ENDOCRINE AND METABOLIC PROFILE IN HOLSTEIN AND RED HOLSTEIN HEIFERS DURING PERIPARTAL PERIOD

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The aim of this stady was to examine the relationship between triiodothyronine (T_3), thyroxin (T_4), insulin-like growth factor-I (IGF-I) and blood serum liver function biomarkers concentrations in Holstein and Red Holstein heifers during the peripartal period. In the first experimental group (I) were heifers of Holstein Friesian breed (n=10), and in the second experimental group (II) were Red Holstein heifers (n=10). Blood samples were taken from the vena coccygea three times during the short peripartal period (5 days before delivery, at delivery and 5 days after delivery), and blood serum samples were separated after spontaneous coagulation. Blood serum T_3, T_4 and IGF-I concentrations were determinated using radioimmunoassay (RIA). Blood serum concentrations of glucose (G), total protein (TP), albumin, (Alb), urea, total bilirubin (tBil) and aspartate aminotrasferase (AST) were determined using commercial ELISA kits. The statistical significance between means was determined using Student t-test.

Blood serum T_3 concentration in Group II was significantly lower after delivery, and T4 concentration was significantly higher before delivery (p<0.05), compared to Group I. Mean blood serum IGF-I concentrations between the two experimental groups were not significantly different at all time intervals. Relationships between the determined hormones showed a median positive correlation between T_3, and T_4 (r=0.74) in Group I and highly positive correlation in Group II (r=0.81). Blood serum thyroid gland hormones and IGF-I concentrations in healthy heifers during peripartal period vary considerably, the lowest values being determined immediately after calving in both experimental groups.

Blood serum G, TP, Alb and urea concentrations were significantly higher in Group I at all investigated time intervals. Blood serum tBil concentration was significantly higher after partus in Group I (11.02 ± 3.33 : 8.49 ± 3.35 µmol/L, Group I vs. II, respectively), and AST activity was not significantly different at all intervals.

Key words: Holstein heifers, endocrine and metabolic profile, peripartal period

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INTRODUCTION

The most significant changes of physiological nature in dairy cows occur during the period of 3 weeks before and after calving, so-called "transition period" (Grummer, 1995). Those changes start during the dry period, exacerbate in the calving period and continue at the beginning of lactation. They represent an adaptation process of dairy cows to increased metabolic demands of the mammary gland. Precisely, the intensification of important metabolic processes and increased energy needs appear during this period, while intake of nutrients is insufficient. The adaptation process is also characterized by the rapid increase of peripheral utilization of different hormones, especially thyroid, pituitary and adrenal glands products. A hormonal status test i.e., dynamics of hormones in charge of regulation of energy metabolism, is significant for further explanation of the relationship between homeostasis and homeoresis in heifers during peripartal period. In the situation of negative energy balance (NEB), the organism uses its own energy stores, first from body fat depot, where their increased mobilization occurs (Holtenius, 1989; Grummer, 1993; Kovačević, 2004). Increased lipid stores mobilization and high energy consumption in the mammary gland usually results in increased blood serum free fatty acids concentration, hypoglycemia and hyperketonemia, the situation that could easily end up as a production disease such as ketosis, fatty liver syndrome or puerperal paresis (Goff and Horst, 1997).

Thyroid gland hormones are important in maintaining lactation and could play a major role in regulating homeoretic adaptation responses. The higher blood serum thyroxin concentration in pregnant heifers with lower production ability suggests a negative relationship of thyroxin to milk yield potential (Bitman *et al*., 1984). Restricted energy intake could be an important factor in reducing blood serum triiodothyronine concentration during the peripartal period (Pethes *et al*., 1985). There is also a significant effect of weather conditions on the thyroid gland hormones blood serum levels during different lactation phases (Aceves *et al*., 1985). Nixon *et al*. (1988) determined free and total thyroid hormones in the serum of Holstein cows and concluded that free and total T_4 and T_3 are low in early lactation due to the high metabolic demands form peak milk production. In his study Tirrats (1997) showed that plasma thyroxin level was significantly lower during early stage of lactation compared with later stages, but increased as the stage of lactation progressed. Stojić *et al*. (2001) reported that heifers in the early lactation period two to three days after calving had decreased concentrations of T_3 and T_4 relative to the days before calving. Positive correlation was established between thyroid hormones in blood and energy balance (Reist *et al.,* 2002).

Insulin-like growth factor (IGF-I) is synthesized in the liver under the influence of somatotropin (STH) (Jones *et al*., 1995), which is the basics of the STH-IGF-I axis. Early lactation in dairy cows is characterized by prolonged NEB during which the liver becomes insensitive to the STH stimulation (Vicini *et al*., 1991), leading to a marked reduction of plasma IGF-I concentration. Decrease of IGF-I concentration starts two weeks before parturition, approximately at the time when plasma insulin gradually decreases. At the same time plasma somatotropin level is changing contrary to plasma insulin and IGF-I concentrations (Bell, 1995).

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This situation when STH can not stimulate liver IGF-I synthesis and secretion has been termed "resistance to STH" (Donaghy *et al*., 1996).

Blood serum total protein, albumin and urea concentrations represent not just indicators of protein metabolism (Kida, 2002), but also liver function biomarkers (Kaneko, 1989). A more specific liver function biomarker in the blood serum is AST activity (Kaneko, 1989; Garry *et al.*, 1994; Rehage *et al*., 1996; Kida, 2002) and among most commonly used hepatic status indicators is total blood serum bilirubin concentration (Rehage *et al*., 1996; Kalaitzakis *et al.*, 2007). Blood serum glucose concentration is an energy metabolism indicator, but it can also be used as a liver function biomarker, since hepatic gluconeogenesis is the major contributor to the blood glucose homeostasis in dairy cows (Kida, 2002).

The aim of the present study was to determine blood serum thyroid hormones, IGF-I and liver function biomarkers concentration in two breeds of Holstein heifers during the peripartal period.

MATERIALS AND METHODS

Experimental animals. Total number of 20 healthy heifers were divided into two experimental groups: 1) the first experimental group (I) were Holstein-Friesian heifers (n=10), and 2) the second experimental group (II) were Red Holstein heifers (n=10). All experimental animals were born and bred on the farm, had normal calving (eutocia) and one live calf (heifers with twins were excluded). The urine ketonic tests have been used (Urocomb stripes and Rother-a test) to exclude the diagnosis of ketosis. The experiment was conducted during the spring time (April-May), with an average environmental temperature between 18-24°C, and equal diet composition.

Blood sampling. Blood samples were collected three times during the short peripartal period (5 days before partus, at the parturation day and 5 days post partum) between 10 and 12 a.m. (about 5 hours after milking and feeding) by vacutainer systems from the *vena coccygea* (one test tube of blood per sampling, approximately 10 mL of blood). The blood samples were allowed to clot spontaneously (approximately 15 min) at room temperature. The serum was then decanted at 1 000 g and stored at −18°C until analyzed.

Endocrine parameters determination. Thyroid gland hormones (T_3 and T_4) and IGF-I blood serum concentrations were determined by radioimmunoassay (RIA) method, using commercial RIA-kits (INEP, Zemun, Serbia).

Blood serum liver function biomarkers determination. Blood serum glucose (G), total protein (TP), albumin (Alb), urea (BUN), total bilirubin (tBil) and aspartate aminotransferase (AST) activity were determined using commercial ELISA kits (Reanal Finechemical Co.) on biochemical analyser Basic Secoman.

Statistical analysis. Statistical significance of the differences between mean values was determined using Student t-test, and correlation coefficient (r), according to Chebish-ev table, was used for the evaluation of the degree of linear correlation between endocrine parameters.

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RESULTS

Mean blood serum T_3 concentrations for Group I at three time intervals were 2.6 ± 0.62, 2.7 ± 0.65 and 2.3 ± 0.41 nmol/L, and for Group II were 2.49 ± 0.52, 2.16 ± 0.78 and 1.74 ± 0.61 nmol/L. Comparing blood serum T_3 concentrations within groups, in experimental Group II, a statistically significant difference ($p<0.01$) was found between the mean at the period after delivery (1.74 ± 0.61 nmol/L) relative to the periods before it. Mean blood serum T_3 concentration in Group II after delivery (1.74 ± 0.61 nmol/L) was also significantly lower ($p<0.05$) compared to the mean T_3 value in Group I after delivery (2.3 ± 0.41 nmol/L).

Mean blood serum T_4 concentrations for Group I at three time intervals were 35.70 ± 9.96, 39.70 ± 12.96 and 32.2 ± 7.94 nmol/L, while for Group II were 58.45 ± 12.86, 49.9 ± 22.42 and 43.91 ± 16.89 nmol/L. Mean blood serum T_4 concentrations decreased after delivery in both experimental groups, but there were no significant differences between means within groups. Mean blood serum T_4 concentration in Group II before delivery (58.45 ± 12.86 nmol/L) was significantly higher ($p<0.01$) compared to the mean T_4 concentrations at all time intervals in Group I.

Blood serum T_3, T_4 and IGF-I concentrations in Holstein-Friesian (Group I) and Red Holstein heifers (Group II) during the peripartal period are presented in Table 1.

Table 1. Blood serum T_3, T_4 and IGF-I concentrations (mean$\pm$SD) in the Holstein-Friesian (Group I) and Red Holstein heifers (Group II) during the peripartal period

Time of testing	Group	T_3 (nmol/L)	T_4 (nmol/L)	IGF-I (nmol/L)
Before delivery	I (n=10)	2.60 ± 0.62[NS]	35.70 ± 9.96**	18.17 ± 5.17[NS]
	II (n=10)	2.49 ± 0.52	58.45 ± 12.86	18.64 ± 5.43
Delivery	I (n=10)	2.70 ± 0.65[NS]	39.70 ± 12.96[NS]	19.42 ± 8.35[NS]
	II (n=10)	2.16 ± 0.78	49.90 ± 22.42	14.15 ± 3.83
After delivery	I (n=10)	2.31 ± 0.4*	32.20 ± 7.94[NS]	12.53 ± 3.01[NS]
	II (n=10)	1.74 ± 0.61	43.91 ± 16.89	10.46 ± 3.27

Legend : NS – not significant; **$p<0.01$, *$p<0.05$.

Blood serum T_3, T_4 and IGF-I concentrations were lower after delivery compared to the values before it and at parturition in both experimental groups. Statistical analysis of the differences between mean thyroid hormone values in two groups reviled two important facts: 1) mean blood serum T_3 concentration after delivery was significantly lower in Group II compared to Group I (1.74 ± 0.61: 2.31 ± 0.41 nmol/L, $p<0.05$), and 2) mean blood serum T_4 concentration was significantly higher before parturition in Group II compared to Group I. Although there were no significant differences between mean blood serum IGF-I values in two groups of heifers, IGF-I values were lower at parturition and after it in Group II (Red Holstein).

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The degree of correlation between mean blood serum T_3, T_4 and IGF-I concentrations in two breeds of Holstein heifers during the peripartal period is presented in Table 2.

Table 2. The degree of correlation between mean blood serum T_3, T_4 and IGF-I concentrations in two breeds of Holstein heifers during the peripartal period

		Group I					Group II		
		T_3	T_4	IGF-I			T_3	T_4	IGF-I
T_3	r	-	0.745*	0.120	T_3	r	-	0.812*	0.402
T_4	r	0.745*	-	0.139	T_4	r	0.812*	-	0.148
IGF-I	r	0.120	0.139	-	IGF-I	r	0.402	0.148	-

There was a positive correlation between mean blood serum T_3 and T_4 concentrations in Group I (r=0.74) and high positive correlation (r=0.81) in Group II. There was a low degree of correlation between IGF-I and thyroid hormone concentrations in both groups of heifers.

Results of blood serum liver function biomarkers are presented in Table 3.

Table 3. Blood serum concentrations of G, TP, Alb, Urea, tBil and AST activity (mean±SD) in two breeds of Holstein heifers during the peripartal period

Time of testing	Group	G (mmolLI)	TP (gLI)	Alb (g/L)	Urea (mmol/L)	tBil (μmol/L)	AST (U/L)
Before delivery	I (n=10)	1.79± 0.93[NS]	77.88± 5.92**	39.51± 3.48**	7.72± 2.61*	8.28± 2.75[NS]	67.16± 22.91[NS]
	II (n=10)	2.52± 0.99	62.81± 4.16	29.20± 4.96	5.31± 1.50	6.81± 2.15	70.96± 44.78
Delivery	I (n=10)	2.25± 1.38[NS]	78.80± 6.16**	37.32± 6.77**	9.60± 3.60**	9.85± 1.80[NS]	84.45± 24.64[NS]
	II (n=10)	2.82± 1.28	57.20± 9.83	26.82± 6.45	4.98± 1.33	7.89± 2.60	91.66± 68.15
After delivery	I (n=10)	2.78± 1.46[NS]	78.69± 6.30**	39.08± 4.06**	8.58± 2.90**	11.02± 3.33*	78.63± 20.28[NS]
	II (n=10)	2.76± 0.77	64.62± 5.77	29.09± 4.26	4.67± 0.83	8.49± 3.35	119.48± 105.40

Legend : NS – not significant; **p<0.01, *p<.05

There were no significant differences in the blood serum glucose concentration between groups during the peripartal period. However, data presented in Table 3. indicates two important facts: 1) blood serum glucose concentration in Group I before delivery is lower than the physiological value for dairy cows (2.52-4.16 mmol/L, Kaneko, 1989), 2) there is no decrease in blood serum glucose concentration in heifers after delivery.

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Blood serum total protein and albumin concentrations were significantly higher in Group I at all investigation periods, but they were within the physiological limits. Similar relationship is evident for urea, with highest value determined in Group I at parturition (9.60±3.60 mmol/L).

Blood serum tBil values were continously higher in Group I, with a significant difference between means determined after partus (11.02±3.33:8.49±3.35, $p<0.05$). There were no significant differences in blood serum AST activity between groups in all investigation periods. However, blood serum AST activity increased after partus in Group II, with a high degree of individual variation (119.48±105.40 U/L, Group II after partus).

DISCUSSION

Thyroid gland hormones concentration in the heifers' blood serum during the short peripartal period was lowest after calving in both groups, and is in accordance with research results (Đoković, 1998; Kovačević, 2000; Nikolić *et al.*, 2001; Kovačević, 2004).

In experimental Group I, higher mean blood serum T_3 concentrations were noticed at all time intervals relative to the concentration of this hormone in Group II, while heifers in Group II had higher concentrations of T_4 hormone.

These findings are in accordance with those of Stojić *et al*. (2001), who reported that heifers in the early lactation period two to three days after calving have decreased concentrations of T_3 and T_4 relative to the days before calving. Food, environment and especially temperature influence the concentration of these hormones (McGuire, 1998). However, Grum *et al*., (1996) stated that T_3 and T_4 values in the blood decrease in the case of negative energy balance. Adding fat in meals results in the increase of T_3, while food rich in concentrate has no influence on T_3, but results in the increase of T_4. Triiodothyronine and thyroxin concentration in the blood plasma of dairy cows is significantly lower during lactation relative to the period when the milk gland is inactive. However, values of these hormones in the blood are always lower in cows with higher milk production (Blum *et al*., 1983).

Many authors have confirmed that T_3 and T_4 concentration in the blood serum of cows immediately after calving decreases, relative to their concentration in the dry period (Đoković, 1998; Kovačević, 2000; Nikolić *et al*., 2001). Under normal setting conditions, cows in the early lactation period have significantly lower concentrations of thyroid hormones in the blood plasma (Aceves *et al.*, 1985). They explain this phenomenon with the fact that animals do not get enough energy during the period of parturition, i.e., they are in negative energy balance (Hart *et al*., 1978; Pethes *et al*., 1985; Đoković, 1998, Gvozdić *et al*., 2006). Nikolić *et al*. (1997, 2001) found that during high pregnancy there is an adequate status of thyroid hormones, while at the beginning of lactation a significant decrease of their concentration occurs in one third of the tested cows. In such conditions in cows with high milk production there is an intensive and uncontrollable lipomobilisation, which results in the violation of liver morphology and functional integrity. It eventually leads to "liver fattening", often accompanied by metabolic

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diseases such as ketosis, or in more difficult cases, hepatic coma (Jorritsma, 2003).

Cows with higher milk production during late pregnancy, early lactation and in the second month of lactation have a considerably lower concentration of blood serum thyroid hormones relative to cows with lower milk production (Kovačević, 2004). Thyroid gland hormones in the blood serum of pregnant heifers with different genetic predispositions for milk production (685 kg per lactation) were lower in the morning than in the afternoon in both groups. There was no difference in the concentration of T_3 between the two groups, while T_4 in the blood serum of heifers predisposed for lower milk production was considerably higher than in the other group (Bitman *et al*., 1984).

In our research the intermediate to high positive correlation between T_3 and T_4 concentration in the blood serum of heifers in the peripartal period (r=0.745 and r=0.812, Group I and II, respectively) was determined, which is in accordance with the studies done by Kunz *et al*. (1985) and Kovačević (2004).

The results of blood serum IGF-I concentration in heifers during peripartal period in our research are in accordance with the findings of Bishop (1994) and Bugarski (2002), who pointed that cows in the negative energy balance have lowered IGF-I blood serum concentration. Blood serum IGF-I concentration in dairy cattle is stable during the day and does not change significantly even in conditions when animals are hungry, and hormone secretion is distinctly pulsatory (Glyckman, 1987). However, Wathes *et al*. (2007) suggested that diet has an important influence on the IGF-I level before delivery.

Longer monitoring of blood IGF-I concentration in cows has proved that it is variable depending on the lactation degree. It is lowest at the very beginning of lactation and gradually increases, reaching highest values during the dry period (Sharma *et al*., 1994; Grum *et al*., 1996). Lowest blood IGF-I values after calving may be related to the negative energy balance in the beginning of lactation, resulting from the decrease of IGF-I synthesis in the liver, as well as from its active movement from the blood to colostrum, where its value can be increased even up to 20 times relative to its concentration in the blood (Sharma *et al*., 1994; Hadsell *et al*., 1993). As IGF-I concentration in the blood is lowest in the early lactation period, when blood growth hormone concentration is highest, the exact role of IGF-I in early lactation is not quite clear; therefore, galactopoietics' significance is attributed to it only in the period of late lactation (Sharma *et al*., 1994).

Blood serum glucose concentration is an important indicator of energy metabolism and in ruminants over 3 months age it is entirely dependant on the endogenous synthesis from non-carbohydrate sources by hepatic gluconeogenesis (Radojičić, 1995). Requirement for glucose in dairy cows increases considerably during pregnancy and lactation. In the pregnant animal the fetus and uterus utilize glucose as a major source of energy and in lactation large quantities of glucose from the blood are used by the mammary gland for lactose synthesis (Bell, 1995). The reference range values for blood serum glucose concentration in heifers and cows are 2.5-4.16 mmol/L (Kaneko, 1989). Blood serum glucose concentration in Group I before delivery (1.79± 0.93 mmol/L) was below reference values and possible explanations are as

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follows: 1) low level of highly digestible carbohydrates in the diet, 2) relative decrease of hepatic gluconeogenesis or 3) an increase of erythrocytes glucose utilization due to the prolonged blood samples transport time.

Blood serum total protein and albumin concentrations were significantly higher in heifers in Group I in all investigated periods, but the values were within physiological variation interval limits (Kaneko, 1989). A similar relationship is evident for urea concentration, with the highest value determined in Group I at parturition (9.60±3.60 mmol/L).

Blood serum tBil values were continously higher in Group I, with a significant difference between means determined after partus (11.02±3.33:8.49±3.35, $p<0.05$). There were no significant differences in blood serum AST activity between groups at all investigated periods. However, blood serum AST activity increased after partus in Group II, with a high degree of individual variation (119.48±105.40 U/L, Group II after partus). The blood serum tBil and albumine concentrations, and AST activity are good parameters for the evaluation of liver function status in dairy cows, especially in the peripartal period (Brugere and Brugere, 1987; Radojicic *et al*., 2007).

Our research results indicate that lower thyroid gland hormones and IGF-I concentration in the blood serum of heifers immediately after calving represent one aspect of organism adaptation to the negative energy balance at the beginning of the first lactation. There is a positive correlation between the degree of negative energy balance and the decrease of the level of thyroid gland hormones. Concentrations of total bilirubin, urea, albumin, and activity of AST are valide liver function biomarkers and good parameters of metabolic status of dairy cows in the first peripartal period.

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ENDOKRINI I METABOLIČKI PROFIL JUNICA HOLŠTAJNA I CRVENOG HOLŠTAJNA U PERIPARTALNOM PERIODU

KASAGIĆ D, RADOJIČIĆ BILJANA, GVOZDIĆ D, MIRILOVIĆ M i MATARUGIĆ D

SADRŽAJ

Cilj ovih istraživanja je bio određivanje koncentracije triijodtironina (T_3), tiroksina (T_4), insulinu sličnog faktora rasta-I (IGF-I) i biomarkera, pokazatelja funkcionalnog stanja jetre u krvnom serumu dva varijeteta junica Holštajn rase u peripartalnom periodu. Prvu eksperimentalnu grupu (I) činile su junice crno belog holštajna (n=10), a drugu grupu (II) junice crvenog holštajna (n=10). Krv za analize je uzimana iz repne vene, tri puta u peripartalnom periodu (5 dana pre partusa, na dan partusa i do 5 dana posle partusa), a krvni serum je odvajan nakon spontane koagulacije. U krvnom serumu je radioimunološkom metodom (RIA, INEP-Zemun) određivanja koncentracija trijodtironina (T_3), tiroksina (T_4) i insulinu sličnog faktora rasta-I (IGF-I). Koncentracija glukoze (G), ukupnih proteina (TP), albumina, (Alb), ureje, ukupnog bilirubina (tBil) i aktivnost aspartat aminotrasferaze (AST) je određivana upotrebom komercijalnih ELISA kitova. Statistička značajnost razlika srednjih vrednosti ispitivanih endokrinih i metaboličkih parametara je određena Studentovim t-testom.

Dobijeni rezultati ukazuju na vrlo različite koncentracije hormona štitne žlezde u krvnom serumu junica oba varijeteta holštajna pre i posle partusa. Koncentracija T_3 je bila statistički značajno niža u grupi II nakon partusa, a T_4 značajno viša pre partusa ($p < 0.05$) u odnosu na nivo hormona u grupi I. Za prosečne vrednosti koncentracija IGF-I, između isptivanih grupa nije ustanovljena statistički značajna razlike ni u jednom od tri perioda ispitivanja. Utvrđena je srednje pozitivna korelacija između koncentracije T_3 i T_4 (r=0,74) u krvnom serumu junica grupe I, i vrlo visoka pozitivna korelacija u grupi II (r=0,81). Koncentracija TP, Alb i ureje bila je statistički značajno viša kod junica grupe I u sva tri perioda ispitivanja. Nivo tBil u krvnom serumu je takođe bio povišen kod junica grupe I u sva tri perioda ispitivanja, ali su statistički značajne razlike između srednjih vrednosti ustanovljene samo u periodu nakon partusa. Aktivnost AST u krvnom serumu junica oba varijeteta značajno je varirala u svim periodima ispitivanja i nisu ustanovljene statistički značajne razlike između srednjih vrednosti.