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RESEARCH ARTICLE

Relationship between Blood Metabolic Hormones, Metabolites and Energy Balance in Simmental Dairy Cows during Peripartum period and Lactation

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ABSTRACT

The objective of the present study was to investigate the metabolic and endocrine status in Simmental dairy cows during peripartum period and mid lactation based on the relationships between blood growth hormone (GH), insulin, triiodothyrinine (T3), thyroxine (T4), glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total lipid (TL), triglycerides (TG), total cholesterol, total protein (TP), albumin (ALB), urea and energy balance (EB)**.** Fifteen late pregnant, 15 early lactation and 15 mid lactation cows were chosen for the analysis. Blood metabolic hormones, metabolites and EB were recorded. Early lactation cows had higher serum concentrations of GH, NEFA, BHB, TL and lower blood serum concentrations of T3, glucose, TG, total cholesterol, albumin and urea compared to late pregnant and/or mid lactation cows ($P<0.05$). Correlations between most hormones and metabolites were dependent ($P<0.05$) on the EB of cows, but this factor becomes unimportant under certain circumstances, depending on lactation period. Accordingly, correlations between insulin, BHB or NEFA and other parameters were not solely dependent on the EB, which may be associated with insulin resistance and fatty liver developing in cows.

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INTRODUCTION

Adaptation of the endocrine system during the peripartal period is crucial to maintaining metabolic balance (Bauman and Currie, 1980). Growth hormone (GH) concentration increases during this period and is accompanied by an increase in IGF and IGF binding proteins in mammary secretions, suggesting a role of these factors in mammogenesis and lactogenesis (Tucker, 1994; Lucy *et al.,* 2001; Butler *et al.,* 2003). When puerperal cows are in a negative EB (NEB), GH stimulates lipolysis and milk yield (Bauman and Vernon, 1993). Similarly, plasma concentrations of insulin, another homeorhetic hormone, would be changed by prepartum nutrition and this would affect nutrient supply to the udder. Insulin plays a role in the adaptation of organic matter metabolism in dairy cows during the peripartal period and lactation, particularly in terms of nutrient redistribution and partitioning towards the mammary gland, a phenomenon termed as insulin resistance (Tucker, 1994; Balogh *et al.,* 2008).

Blood levels of thyroid hormones in peripartal cows decrease, particularly in early lactation, when body reserves are mobilized for high milk production (Tiirats, 1997; Huszenicza *et al.,* 2002). Blood T3 and T4 levels are indicators of adaptation (homeorhetic adaptation) to NEB, until EB is attained and correlates positively with EB (Reist *et al*., 2002). NEB, lipomobilization and hypothyreoidism at the start of lactation in dairy cows involve a serious risk of carbohydrate and lipid metabolism disorder, resulting from reduced energy metabolism and oxidation.

The degree of NEB in early lactation and the recovery rate from NEB are very importance for health and productivity (Reist *et al.,* 2002; Remppis *et al.,* 2011). During the early weeks of lactation, the metabolic demands of high milk production almost necessarily lead to NEB, involving dramatic changes in blood metabolites. As a result, dry mater intake (DMI) is reduced, leading to large increases in NEFA and BHB during peripartal period and NEFA are accumulated as TG in the liver cells (Sevinc *et al.,* 2003). However, under steatotic conditions, endogenous liver synthesis declines, resulting in reduced levels of blood glucose, TP, albumin, globulin, total cholesterol, TG and urea (Sevinc *et al.,* 2003; Djoković *et al.,* 2011; Chamberlin *et al.,* 2013; Butt *et al*., 2014; Bilbar *et al*., 2014).

The objective of the present study was to investigate the metabolic and endocrine status in Simmental cows during peripartal period and mid lactation based on the relationships between levels of blood metabolic hormones, metabolites and EB**.** The effect of the energy balance of the cows on the correlation between hormones and metabolites were examined.

MATERIALS AND METHODS

Animals, diets and study protocol: This experiment involved a dairy Simmental herd with a preceding lactation yield of about 6500 L (Farm Ćurčić-Miličić, Mrsać, Kraljevo, Central Serbia, November 2013). Fifteen late pregnant, 15 early lactation and 15 mid lactation cows were chosen for the analysis. Blood was sampled from 25 to 1 (13±9) days before partus, in the first month of lactation (16±9 days), and in mid lactation cows between 3 to 5 months of lactation (115±29 days). The cows exhibited a body condition score (BCS) of 3.85±0.65 (late pregnancy), 3.57±0.55 (early lactation) and 3.37±0.74 (mid lactation) (Ferguson *et al.,* 1994). They were kept under tie-stall conditions.

Total mixed rations (TMR) with different levels of ME were offered to experimental cows twice daily. Meals for the cows were based on lucerne hay, maize silage and concentrate. Chemical components of meals meet the needs of cows in dry period and different period of lactation. The feed consumption was recorded daily to evaluate production efficiency of experimental groups. Diet was suited to the energy requirements of late pregnancy, early and mid-lactation cows. Weende methodology was used for the chemical analysis of the feed (da Silva and Walter, 2012). Energy balance was calculated according to NRC recommendation (2001). Consumed dry matter intake and actual energy balance were calculated as a difference between DMI and NEL of the ration offered and DMI and NEL of the rest of the ration after feeding.

Blood collection and analysis: Blood samples were collected at 10 a.m. by puncture of the jugular vein, serum were harvested and stored at -20°C. Blood samples collected on fluoride were centrifuged, and plasma was measured for glucose values. Serum concentrations of GH, insulin, T3 and T4 were determined by ELISA methods (Endocrine Technologies Inc. CA, USA) using Humareader Single plus (Human, Germany). Different colorimetric techniques and Cobas Mira (Roche, Belgium) and Gilford Stasar III (Gilford, USA) spectrophotometers were used to measure blood metabolites: Fortress kits (USA) for BHB and TL, Randox kits (United Kingdom) for NEFA level, Human kits (Germany) for glucose and total cholesterol, Biosystem kits (Spain) for albumin and urea, and Elitech kits (France) for TP and TG.

Statistical analysis: Data were subjected to statistical analysis using the Statgraphic Centurion software Stat point Technologies Inc. Warrenton, Va, Virginia, USA). Difference in the concentration of hormones and metabolites between three periods of lactation was calculated using ANOVA and posthock LSD test. Regression analysis of b parameters (b=0) was performed to evaluate the intensity of change in the endocrine and metabolic profile as a function of energy balance. Finally, correlation and partial correlation between metabolic parameters were evaluated by Pearson correlation analysis. Partial correlation analysis was used to examine the correlation between endocrine and metabolic parameters with the effects of EB removed.

RESULTS

Results on blood hormones, metabolites, EB, DMI and MY for cows of three groups are shown in Table 1. It shows significant changes in most blood metabolic hormones and metabolites across the experimental groups. Biochemical testing of serum showed significantly higher values ($P<0.05$) of GH, NEFA, TL and BHB, and lower ($P<0.05$) T3, glucose, TG, total cholesterol, albumin and urea values in early lactation cows compared to late pregnant and/or mid lactation cows. Non-significant differences were observed in the serum values of T4, insulin and TP among three groups. Cows in early lactation showed NEB compared to cows in dry period and mid lactation ($P<0.01$). DMI was lower in periparturient period compared to mid lactation ($P<0.01$). Significantly higher MY was found in mid lactation ($P<0.01$) compared to early lactation.

Changes in metabolic hormones and metabolites as a function of EB calculated for all cows in the present study are shown in Table 2. Regression analysis showed that EB was significantly negatively correlated with NEFA and BHB ($P<0.01$), and positively with glucose ($P<0.01$), TG, total cholesterol, urea ($P<0.05$), T3 ($P<0.01$), T4 ($P<0.05$) and insulin ($P<0.01$).

The correlation coefficient between hormones and metabolites summarised for all three experimental periods (Table 3) showed significantly negative ($P<0.05$) correlations (GH and albumin, BHB and glucose, BHB and TG, NEFA and glucose, total cholesterol and NEFA and urea and NEFA) or significantly positive ($P<0.05$) correlations (insulin and T3, T3 and T4, GH and NEFA, NEFA and BHB, glucose and TG, glucose and albumin, glucose and urea, albumin and TG) among the tested parameters.

Relationships between metabolites resulting from the effect of the EB during the dry period (Table 4) were observed between: glucose and NEFA, total cholesterol and TG, thyroid hormones and lipid status indicators, NEFA and GH. In addition, many important correlations were found between the metabolites remaining after the removal of the EB value, which suggests that there may be other factors that affect these relationships, primarily those between insulin and metabolites (NEFA, TG, BHB). Moreover, insulin correlated with T3 and T4 and GH, and maintained the correlation regardless of the energy balance.

In early lactation (Table 5) many important correlations between metabolites depend on EB, but correlations between BHB and metabolites were not dependent solely on this factor. Likewise, the correlations between insulin and glucose, NEFA, BHB and GH were not dependent on EB.

In mid-lactation (Table 6), almost all relationships depended on EB, whereas those between NEFA and other parameters did not show exclusive dependence on this factor.

Table 1: Blood metabolic hormones and metabolites (mean±SD) in late pregnant, early and mid-lactation dairy cows (n=15 in each group)

Parameters	Late pregnant cows	Early lactation cows	Mid lactation cows
GH (ng/mL)	11.74±8.67^{a}	17.13±3.87^{b}	11.45±4.42^{a}
Insulin (ng/mL)	0.55±0.44^{a}	0.39±0.21^{a}	0.65±0.47^{a}
T3 (ng/mL)	0.77±0.36^{a}	0.73±0.41^{a}	1.29±1.01^{b}
T4 (ng/mL)	32.70±13.67^{a}	31.93±18.30^{a}	33.06±17.04^{a}
Glucose (mmol/L)	3.35±0.32^{a}	2.29±0.48^{b}	2.75±0.43^{c}
BHB (mmol/L)	1.17±0.36^{a}	1.59±0.25^{b}	0.91±0.16^{a}
NEFA (mmol/L)	0.17±0.06^{a}	0.40±0.28^{b}	0.13±0.04^{a}
TL (g/L)	3.68±0.78^{a}	7.49±1.42^{b}	5.98±0.85^{c}
TG (mmol/L)	0.28±0.07^{a}	0.12±0.02^{b}	0.15±0.04^{b}
T. cholesterol (mmol/L)	3.30±0.80^{a}	3.48±1.07^{a}	5.35±1.43^{b}
TP (g/L)	76.77±4.58^{a}	8.89±4.92^{a}	75.27±4.50^{a}
Albumin (g/L)	41.87±7.29^{a}	4.61±3.56^{b}	37.57±3.15^{a}
Urea (mmol/L)	5.26±1.37^{a}	3.60±1.06^{b}	5.33±0.95^{a}
Dry Matter (DM) (kg)	12±0.9^{A}	13±2.1^{A}	21±2.5^{B}
EB (MJ/day)	9.6±3.6^{A}	-6.5±9.4^{B}	6±7.3^{A}
MY (L/day)	/	14±2,5^{B}	23±4,4^{C}

Mean values within a row with no common superscript differ significantly. Small letter (P<0.05), capital letter (P<0.01).

DISCUSSION

Blood metabolic hormones, metabolites and EB in late pregnant, early lactation and mid lactation cows were compared in this study. The peripartal and early lactation periods were considered as time periods that have the potential to enhance lactation performance (Lucy *et al.,* 2001). In the current study, early lactation cows had significantly higher GH levels than late pregnant and mid lactation cows. GH dramatically increases lipid mobilization from the adipose tissue, and increases blood NEFA and BHB in early lactation cows (Tucker, 1994; Jindal and Ludri, 1994). In this study, GH was significantly positively correlated with NEFA, but negatively with EB. 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 in fat lipomobilization, and stimulates MY in dairy cows during lactation.

Growth hormone reduces the action of insulin, restricts lipogenic enzyme activity, and reduces glucose utilization (Balogh et al., 2008). In this study, blood insulin levels were non-significantly lower in early lactation cows than in late-pregnant and mid lactation cows and a positive significant correlation was established between insulin and EB. The decrease in blood insulin levels (insulin insufficiency) under NEB, reduced DMI and high blood GH values cause an increased uncontrolled mobilization of NEFA from body reserves and ketogenesis in the liver (Jindal and Ludri, 1994; Butler *et al.,* 2003).

Thyroid hormones are of importance in adapting the endocrine system during lactation, since their very low blood levels in peripartal cows lead to a decrease in energy metabolism, mobilization of body fat reserves and their partitioning toward high milk production (Tiirats, 1997; Huszenicza *et al.,* 2002; Khatri and Bhutto, 2014). Blood levels of T3 and T4 in this experiment were lower in puerperal cows than in late pregnant and mid lactation cows, and exhibited a generally significantly positive correlation with EB, but a negative non-significant correlation with NEFA and BHB. These findings are consistent with those of other authors (Jindal and Ludri, 1994; Tiirats, 1997; Eppinga *et al.,* 1999; Capuco *et al.,* 2001; Huszenicza *et al.,* 2002) suggesting that blood levels of thyroid hormones decrease in puerperal cows, particularly in those suffering from metabolic disorders.

NEFA, TL and BHB levels were significantly higher in early lactation cows than in late pregnant and mid lactation cows. A significant negative correlation was found between NEFA and BHB with EB. Blood NEFA and BHB levels showed a significant positive correlation with each other, but a significant negative correlation with blood glucose. Reist *et al.* (2002) observed a strong negative correlation between blood NEFA and BHB levels and EB in early lactation dairy cows. These correlations show that under NEB, increased blood levels of GH, NEFA and BHB are important in terms of precursor supply for milk synthesis during lactation.

Serum levels of glucose, TG, total cholesterol, TP, albumin and urea are indicators of hepatic function (Sevinc *et al.,* 2003; Djoković *et al.,* 2011; Chamberlin *et al.,* 2013); their decrease may suggest fat infiltration in the liver. In this study, glycaemia and serum levels of TG, total cholesterol, albumin and urea were significantly lower in early lactation cows than in late pregnant and/or mid lactation cows. A significant positive correlation of glucose, TG, total cholesterol and urea levels was found between them and with EB. This study showed that fat infiltration of the liver can develop in early lactation cows. Possible changes in the liver function may have a harmful effect on their metabolism and a negative effect on milk production or reproduction.

Correlations between most metabolites are dependent on EB in cows, but this factor becomes unimportant under certain circumstances. Accordingly, the correlations between insulin, BHB and NEFA, and metabolites do not depend solely on EB, and they can be attributed to the development of insulin resistance and fatty liver in cows. Pronounced ketogenic insulin resistance and fatty liver are metabolic decompensation processes in cows that are not sufficiently adapted to high milk production. It is due to metabolic decompensation that relationships between metabolites cannot be governed through EB control. This is supported by the fact that classifying cows according to insulin, NEFA or BHB levels can be of considerable help in detecting cows that would show certain metabolic changes in early lactation or develop a metabolic disease and, thus, produce less milk (Hachenberg *et al.,* 2007; Kessel *et al.,* 2008; Ospina *et al.,* 2010, Cincović et *al.,* 2012), due to poor adaptation to homeorhetic processes.

Conclusion: Correlations between most hormones and metabolites are dependent on the EB of cows, but this factor becomes unimportant under certain circumstances, depending on lactation period. Accordingly, correlations between insulin, BHB or NEFA and other parameters are not solely dependent on the EB, which may be associated with insulin resistance and fatty liver developing in cows.

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Table 2: Regression analysis for significant correlations for all three measurement periods, values and testing of b=0

	glucose	T. chol.	TG	TL	NEFA	BHB	TP	Alb.	Urea	T3	T4	insulin	GH
EB	0.14±0.04**	1.17±0.6*	0.12±0.06*	NS	-0.07±0.02**	-0.21±0.08**	NS	NS	1.26±0.6*	0.93±0.36**	3.4±1.9*	0.06±0.02**	NS

Legend: Significant correlations (P<0.05) and (P<0.01) are marked with an asterisk (*) and (**). NS: non-significant.

Table 3: Correlation coefficients for the biochemical metabolites calculated for all cows

	Chol	TG	TLip	NEFA	BHB	TProt	Alb	Urea	T3	T4	Insulin	GH
Glu	0.07	0.63*	0.08	-0.35*	-0.45*	-0.02	0.44*	0.32*	-0.01	-0.03	-0.11	-0.22
Chol		-0.12	0.23	-0.40*	-0.39*	-0.02	0.21	0.17	-0.01	-0.10	0.22	-0.28
TG			-0.11	-0.21	-0.32*	-0.01	0.39*	0.28	-0.12	-0.06	0.14	-0.10
TLip				-0.12	-0.04	0.03	-0.13	-0.16	-0.08	-0.15	0.08	0.28
NEFA					0.39*	0.30*	-0.27	-0.33*	-0.21	-0.18	-0.23	0.35*
BHB						-0.09	-0.19	-0.53*	-0.19	-0.05	-0.20	0.28
TProt							0.06	-0.19	0.21	-0.01	-0.18	-0.19
Alb								0.24	-0.20	0.07	-0.05	-0.39*
Urea									-0.07	-0.16	0.09	-0.23
T3										0.31*	0.37*	-0.08
T4											0.08	-0.16
Insulin												0.16

Significant correlations (P<0.05) and (P<0.01) are marked with an asterisk (*) and (**), respectively.

Table 4: Correlation coefficients between endocrine and metabolic parameters in cows during the dry period

	Chol.	TG	TLip	NEFA	BHB	TP	Albu.	Urea	T3	T4	Insulin	GH
Glucose	0.12 [a]	0.18	0.25	-0.37*	-0.18	0.11	0.14	0.09	0.18	0.2	0.35*	0.19
	0.16 [b]	0.17	0.26	0.26	-0.16	0.11	0.12	0.10	0.21	0.14	0.31*	0.22
Chol.		0.29*	0.14	0.09	-0.21	0.19	0.11	0.17	0.31*	0.34*	0.42**	0.22
		0.17	0.19	0.09	-0.19	0.15	0.14	0.19	0.23	0.17	0.26	0.19
TG			0.22	0.19	0.23	0.14	0.16	0.09	0.35*	0.29*	0.31*	0.11
			0.21	0.23	0.16	0.12	0.16	0.11	0.17	0.21	0.36*	0.14
TL				0.11	0.07	0.13	0.16	0.17	0.25	0.31*	0.14	0.09
				0.13	0.08	0.13	0.15	0.14	0.21	0.26	0.19	0.11
NEFA					0.21	0.07	0.14	0.18	-0.36*	-0.34*	-0.39**	0.31
					0.22	0.09	0.18	0.19	-0.20	-0.26	-0.43**	0.26
BHB						0.14	0.12	0.18	-0.39*	-0.37*	-0.36*	0.19
						0.15	0.16	0.14	-0.24	-0.26	-0.38*	0.18
TP							0.35*	0.15	0.2	0.17	0.16	0.2
							0.41**	0.19	0.17	0.17	0.15	0.2
Albumin								0.14	0.19	0.08	0.13	0.17
								0.15	0.11	0.09	0.14	0.2
Urea									0.21	0.16	0.11	0.18
									0.17	0.15	0.13	0.2
T3										0.46**	0.31*	0.19
										0.47**	0.3*	0.22
T4											0.3*	0.23
											0.29*	0.24
Insulin												-0.29*
												-0.33*

[a]correlation between parameters controlled by energy balance, [b] correlation between parameters after exclusion of energy balance; Significant correlations (P<0.05) and (P<0.01) are marked with an asterisk (*) and (**).

Table 5: Correlation coefficients between endocrine and metabolic parameters in cows in early lactation

	Chol	TG	TLip	NEFA	BHB	TP	Album	Urea	T3	T4	Insulin	GH
Gluc	0.33* [a]	0.31*	0.22	-0.45**	-0,49**	0.14	0.29*	0.37*	0.19	0.14	0.41**	0.37*
	0.27[b]	0.29*	0.23	0.25	-0.46**	0.18	0.21	0.26	0.2	0.16	0.44**	0.14
Chol		0.14	0.11	-0.48**	-0.44*	0.12	0.21	0.14	0.37*	0.31*	0.19	-0.12
		0.13	0.12	-0.23	-0.41*	0.14	0.19	0.16	0.35*	0.32*	0.16	-0.13
TG			0.18	-0.45**	-0.48**	0.17	-0.38*	0.24	0.41**	0.31*	0.31*	-0.17
			0.21	0.26	-0.45**	0.15	-0.36*	0.21	0.37**	0.33*	-0.26	-0.15
TL				0.19	-0.21	0.11	-0.11	0.16	0.22	0.16	0.11	0.11
				0.15	-0.23	0.13	-0.12	0.15	0.23	0.15	0.09	0.08
NEFA					-0.51**	-0.27*	-0.34*	0.39**	-0.35*	-0.35*	-0.49**	0.46**
					-0.47**	-0.23	-0.38*	0.19	-0.22	-0.19	-0.5**	0.44**
BHB						-0.22	-0.33*	-0.35*	-0.46**	-0.41**	-0,32*	0.25
						-0.23	-0.37*	0.26	-0.48**	-0.37*	-0.3*	0.21
TP							0.41**	0.13	-0.12	-0.09	0.14	-0.12
							0.4**	0.12	-0.14	-0.07	0.11	-0.13
Alb								-0.31*	0.11	0.24	0.21	-0.09
								0.22	0.12	0.19	0.19	-0.1
Urea									0.11	0.09	-0.14	0.09
									0.15	0.1	-0.11	0.09
T3										0.55**	0.19	-0.11
										0.45**	0.18	-0.13
T4											0.21	-0.14
											0.2	-0.12
Insulin												-0.32*
												-0.3*

[a]correlation between parameters controlled by energy balance, [b] correlation between parameters after exclusion of energy balance; Significant correlations (P<0.05) and (P<0.01) are marked with an asterisk (*) and (**).

Table 6: Correlation coefficients between endocrine and metabolic parameters in mid-lactation cows

	Chol	TG	TLip	NEFA	BHB	TP	Album	Urea	T3	T4	Insulin	GH
Glu	0.35*[a]	0.29*	0.31*	-0.36*	-0.31*	0.12	0.28*	0.33*	0.12	0.11	0.36*	0.18
	0.23[b]	0.18	0.22	-0.35*	-0.26	0.15	0.23	0.21	0.19	0.14	0.21	0.17
Chol		0.14	0.11	-0.34*	-0.14	0.14	0.11	0.15	0.35*	0.32*	0.14	0.11
		0.16	0.13	-0.25	-0.12	0.16	0.15	0.15	0.21	0.19	0.15	0.12
TG			0.11	-0.37*	-0.14	-0.09	0.14	0.11	0.39**	0.29	0.16	0.19
			0.13	-0.24	-0.15	-0.07	0.17	0.12	0.26	0.22	0.3	0.16
TLip				-0.21	-0.17	0.11	0.09	0.21	0.34*	0.31*	0.13	0.09
				-0.14	-0.16	0.12	0.07	0.19	0.25	0.26	0.14	0.07
NEFA					0.11	-0.09	-0.34*	-0.36*	-0.4**	-0.38**	-0.4**	0.14
					0.15	-0.09	-0.19	-0.35*	-0.21	-0.19	-0.35*	0.15
BHB						0.14	-0.17	-0.12	-0.11	-0.12	-0.28*	0.13
						0.17	-0.15	-0.14	-0.15	-0.12	-0.2	0.12
TProt							0.47**	0.38*	0.12	0.09	0.19	0.14
							0.44**	0.25	0.14	0.11	0.17	0.13
Alb								0.34*	0.17	0.12	0.21	0.19
								0.22	0.17	0.14	0.23	0.16
Urea									0.2	0.15	0.14	0.18
									0.21	0.16	0.14	0.17
T3										0.49**	0.18	0.22
										0.46**	0.19	0.23
T4											0.16	0.22
											0.17	0.23
Insulin												-0.16
												-0.17

[a]Correlation between parameters controlled by energy balance, [b] correlation between parameters after exclusion of energy balance. Significant correlations (P<0.05) and (P<0.01) are marked with an asterisk (*) and (**).

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