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LEVELS OF INSULIN, INSULIN-LIKE GROWTH FACTOR-I AND THYROID HORMONES IN RELATION TO THE BODY CONDITION SCORE CHANGES IN PERIPARTURIENT DAIRY COWS

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The objective of this study was to determine the levels of insulin, insulin like growth factor I (IGF-I) and thyroid hormones in relation to the body condition score (BCS) of periparturient dairy cows. The study was carried out on twenty Holstein-Friesian dairy cows with average milk production of 7000 L/305 days in the previous lactation, parity ranging from 2-4. All cows were BCS scored during the early dry period, 7±3 days before and after parturition. Based on the BCS at the early dry period, cows were divided in two groups: cows with high BCS (3.75-4.25, HBCS, n=10), and cows with moderate BCS (2.75-3.75, MBCS, n=10). Blood samples were taken at the time of BCS evaluation. Concentrations of insulin, IGF-I, triiodothyronine (T₃) and thyroxine (T₄) were determined by radioimmunoassay (RIA, INEP-Zemun, Serbia). Statistical differences between mean values were determined using Student t-test (p<0.01).

Mean BCS values at early dry period were significantly different compared to two other periods (p<0.01) within both groups, but BCS was similar in both groups at 7±3 days after calving (2.69±0.67:2.62±0.27, p>0.05). IGF-I level in HBCS cows at days 7±3 before calving was significantly higher (16.28±3.07:11.76±2.28, p<0.01), with a reverse relationship after calving (3.77±1.64:8.46±2.37, p<0.01). Insulin level was significantly lower at 7±3 days before calving in HBCS cows (16.26±4.60:20.18±4.96mIU/L, p<0.05). Thyroid hormones levels were significantly lower in HBCS group et all examined periods.

Key words: body condition score, dairy cow, insulin, IGF-I, thyroid hormones

INTRODUCTION

Body condition score (BCS) reflects the energy balance during pregnancy and lactation, especially during the transition period. The transition period is particularly important for health and subsequent performance of dairy cows which

are exposed to dramatic physiological changes and metabolic stress (Goff and Horst, 1997). BCS during early lactation decreases due to negative energy balance (NEB). High yielding dairy cows in early lactation do not consume sufficient feed to meet their energy requirements for maintenance and milk secretion. Consequently, most dairy cows enter into a period of NEB during the early lactation period. To cope with this shortfall in nutrient balance, fat and protein stored in body reserves are mobilized and used for the required physiological functions. A relationship between BCS and incidence of metabolic disease has been widely reported. Many scientists recommended BCS of cows as a useful indicator for the evaluation of energy status in dairy cows. BCS may be excessive due to unrestricted feeding during the dry period. This type of feeding represents a great risk for the appearance of obese cows in the population. Overconditioned dry cows are more prone to metabolic disorders.

Body condition score (BCS) at calving has been shown to have a marked effect on energy balance in early lactation (Wathes *et al.*, 2007; Šamanc *et al.*, 2010). Unrestricted feed consumption with increased energy intake in dairy cows during the dry period could lead to over condition and syndrome called "fat cow syndrome". In many cases the degree of NEB is intolerable and the organism adaptation ability is overcome resulting in health disorders.

Genetic selection for increased milk production has been associated with changes in key metabolic hormones that regulate metabolism by homeostasis and homeorhesis. A decrease in insulin 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 levels favor gluconeogenesis and lipolysis (Herdt, 2000). Concentration of IGF-I decreases at calving (Meikle *et al.*, 2004). Insulin and IGF-I are also putative mediators of nutritional status (Bossis *et al.*, 2000) and their peripheral concentrations are positively correlated (McGuire *et al.*, 1992). In cattle maintained in a negative energy balance, insulin and IGF-I are low (Roche *et al.*, 2000). It is interesting to note that IGF-I profiles reflected more likely the changes in BCS, in contrast with insulin profiles, which were associated more with the day-to-day effects of the nutrition (Adrien *et al.*, 2012). The regulation of each hormone individually may vary according to metabolic status, and the direction of the changes in body weight. A positive correlation among IGF-I, insulin, and BCS was reported previously in heifers and beef cows (Vizcarra *et al.*, 1998). However, in the study of León *et al.*, (2004), as heifers achieved a higher BCS, the rate of increase in insulin and IGF-I differed. Total circulating IGF-I concentrations increased steadily between BCS 2 to 4 and plateaued after BCS 4. This showed the differences in the mechanisms regulating the production of IGF-I and insulin.

Positive correlation was established between thyroid hormones in blood and energy balance. Under the condition of NEB and high lipid mobilization, the concentration of thyroid hormones was reduced in the blood (Reist *et al.*, 2002). There are also evidences that thyroid hormones levels in dairy cows with NEB could be relatively low, possibly contributing to the occurrence of fatty liver (Đoković *et al.*, 2007; Šamanc *et al.*, 2010). The aim of this work to investigate the relationship between blood levels of insulin, IGF-I, thyroid hormones and BCS changes in dairy cows during the periparturient period.

MATERIALS AND METHODS

Animals

The study was carried out on twenty Holstein-Friesian dairy cows with average milk production of 7000 L/305 days in the previous lactation, parity ranging from 2-4. All cows were scored for body condition (BCS) during the early dry period, 7 ± 3 days before and after parturition, according to the system provided by Elanco Animal Health Buletin AI 8478. BCS was determined on a five-point scale (1=emaciated, to 5=obese), it was always performed by the same trained operative by visualizing and palpating individual body parts of the spinal column (chin, loin, and rump), the *cranial coccygeal vertebrae* (tail head), the *tuber ischia* (pin bones), the tuber sacral (hip or hook bones), and the thigh region. In a five years period before our study the dairy herd had a problem with obese cows. According to BCS, cows were divided into two groups based on the BCS at the early dry period: group of cows with high BCS (BCS= 3.75- 4.25, HBCS, n=10), with, and group of moderate BCS cows (BCS=2.75-3.75, MBCS, n=10).

Blood serum sampling and radioimmunoassay

Blood samples were taken from the jugular vein, at 4-6 hours after the first daily meal, three times during the study (at early dry period, 7 ± 3 days before and after calving). Blood serum was separated after spontaneous coagulation at room temperature, centrifuged at 3000 rpm, and stored in polypropylene microtubes at -20°C until analysis. Insulin, insulin-like growth factor I (IGF-I), triiodothyronine (T_3), and thyroxine (T_4) blood serum concentrations were determined by radioimmunoassay, using commercial RIA kits (INEP-Zemun, Serbia). Intra-assay coefficients of variation (CV) ranged from 3.1% to 7.2%.

Statistical analysis

Results are expressed as means \pm SD. Student t-test was applied to identify differences between mean values. The differences were considered significant at $p<0.05$ and $p<0.01$.

RESULTS

The mean body condition score values of cows included in the study (N=20) at the early dry period, 7 ± 3 days before and after calving with statistical parameters are presented in Table 1.

The BCS values in both groups increased during the dry period and the highest value was recorded in HBCS cows at 7 ± 3 days before parturition (4.56 ± 0.39). However, BCS values were similar in both groups at early puerperium (2.69 ± 0.67 : 2.62 ± 0.27 ; $p>0.05$, HBCS:MBCS, respectively). HBCS cows showed higher level of BCS reduction between 7 ± 3 days before and after calving, compared to MBCS group (1.87 : 1.27 , HBCS:MBCS, respectively).

Table 1. The mean BCS values of cows included in the study (N=20) and statistical parameters

Statistical parameters	Body condition score (BCS)					
	dry period HBCS		early lactation HBCS	dry period MBCS		early lactation MBCS
	early dry period	-7±3 day	+7±3 day	early dry period	-7±3 day	+7±3 day
X	3.92 ^b	4.56 ^a	2.69 ^{d,e}	3.17 ^d	3.89 ^{b,c}	2.62 ^e
SD	0.41	0.39	0.67	0.52	0.43	0.27
SE	0.12	0.12	0.21	0.16	0.13	0.08
CV %	10.45	8.55	24.96	16.40	11.05	10.30
IV	3.75-4.25	4.25-4.75	2.0-3.50	2.75-3.75	3.5-4.25	2.25-3.00

Legend: ^{a,b,c,d,e}Means in the same row with different superscripts are significantly different (p<0.01).

The results of blood serum IGF-I concentration and statistical parameters of cows included in the study are presented in Table 2.

Table 2. Blood serum IGF-I concentration (nmol/L) and statistical parameters of cows included in the study (N=20)

Statistical parameters	IGF-I (nmol/L)					
	dry period HBCS		early lactation HBCS	dry period MBCS		early lactation MBCS
	earlydry period	-7±3 day	+7±3 day	earlydry period	-7±3 day	+7±3 day
X	¹ 15.34 ^{NS}	¹ 16.28 ^{**}	¹ 3.77 ^{**}	13.15	11.76	8.46
SD	3.69	3.07	1.64	3.56	2.28	2.37
CV %	24.00	18.86	43.50	27.10	19.39	28.01
SE	1.23	1.02	0.54	1.18	0.76	0.79
IV	8.40-20.1	10.04-20.94	1.78-7.16	7.99-19.01	8.86-16.16	5.10-12.18

Legend: ¹Mean compared to the same time period in MBCS cows; **- p<0.01.

Data presented in Table 2 indicate that there is no significant difference between mean IGFI concentrations in the early dry period (15.34±3.69:13.15±3.56 nmol/L, p>0.05). Blood serum IGF-I concentrations decreased after parturition in both groups of cows, with a more pronounced decrease in HBCS cows. It is interesting that mean blood serum IGF-I level was significantly higher at 7±3 days before calving in HBCS dairy cows (16.28±3.07:11.76±2.28, nmol/L,

$p < 0.01$), with a reverse relationship at 7 ± 3 days after calving ($3.77 \pm 1.64: 8.46 \pm 2.37$ nmol/l, $p < 0.01$).

Blood serum insulin concentrations (mIU/l) and statistical parameters of cows included in the study (N=20) are presented in Table 3.

Table 3. Blood serum insulin concentrations (mIU/l) and statistical parameters of cows included in the study (N=20)

Statistical parameters	Insulin (mIU/L)					
	dry period HBCS		early lactation HBCS	dry period MBCS		early lactation MBCS
	early dry period	-7±3 day	+7±3 day	early dry period	-7±3 day	+7±3 day
X	¹ 36.83 ^{NS}	¹ 16.26*	¹ 11.69 ^{NS}	30.86	20.18	14.17
SD	11.08	4.60	3.14	5.77	4.96	3.52
SE	3.69	1.53	1.40	1.92	1.65	1.17
CV %	30.00	28.30	26.90	18.70	24.58	24.87
IV	20.50-49.60	9.50-23.70	7.10-18.40	18.19-40.10	12.80-28.60	9.60-21.60

Legend: ¹Mean compared to the same time period in MBCS; *- $p < 0.05$.

Table 4. Blood serum triiodothyronine (T₃) concentrations (nmol/L) and statistical parameters of cows included in the study (N=20)

Statistical parameters	T ₃ (nmol/L)					
	dry period HBCS		early lactation HBCS	dry period MBCS		early lactation MBCS
	early dry period	-7±3 day	+7±3 day	early dry period	-7±3 day	+7±3 day
X	¹ 1.79**	¹ 1.80*	¹ 1.37**	2.56	2.17	1.80
SD	0.41	0.22	0.29	0.55	0.45	0.43
SE	0.14	0.07	0.10	0.18	0.87	0.14
CV %	23.00	12.49	21.52	21.44	11.43	23.92
IV	1.11 - 2.48	1.48 - 2.12	1.01 - 2.00	1.57 - 3.67	1.69 - 2.46	1.44 - 2.99

Legend: ¹Mean compared to the same period in MBCS; *- $p < 0.05$ and **- $p < 0.01$.

In both groups of cows insulin concentrations decreased from the early dry period to 7 ± 3 days after calving. There was no significant difference in insulin level between groups in the early dry period. A statistically significant difference in insulin concentration was established between groups at 7 ± 3 days before calving ($16.26 \pm 4.60: 20.18 \pm 4.96$ mIU/L, $p < 0.05$, HBCS:MBCS, respectively). In the early lactation period there was no significant difference in blood serum insulin level

between groups (11.69 ± 3.14 : 14.17 ± 3.52 mIU/L, $p > 0.05$, HBCS:MBCS, respectively).

Blood serum triiodothyronine (T_3) concentration and statistical parameters of cows included in the study ($N=20$) are presented in Table 4.

Data presented in Table 4 indicate that blood serum T_3 level was significantly lower in HBCS cows in all examined periods compared to the MBCS cows. The lowest T_3 level was observed at 7 ± 3 days after calving in HBCS dairy cows (1.37 ± 0.29 nmol/L).

Blood serum thyroxine (T_4) concentration and statistical parameters of cows included in the study ($N=20$) are presented in the Table 5.

Table 5. Blood serum thyroxine (T_4) concentrations (nmol/L) and statistical parameters of cows included in the study ($N=20$)

Statistical parameters	T_4 (nmol/L)					
	dry period HBCS		early lactation HBCS	dry period MBCS		early lactation MBCS
	early dry period	-7 \pm 3 day	+7 \pm 3 day	early dry period	-7 \pm 3 day	+7 \pm 3 day
X	¹ 29.90**	¹ 24.50**	¹ 21.44**	39.14	33.13	28.21
SD	7.76	7.48	5.84	7.61	7.03	5.48
SE	2.92	2.49	1.94	2.87	2.67	1.83
CV %	25.95	30.54	27.22	19.44	21.22	19.41
IV	19.10-38.40	15.80-36.10	15.30-30.10	24.20-48.40	19.10-44.10	22.10-40.30

Legend: ¹Mean compared to the same period in MBCS: cows; **- $p < 0.01$.

Data presented in Table 5 indicate that blood serum T_4 level was significantly lower in HBCS cows in all examined periods compared to MBCS cows. The lowest T_4 level was observed at 7 ± 3 days after calving in HBCS dairy cows (21.44 ± 5.84 nmol/L).

DISCUSSION

Dairy cows, like other mammals, undergo a normal cycle of body energy storage and mobilization, with increased body fat storage during mid-gestation and increased body fat mobilization during early lactation. Many scientists recommended BCS of cows as a useful indicator for evaluation of the energy status in dairy cows (Gearhart *et al.*, 1990; Stockdale, 2001; Roche *et al.*, 2007; 2009). BCS of cows at calving may be excessive due to unrestricted feeding during the dry period. This type of feeding represents a great risk for the occurrence of obese cows in the population. Over conditioned dry cows are more prone to metabolic disorders (Drackley *et al.*, 2005; LeBlanc *et al.*, 2010). Cows that have a higher BCS during the dry period tend to have a slower increase in dry

matter intake during the first weeks post partum, after the drop in feed intake just prior to parturition (Drackley *et al.*, 2005). This means that high BCS during the dry period, results on average in a more severe and probably also longer lasting NEB in these cows and should therefore be regarded as a risk factor for non adaptation.

However, the process of adaptation to the NEB in dairy cows usually is accompanied with a decrease of blood insulin, IGF-I, and thyroid hormones levels (Jorristma *et al.*, 2003; Hammon *et al.*, 2009; Wathes *et al.*, 2011). Thyroid hormones level during the peripartal period could be related to the postpartal metabolic disorders in Holstein-Friesian dairy cows (Nikolić *et al.*, 2003; Van Knegselet *et al.*, 2007). It has been observed that thyroid hormones concentrations decrease during the periparturient period due to the adaptation of dairy cows organism to negative energy balance and initiation of lactation (Blum *et al.*, 1983; Nixon *et al.*, 1988; Nikolić *et al.*, 1997; Tiirats, 1997; Pezzi *et al.*, 2003). Our results indicate that BCS in both groups increased during the dry period and was highest in HBCS at 7 ± 3 days before calving (4.56 ± 0.39 , Table 1). The HBCS cows showed higher level of BCS reduction from days 7 ± 3 before and after calving, compared to MBCS group (1.87:1.27, HBCS:MBCS, respectively). An optimum calving BCS in Holstein-Friesian dairy cows is between 3.0-3.25 (5-point scale) (Roche *et al.*, 2009). Lower calving BCS is associated with reduced production and reproduction, whereas calving BCS ≥ 3.5 (5-point scale) is associated with a reduction in early lactation dry matter intake and milk production and an increased risk of metabolic disorders (Allbrahim *et al.*, 2010). High rates of body condition score (BCS) loss in the early postpartum period are associated with a severe negative energy balance status, alterations in blood metabolites and hormone profiles (Wathes *et al.*, 2007a; Leroy *et al.*, 2008).

Changes in insulin and IGF-I play an important role in the metabolic adaptation of cattle to changes in weight and body condition (Leon *et al.*, 2004). Our data indicate that hormonal status (insulin, IGF-I, T_3 and T_4) of HBCS cows was significantly different from MBCS cows in the dry period and early puerperium. Mean IGF-I concentration in the blood serum at day 7 ± 3 before calving in HBCS cows was significantly higher compared to MBCS group ($16.28 \pm 3.07:11.76 \pm 2.28$, $p < 0.01$). However, HBCS cows showed more pronounced BCS loss between 7 ± 3 before and after calving, with lower IGF-I concentration at day 7 ± 3 after calving ($3.77 \pm 1.64:8.46 \pm 2.37$, $p < 0.01$, HBCS:MBCS, respectively). There was also a decrease in IGF-I level as BCS declined in both groups. NEB in early lactation is usually associated with low serum IGF-I level, resulting from depressed synthesis of IGF-I by the liver (Roche *et al.*, 2000; Radcliff *et al.*, 2003). The liver produces IGF-I in response to growth hormone (GH) stimulation. Feed restriction of animals provoked a decline in circulating concentrations of IGF-I, despite elevated concentrations of GH (Yambayamba *et al.*, 1996; Looor *et al.*, 2007). This lack of response of the liver is caused by reduced insulin and thus IGF-I concentrations are low (McGuire *et al.*, 1992). Our data regarding IGF-I profile in HBCS dairy cows reflected the changes in BCS, in accordance with data presented by Adrien *et al.*, (2012). Similar but less pronounced IGF-I profile changes are recorded in MBCS group. Our findings

correspond with finding by Kessel *et al.*, (2008). The adaptive ability of cows during early lactation is based on a variety of metabolic and endocrine variables.

Blood insulin concentration decreases during late pregnancy, reaching its lowest level during the early lactation period (Bossis *et al.*, 2000; Wathes *et al.*, 2007). The decrease in blood insulin level is accompanied with an increased adipose tissue lipid mobilization (Hammon *et al.*, 2009). Cows that experience a severe NEB post partum and acquire TAG accumulation in the liver were reported to have lower insulin concentrations. Our data also indicate that insulin blood level in both groups decreases from the early dry period towards early lactation. However, insulin level was significantly lower in HBCS cows at 7 ± 3 days before calving (16.26 ± 4.60 : 20.18 ± 4.96 , $p < 0.05$, HBCS:MBCS, respectively). This result could indicate a more pronounced metabolic adaptation process starting in HBCS cows as early as 7 ± 3 days before calving.

Cows with high BCS before calving had greater risks of metabolic problems because of excessive mobilization of body reserves. Cows with optimal BCS at calving (moderate body condition) 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. Insulin and IGF-I are putative indicators of nutritional status and their peripheral concentrations are positively correlated (McGuire *et al.*, 1992). Our results also confirmed higher insulin level at 7 ± 3 days after calving in cows with moderate initial BCS score. Additionally, feed deprivation had a large effect on insulin secretion. Overall, effects of feed deprivation were larger than effects of plane of nutrition (Schoenberg *et al.*, 2012)

There is evidence that thyroid hormones are positively correlated with energy balance in dairy cows during the early lactation period (Stojić *et al.*, 2001). Dairy cows with fatty liver are hypothyroid prior to development of the condition due to lower T_4 concentrations, and had significantly lower concentration of T_3 and higher T_3/T_4 ratios than cows with mild and moderate fatty liver (Šamanc *et al.*, 2010). Decreased thyroid hormones level from dry to early lactation period in both groups in our research could represent an adaptive mechanism to NEB in dairy cows. Our results showed that thyroid gland hormones level was significantly lower at all examined periods in HBCS cows (Table 4 and 5). This finding could also be explained by the lower level of liver's 5'-deiodinase activity (Pezzy *et al.*, 2003) in HBCS dairy cows, resulting from possibly increased liver fat accumulation (Đoković *et al.*, 2007; Kirovski *et al.*, 2011).

In general, blood levels of insulin, IGF-I, T_3 and T_4 were significantly lower in over conditioned cows (HBCS group), and concentrations of IGF-I and T_3 were significantly lower than physiological values. Reduction of insulin, IGF-I, T_3 and T_4 levels was less pronounced in MBCS group and mean values were not out of the physiological range. There was a good agreement between BCS and hormones related to energy metabolism in moderate BCS cows.

Routine, proactive actions, observations, or analysis are intended to accurately and efficiently provide early detection of problems, to provide an opportunity for investigation and intervention in order to limit the consequences and costs of health problems and reduced animal performance or welfare. An

improved mechanistic understanding of both physiological and pathological changes during the periparturient period may facilitate development of better feeding and management strategies.

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**NIVO INSULINA, INSULINU SLIČNOG FAKTORA RASTA–I I TIREOIDNIH HORMONA
U ODNOSU NA PROMENE TELESNE KONDICIJE (OTK) U PERIPARTALNOM
PERIODU KOD KRAVA**

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SADRŽAJ

Cilj ovog rada je bio da se odredi nivo insulina, insulinu sličnog faktora rasta IGF-I, trijodtironina (T3) i tiroksina (T4) u krvi u odnosu na promene telesne kondicije kod krava u peripartalnom periodu. Za ogled je odabrano 20 krava holštajn - frizijske rase između 2-4 laktacije i čija je proizvodnja mleka u predhodnoj laktaciji iznosila 7000 L za 305 dana. Telesna kondicija (OTK) je kod svih krava određivana na početku zasušenja, 7 ± 3 dana pre i 7 ± 3 posle teljenja. Na osnovu OTK na početku zasušenja, krave su bile podeljene u dve grupe: krave sa visokom OTK (3,75-4,25, n=10) i krave sa optimalnom OTK (2,75-3,75, n=10). Uzorci krvi su prikupljeni u vreme određivanja OTK. Koncentracija insulina, IGF-I, trijodtironina (T3) i tiroksina (T4) određivana je RIA metodom (INEP-Zemun, Serbia). Statistička značajnost razlika između srednjih vrednosti procenjivana je Studentovim t-testom. Srednje vrednosti OTK u ranom periodu zasušenja bile su statistički značajno različite u odnosu na druga dva perioda ($p < 0,01$) kod obe grupe, ali je OTK kod obe grupe bila slična 7 ± 3 posle teljenja ($2,69 \pm 0,67$: $2,62 \pm 0,27$, $p > 0,05$). Koncentracija IGF-I bila je statistički značajno viša kod krava sa visokom OTK, 7 ± 3 dana pre teljenja ($16,28 \pm 3,07$: $11,76 \pm 2,28$, $p < 0,01$), sa obrnutim odnosom 7 ± 3 dana posle teljenja ($3,77 \pm 1,64$: $8,46 \pm 2,37$, $p < 0,01$). Koncentracija insulina je bila značajno niža 7 ± 3 dana pre teljenja kod krava sa visokom OTK ($16,26 \pm 4,60$: $20,18 \pm 4,96$ mIU/L, $p < 0,05$). Nivo tireoidnih hormona je bio značajno niži kod krava sa visokom OTK u svim ispitivanim periodima.