatosis, free fatty acids accumulate in the liver parenchyma, and it has been demonstrated that some fatty acids inhibit type-I liver 5'-deiodinase activity. In accordance, Pezzy et al. (2003) assume that in dairy cows in early lactation, the state of hypothyroidism is present and it is the cause of the liver's decreased 5'-deiodinase activity or the secretion of thyroid hormones in milk. Decreased thyroid hormones level from dry to early lactation period in our research could represent an adaptive mechanism to NEB in dairy cows. In this study, significantly correlations were determined between plasma ghrelin and other measured parameters. But, no significant correlation was found between ghrelin and glucose, indicating that glucose values may not be considered as an adequate indicator of negative energy balance in dairy cows. These results confirm the fact that under negative energy balance and high lipomobilization, increased blood ghrelin, BHB and NEFA, and decreased blood values of leptin, TG, T3 and T4.

Conclusions

In conclusion, the energy deficit of periparturient cows causes a sustained elevation in serum ghrelin and reduction in serum leptin. This elevation and 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.

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