Macedonian Journal of Animal Science, Vol. **6**, No. 2, pp. 79–84 (2016)
218 In print: ISSN 1857 – 6907
Received: September 20, 2016 On line: ISSN 1857 – 7709
Accepted: November 15, 2016 UDC: 636.2.082.4.09:612.43

Original scientific paper

RELATIONSHIPIS BETWEEN BLOOD HORMONES AND METABOLITES IN SIMMENTAL DAIRY COWS DURING TRANSITION PERIOD AND MID LACTATION

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The main purpose of this article, was investigation metabolic and endocrine status in Simmental dairy cows during transition period and mid lactation, based on the relationships between blood growth hormone (GH), insulin, triiodothironine (T3), thyroxine (T4), glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and triglycerides (TG)**.** For the analysis were chosen 45 Simmental cows divided in three equal groups and each group was consist of fifteen cows: Group 1 was late pregnant cows, group 2 was early lactation cows and group 3 was mid lactation cows. Blood metabolic hormones as well as metabolites were analyzed and obtained results were recorded. Second group of early lactation cows have significant higher blood serum concentrations of GH ($p<0.05$), NEFA ($p<0.05$) and BHB ($p<0.05$) and significant lower blood serum concentrations of insulin ($p>0.05$), T3 ($p<0.05$), T4 ($p>0.05$), glucose ($p<0.05$) and TG ($p<0.05$) compared with the first group of late pregnancy cows and third group of mid lactation cows. Significant positive correlations ($p<0.05$) were noticeable between GH and NEFA, T3 and insulin, T3 and T4, glucose and T3, glucose and TG and negative ($p<0.05$) between glucose and NEFA and glucose and BHB. These hormonal and metabolic changes can be useful indicators of the metabolic and endocrine status of dairy cows.

Key words: Simmental dairy cows; blood hormones; blood metabolites

ОДНОС МЕЃУ ХОРМОНИТЕ НА КРВТА И МЕТАБОЛИТИТЕ КАЈ СИМЕНТАЛСКИ МЛЕЧНИ КРАВИ ЗА ВРЕМЕ НА ПРЕОДНИОТ ПЕРИОД И СРЕДИНА НА ЛАКТАЦИЈАТА

Главна цел на овој труд е проучување на метаболитичкиот и ендокриниот статус на сименталски млечни крави за време на преодниот период и средина на лактацијата, врз основа на односот помеѓу крвниот хормон за раст (GH), инсулинот, тријодотиронинот (T3), тироксинот (T4), глукозата, бета-хидроксибутиратот (BHB), неестерифицираните масни киселини (NEFA) и триглицеридите (TG). За анализа беа избрани 45 симентални крави, поделени во три еднакви групи при што секоја група се состоеше од петнаесет крави: група 1 беа крави во доцна стелност, група 2 беа крави во рана лактација и група 3 беа крави во средина од лактацијата. Беа анализирани метаболитичките хормони на крвта и метаболитите, а добиените резултати беа евидентирани. Втората група на крави во рана лактација имаа значајно повисоки концентрации на GH во крвта ($p<0.05$), NEFA ($p<0.05$) и BHB ($p<0.05$) и значајно пониски серумски концентрации на инсулин во крвта ($p>0.05$), T3 ($p<0.05$), T4 ($p>0.05$), глукоза ($p<0.05$) и TG ($p<0.05$) во споредба со првата група на крави во доцна бременост и третата група на крави во средина од лактацијата. Значајно позитивни корелации ($p<0.05$) беа забележани меѓу GH и NEFA, T3 и инсулин, T3 и T4, глукоза и T3, глукоза и TG и негативна

(p<0.05) меѓу глукоза и NEFA и глукоза и BHB. Овие хормонални и метаболички промени може да бидат корисни индикатори за метаболитичкиот и ендокриниот статус на млечните крави.

Клучни зборови: сименталски млечни крави; крвни хормони; крвни метаболити

INTRODUCTION

Major changes in the hormonal regulation of metabolic functions in Simmental cows occur during the transitional period and early lactation. Parturition and lactogenesis are accompanied by many physiological changes that alleviate metabolic disturbance during critical period in order to maintenance homeostasis (Bauman and Currie, 1980). Adaptation of the endocrine system during the transitional period is the key factor in maintaining metabolic balance (Bauman and Currie, 1980; Aceves et al., 1985). GH stimulates lipolysis and lipid mobilization of adipose tissue during the negative energy balance (NEB) in postpartal period in Simmental cows, supplying energy for milk production. (Bauman and Vernon, 1993; Lucy et al., 2001). Simultaneously, plasma concentrations of insulin as another homeorhetic hormone, would be decreased in peripartal period, so nutrient supply to the udder will be affected (insulin resistence) (Bonczek et al., 1988; Tucker, 1994; Bell, 1995; Butler et al., 2003; Balogh et al., 2008). Thyroid hormones, primarily triiodothyronine (T3), play an important role in the regulation of energy metabolism. A decrease in thyroid hormone levels occurs in the blood of peripartal cows, particularly during early lactation, when body reserves are mobilized for the production of high amounts of milk (Bonczek et al., 1988; Tiirats, 1997; Huszenicza et al., 2002). NEB, lipomobilization and hypothyreoidism at the onset of lactation in Simmental cows are accompanied with tremendous changes in energy metabolism and oxidation processes, particularly in liver cells. Serum concentration of T3 and T4 are considered as indicators of adaptation (homeoretic adaptation) to NEB in early lactation dairy cows (Reist et al. 2002; Djoković et al. 2007). NEFA is the main blood indicators of lipomobilization in ruminants and BHB is indicator for ketogenesis in liver cells (Oetzel, 2004; Civelek et al., 2011; González et al., 2011). NEB and intensive lipomobilization from body depots induces an increase in both lipogenesis and ketogenesis, a decrease in gluconeogenesis in liver cells, and disturbance in the morphological and functional integrity of hepatocytes, leading to decreased blood levels of glucose, albumin, globulin, total cholesterol, TG and urea (Veenhuizen et al.,1991; Sevinc et al., 2003; Oetzel, 2004; Djoković et al., 2007).

The objective of the present study was to investigate the metabolic and endocrine status in Simmental cows during transition period and mid lactation based on correlation between blood hormones and biochemical metabolites**.**

MATERIALS AND METHODS

The experiment was carried out in Simmental dairy herd which have high genetic merit for milk production (220 Simmental cows). Cows were of similar body mass (600-650 kg), 4-6 years old, an average of 3 lactations with a mean milk yield of 6500 l (calculated over 305 days) in the previous lactation. Clinically healthy cows were divided in three groups from the herd. Group 1 consisted of late pregnant cows (n = 15) from 25 to 1 (13 ± 9) days relative to partus, Group 2 included early lactation cows (n = 15) during the first month of lactation (16 ± 9 days), and Group 3 comprised mid lactation cows (n = 15) between 3 to 5 months of lactation (115 ± 29 days). The body condition scores (BCS) of the test cows were 3.85 ± 0.65 (Group 1, late pregnancy), 3.57 ± 0.55 (Group 2, early lactation) and 3.37 ± 0.74 (Group 3, mid lactation) (Ferguson et al., 1994). The experimental cows were kept in tie-stall barns. Diet and the housing facilities were adapted for research purposes. Diet was appropriate for energy requirement of late pregnancy, early and mid lactation cows. The ingredients and chemical composition of total mixed rations offered to late pregnant, early lactation and mid lactation dairy cows are given in Table 1.

Blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes. After clotting at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully separated and stored at –20°C until analysis. Blood samples collected with fluoride tubes, were immediately centrifuged in the same manner, and glucose concentrations in plasma was analysed. Serum concentrations of GH, insulin, T3 and T4 were determined by ELISA methods (Endocrine Technologies Inc. CA, USA) using Humareader Single plus (Human, Germany).

Table 1

Ingredients and chemical composition of total mixed rations offered to late pregnant, early lactation and mid lactation dairy cows

	Late pregnant cows	Early lactation cows	Mid lactation cows
Grass hay	–	–	5
Lucerne hay (kg)	6	7	7
Maize silage (30% Dry Matter, DM) (kg)	15	20	30
Concentrate (18% crude proteins, CP) (kg)	3	5	8
Dry Matter (DM) (kg)	11.94	16.05	24.82
Net Energy of Lactation (NEL) (MJ)	65.25	87.15	130.23
Crude Protein (CP) (% of DM)	12.55	13.58	13.38
Rumen undegradable protein (RUP) (% of CP)	30.86	35.91	28.33
Fat (% of DM)	3.27	3.09	3.14
Fiber (% of DM)	25.82	23.26	24.33

The following blood metabolites were measured by colorimetric techniques using spectrophotometers Cobas Mira (Roche,Belgium) and Gilford Stasar III (Gilford, USA) according manufactuer instruction: BHB was measured using Fortress kits (USA), NEFA was analyzed using Randox kits (United Kingdom), glucose using Human kits (Germany), TG using Elitech kits (France). The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va, Virginia, USA). The analysis of variance and LSD test were used to evaluate the probability of the significance of the statistical differences between mean parameter values in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when p values were below 0.05.

RESULTS AND DISCUSSION

Homeorhesis is favored since mammary gland utilizes most of the nutrients and metabolites for the milk synthesis, almost regardless of the other body needs (Bauman and Currie 1980). This situation is characterized by NEB, which is the major driving force for substantional endocrine, metabolic and body condition changes. Homeorhesis provoke intense lipid mobilization which characterized by high NEFA concentrations starts within high pregnancy and reaches a maximal intensity in the early lactation. NEFA are preferentially and greatly accumulated as TG in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes and are used for intensive synthesis of ketone bodies in liver cells in ruminant. Ketone bodies (BHBA, acetone and acetoacetate) are intermediate metabolites of oxidation of NEFA (Aceves et al., 1985; Bell, 1995; Drackley, 1999; Cincovic et al., 2012).

Average concentration of variables of blood hormones and biochemical metabolites are examined and correlated in three groups. Results of blood hormones and biochemical metabolites for all examined groups of Simmental cows are shown in Table 2.

Correlations between hormones and biochemical metabolites calculated for all cows in this experiment are given in Table 3.

Results for GH concentrations in the Group 2, (early lactation cows) exhibited significantly increased ($P<0.05$) values, compared with Group 1 (late pregnant) and Group 3 (mid lactation cows). GH dramatically increase the mobilization of lipids from the adipose tissue and increase blood NEFA and BHB in early lactation cows (Tucker, 1994; Jindal and Ludri, 1994). According obtaining results, GH was significant ($P<0.05$) positively correlated with NEFA in this research. These correlations have been reported by other authors (Jindal and Ludri, 1994; Balogh et al., 2008) and show that under NEB conditions, blood GH concentration increases, resulting with fat lipomobilization,

and stimulating milk yield in dairy cows during lactation. (Bonczek et al., 1988; Bauman and Vernon, 1993; Lucy et al., 2001; Butler et al., 2003).

Table 2

Blood metabolic hormones and metabolites in late pregnant, early and mid lactation dairy cows (n=15 in each group).

	Late pregnant cows	Early lactation cows	Mid lactation cows	p-value
GH (ng/ml)	11.74 ± 8.67 [a]	17.13 ± 3.87 [abc]	11.45 ± 4.42 [b]	0.0634
Insulin(ng/ml)	5.52 ± 0.44 [a]	3.93 ± 2.14 [b]	6.54 ± 4.71 [c]	0.1823
T3 (ng/ml)	0.77 ± 0.36 [a]	0.73 ± 0.41 [b]	1.29 ± 1.01 [abc]	0.0466
T4 (ng/ml)	32.70 ± 13.67 [a]	31.93 ± 18.30 [b]	33.06 ± 17.04 [c]	0.9801
Glucose (mmol/l)	3.35 ± 0.32 [a]	2.29 ± 0.48 [abc]	2.75 ± 0.43 [ac]	0.0000
BHB (mmol/l)	1.17 ± 0.36 [a]	1.59 ± 0.25 [abc]	0.91 ± 0.16 [a]	0.0000
NEFA (mmol/l)	0.17 ± 0.06 [a]	0.40 ± 0.28 [abc]	0.13 ± 0.04 [c]	0.0002
TG (mmol/l)	0.28 ± 0.07 [abc]	0.12 ± 0.02 [b]	0.15 ± 0.04 [c]	0.0000

Legend: *Values marked by letters (a,b,c)in one row describe significant differences (p<0.05).*

Table 3

Correlation coefficients between metabolic hormones and metabolites calculated for all cows

	Insulin	T3	T4	glucose	BHB	NEFA	TG
GH	$r = 0.16$	$r = -0.08$	$r = -0.16$	$r = -0.21$	$r = 0.28$	$r = 0.35^{*}$	$r = -0.10$
insulin		$r = 0.37^{*}$	$r = 0.08$	$r = -0.03$	$r = 0.28$	$r = -0.23$	$r = 0.10$
T3			$r = 0.31^{*}$	$r = -0.01$	$r = -0.19$	$r = -0.21$	$r = -0.12$
T4				$r = -0.03$	$r = -0.05$	$r = -0.18$	$r = -0.06$
glucose					$r = -0.45^{*}$	$r = -0.35^{*}$	$r = 0.63^{*}$
BHB						$r = 0.39^{*}$	$r = -0.32^{*}$
NEFA							$r = -0.21$

Legend: *significant correlation (P<0.05) are marked with an asteriks (*)*

Blood insulin values during the same period were nonsignificant (P>0.05) lower in Group 2 (early lactation cows) compared with Group 1 (late-pregnant) and Group 3 (mid lactation cows). Decrease blood insulin concentration under NEB, reduced dry matter intake, while increasing blood GH values, provoke elevation of serum NEFA and BHB. This serum changes of metabolites suggest for reduced anabolic effect of insulin on lipid metabolism resulting with sudden uncontrolled mobilization of NEFA from body reserves and ketogenesis in the liver. Similar results were obtained by other authors (Bonczek et al., 1988; Veenhuizen et al., 1991; Jindal and Ludri, 1994; Butler et al., 2003). Blood levels of thyroid hormones T3 (p<0.05) and T4 (p>0.05) in Group 1 (late pregnancy) and Group 2 (early lactation) were significant lower compared with Group 3 (mid lactation). These results are comply with other authors (Aceves et al., 1985; Jindal and Ludri, 1994; Tilrats, 1997; Huszenicza et al., 2002; Djoković et al., 2007) suggesting that concentration of thyroid hormones are changes as a result of adaptation process of high energy demand of udder and it could be reason for metabolic disorders. During early stage of lactation blood NEFA and BHB were significantly higher (p<0.05) than in late pregnancy and mid lactation cows. BHB values were higher than ref-

erence values in early lactation cows (0.16-0.85 mmol/l), while NEFA values were within physiological range (0.01 - 0.52 mmol/l), (Van Saun, 2004). Blood NEFA and BHB concentrations have significantly ($p<0.05$) positively correlation. Reist et al., (2002) reported a strong negative correlation between blood NEFA and BHB concentrations and NEB in early lactation cows. Blood glucose values in late pregnant and mid lactation cows were within physiological range 2.5 - 4.2 mmol/l (Radostits et al., 2000), while hypoglycemia (2.29 ± 0.48 mmol/l) was find in early lactation cows. Additionally, significant negative correlation ($p<0.05$) was determined between plasma glucose levels with serum levels of NEFA and BHB. Cows in NEB have low rates of glucose and high levels of BHB (Sevinc et al., 2003). Serum levels of glucose, TG, total cholesterol, TP, albumin and urea are indicators of impaired hepatic function (Veenhuizen et al., 1991; Sevinc et al., 2003; Djoković et al., 2007) and decrease concentration of metabolic parameters may imply on fatty liver condition. Present results of serum concentration of glucose and TG in early stage lactation cows, were significantly lower ($p<0.05$) compared with late pregnant and mid lactation cows. Also, significant positive correlation ($p<0.05$) was determined between glucose and TG, which shows that under steatotic conditions, endogenous liver gluconeogenesis synthesis declines, resulting in reduced levels of blood glucose and TG (Sevinc et al., 2003).

This study showed the possibility for fatty liver developing in early stage lactation in the Simmental cows. Possible alterations in the liver function may have deleterious effects on the metabolism of Simmental cows and possibility for adversely impact milk production or reproduction.

4. CONCLUSION

These changes are due to metabolic pathways such as lipolysis and ketogenesis occurring during homeorhetic processes and liver adaptation during early stage lactation in the Simmental cows, which may lead to metabolic disturbance in early stage lactation. These hormonal and metabolic changes can be used as indicators of the metabolic and endocrine status of Simmental cows.

Acknowledgment: This study was financially supported by the Ministry of Education and Science, Republic of Serbia, Project TR 31001.

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