ENDOCRINE AND METABOLIC STATUS IN DAIRY COWS DURING TRANSITION PEROD AND MID LACTATION

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Abstract. The objective of the present study was to evaluate the endocrine and metabolic changes in Simmental dairy cows during the transition period and mid lactation**.** Fifteen late pregnant cows, 15 early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood samples were collected to measure growth hormone (GH), insulin, triiodothyronine (T3) and thyroxine (T4) by ELISA methods and beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, triglycerides (TG), total protein (TP), albumin and urea by different colorimetric techniques. Early lactation cows were found to have higher blood serum concentrations of GH ($P < 0.05$), NEFA ($P < 0.05$) and BHB ($P < 0.05$) and lower blood serum concentrations of insulin ($P > 0.05$), T3 ($P < 0.05$), T4 ($P > 0.05$) , glucose ($P < 0.05$), TG ($P < 0.05$), albumin ($P < 0.05$) and urea ($P < 0.05$) compared to late pregnant and mid lactation cows. Correlation analysis showed that GH levels were negatively correlated with insulin levels ($r=-0.44$; $P < 0.05$) and positively with NEFA levels ($r=0.32$; $P < 0.05$). Insulin levels negatively correlated with NEFA levels ($r=-0.34$; $P < 0.05$) and positively with T3 levels ($r=0.35$; $P < 0.05$). BHB was negatively correlated with glucose ($r=-0.47$; $P < 0.05$) and TG ($r=-0.36$; $P < 0.05$) levels ($r=-0.44$; $P < 0.05$) and positively with NEFA levels ($r= 0.39$; $P < 0.05$). These endocrine and metabolic changes can serve as useful indicators in evaluating the endocrine and metabolic status of dairy cows during lactation.

Keywords: dairy cows, metabolic hormones, metabolites, lactation

Introduction. Parturition and lactogenesis are accompanied by many physiological changes that facilitate the maintenance of homeostasis (Bauman and Currie, 1980). This involves changes in the activity of almost all cells in the organism in order to satisfy the needs of the mammary gland through nutrient redistribution. Adaptation of the endocrine system during the transitional period is the key factor in maintaining metabolic balance (Bauman and Currie, 1980, Acaves *et al.*1985). GH concentration increases at this time; this increase is accompanied by an increase in IGF and IGF binding proteins in mammary secretions, suggesting a role for these factors in mammogenesis and lactogenesis (Tucker,1994, Butler *et al.*2003). However, as one of the homeorhetic complex of hormones, GH would be sensitive to changes in nutrient supply and these might modify its effects on mammary development (Bauman and Vernon, 1993, Batth *et al.* 2012).

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 transitional period and during lactation, particularly in terms of nutrient redistribution and partitioning towards the mammary gland (insulin resistence) (Tucker,1994, Balogh *et al.*2008). Insulin concentrations tend to decrease in early lactation, particularly in higher yielding cows. Thereafter, plasma insulin concentrations are relatively low and remain so throughout lactation, thereby facilitating the mobilization of organic matter from body reserves and its effective use in the synthesis of milk components (Bonczek *et al.*1988, 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 concentrations (hypothyroidism) 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, Kasagić *et al.*2011). T3 and T4 concentrations are considered to be indicators of adaptation (homeoretic adaptation) to negative energy balance (NEB) until energy balance is achieved (Pethes *et al.*1985, Reist *et al.*2002, Djoković *et al.*2007, Remppis

et al.2011). In dairy cows, low T3 and T4 were observed in the first trimester of lactation, even after BHB and NEFA had returned to normal concentrations (Pethes *et al.*1985, Eppinga *et al.*1999).

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 concentrations of glucose, TG, TP, albumin and urea (Veenhuizen *et al.* 1991, Drackley et al.2001, Sevinc *et al*.2003, Dann *et al*.2005, Djoković *et al.*2007, Djoković *et al.*2011, Gonzalez *et al*.2011).

The objective of the present study was to evaluate the endocrine and metabolic changes in Simmental cows during the transition period and mid lactation.

Material and Methods

A total of 45 dairy cows were randomly selected from the same Simmental herd containing 220 cows suffering from several metabolic and reproductive disorders. Group 1 consisted of late pregnant cows (n = 15) from 25 to 1 day (13 ± 9) to partus; Group 2 consisted of early lactation cows (n = 15) in the first month of lactation (16 ± 9 days), and Group 3 included mid lactation cows (n = 15) between 3 to 5 months of lactation (114 ± 28 days). The cows were mid-yielding with a preceding lactation of about 6500 l (late pregnant cows: 6385 ± 872 l, early lactation cows 6488 ± 980 l, and mid lactation cows: 6677 ± 1088 l in previous lactation). The body condition scores (BCS) of the test cows were 3.85 ± 0.65 (late pregnancy), 3.57 ± 0.55 (early lactation) and 3.37 ± 0.74 (mid lactation), according to Ferguson *et al.* (1994). The experimental cows were kept in tie-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the 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.

Table 1. **Ingredients and chemical composition of total mixed rations offered to late pregnant, early lactation and mid lactation dairy cows**

	Late pregnancy	Early lactation	Mid lactation
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)	25.28	23.26	24.33

Blood samples were collected at 10:00 a.m. or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected on fluoride were immediately centrifuged in the same manner and plasmas were assessed for glucose concentrations. The concentrations of GH, insulin, T3 and T4 in the blood serum were determined by ELISA methods (Endocrine Technologies Inc. CA, USA) using Humareader S. The following blood metabolites were measured by different colorimetric techniques using spectrophotometers (Cobas Mira and Gilford Stasar): BHB and NEFA levels were measured using Randox (United Kingdom) kits, glucose was measured using Human kits (Germany), albumin and urea using Biosystem (Spain) kits, TP and TG using Elitech (France) kits (Kvarklab Biochemical Laboratory).

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 was 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 or 0.01.

Results

Results on blood endocrinal and biochemical metabolites for all groups of cows are shown in Table 2.

Table 2 shows significant changes in most blood metabolic hormones and metabolites between the experimental groups of cows in this study. Serum GH concentrations rose ($P < 0.05$) to a peak after calving, then dropped again to an approximately steady level in mid lactation cows. The mean serum insulin concentrations for all animals were not significantly different ($P > 0.05$) throughout the experiment period, due to high individual variability, with lowest insulin level in early lactation cows. Mean serum T3 and T4 concentrations were lower in early lactation cows, with significant differences ($P < 0.05$) observed for T3 values, as compared to the other groups of cows. Biochemical testing for lipids and ketone bodies in the blood serum showed significantly higher values ($P < 0.05$) of NEFA and BHB, and lower ($P < 0.05$) TG values in early lactation cows as compared to late pregnant and mid lactation cows. In addition, serum concentrations of

glucose, albumin and urea of early lactation cows were significantly lower ($P < 0.05$) than those of late pregnant and mid lactation cows. No significant difference ($P > 0.05$) was observed in the serum values of TP between experimental groups of cows.

The correlations among the biochemical metabolites calculated for all cows in this experiment are given in Table 3.

Table 2. **Blood metabolic hormones and metabolites in late pregnant, early and mid lactation dairy cows** (n=15 in each group). **Results are expressed as mean ± standard deviation** (SD). Mean values within a row with no common superscript differ significantly ($P < 0.05$).

Variables	Late pregnant cows	Early lactation cows	Mid lactation cows
GH (ng/ml)	12.50 ±8.67 [a]	16.95 ±4.03 [b]	12.10±4.16 [a]
Insulin(ng/ml)	0.59±0.46 [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)	32.50±13.20 [a]	31.95±18.30 [a]	33.14± 17.06 [a]
Glucose(mmol/l)	3.36±0.30 [a]	2.29±0.48 [b]	2.76±0.43 [a]
BHB(mmol/l)	1.14±0.36 [a]	1.59±0.25 [b]	0.91±0.16 [a]
NEFA(mmol/l)	0.17±0.06 [a]	0.38±0.29 [b]	0.13±0.04 [a]
TG(mmol/l)	0.29±0.07 [a]	0.12±0.02 [b]	0.13±0.04 [b]
TP(g/l)	77.08±4.57 [a]	78.89±4.92 [a]	75.27±4.47 [a]
Albumin(g/l)	42.57±7.53 [a]	34.61±3.56 [b]	37.57±3.15 [a]
Urea(mmol/l)	5.29±1.32 [a]	3.60±1.07 [b]	5.33±0.95 [a]

Table 3. **Correlation coefficients for the biochemical metabolites calculated for all cows in the present study.** Significant correlations ($P < 0.05$) were indicated in bold.

	GH	Insulin	T3	T4	NEFA	BHB	TG	TP	Albu.	Urea
Glucose	r=-0.17	r=0.14	r=-0.04	r=0.02	**r= -0.35**	**r=-0.47**	**r=0.65**	r=0.01	**r=0.46**	**r=0.45**
GH		**r=-0.44**	r=-0.11	r=-0.14	**r=0.32**	r=0.18	r=-0.03	r=-0.19	r=-0.26	r=-0.13
Insulin			r=0.35*	r=0.19	**r=-0.34**	r=-0.20	r=0.14	r=0.14	r=0.02	r=0.17
T3				**r=0.51**	r=-0.29	r=-0.18	r=0.19	r=0.07	r=0.24	r=0.09
T4					r=-0.18	r=-0.05	r=0.07	r=-0.02	r=0.05	r=0.07
NEFA						**r=0.39**	r=-0.21	r=-0.29	r=-0.26	r=-0.13
BHB							**r=-0.36**	r=0.06	r=-0.23	r=-0.03
TG								r=0.04	**r=0.64**	**r=0.61**
TP									r=0.16	r=0.29
Albu.										**r=0.46**

Table 3 shows the correlation coefficients among the biochemical parameters calculated for all cows in this experiment. Significantly negative correlations ($P < 0.05$) were observed between GH and insulin, insulin and NEFA, BHB and glucose, BHB and TG, NEFA and glucose. Significantly positive correlations ($P < 0.05$) were observed between insulin and T3, T3 and T4, GH and NEFA, NEFA and BHB, glucose and TG, glucose and albumin, glucose and urea, albumin and TG, albumin and urea, TG and urea.

Discussion

Blood metabolic hormones and metabolites in late pregnant, early lactation and mid lactation cows were compared in this study. In this study, NEFA and BHB values were significantly higher in early lactation cows ($P < 0.05$) than in late pregnant and mid lactation cows. Our results showed intensive lipomobilization and ketogenesis in cows in early lactation, and agree with other studies (Drackley *et al.*2001,Oetzel, 2004, Dann *et al.*2005, Gonzalez *et al.*2011) which showed that NEB and lipomobilization from body reserves is characterized by high blood levels of NEFA and BHB. Reist *et al.* (2002) reported a strong correlation among blood NEFA and BHB concentrations and EB in early lactation dairy cows. Additionally, in this study, positive correlation was established between NEFA and BHB ($r=0.39$, $P < 0.05$)

GH is a homeorhetic controller of metabolism, shifting the partitioning of nutrients between the various parts of the body during late pregnancy and lactation (Bonczek *et al.*1988, Lucy *et al.*2001, Batth *et al.* 2012). In the present study, early lactation cows exhibited significantly increased ($P < 0.05$) concentrations of GH than late pregnant and early lactation cows. GH dramatically increased the mobilization of lipids from the adipose tissue, and increased blood NEFA and BHB in early lactation cows (Bauman and Vernon, 1993, Tucker, 1994). This condition confirmed a positive correlation between serum GH levels and NEFA levels ($r=0.32$; $P < 0.05$) in this study. Therefore, the net result would be a shift in the availability of these metabolites in the mammary gland where they can be used for milk synthesis (Bonczek *et al.*1988, Tucker, 1994 Butler *et al.*2003, Batth *et al.* 2012).

Insulin performs important homeostatic control in the

regulation of lipid metabolism. GH reduces the ability of insulin to stimulate lipogenesis in adipose tissue. Thus, GH reduces the action of insulin, suppresses lipogenic enzyme activity, and reduces glucose uptake (Balogh *et al.*2008). Blood insulin concentrations during the same period were lower ($P > 0.05$) in early lactation cows than in late pregnant and mid lactation cows, but with no significant differences observed. A negative correlation was determined in this study between serum insulin and NEFA concentrations ($r=-0.34$, $P < 0.05$) and between insulin and GH concentrations ($r=-0.44$, $P < 0.01$). A decrease in blood insulin concentrations at high blood GH values leads to increasing blood NEFA concentrations suggesting that the reduced anabolic effect of insulin on lipid metabolism results in sudden uncontrolled mobilization of NEFA from body reserves. Similar results were obtained by other authors (Bonczek *et al.*1988, Veenhuizen *et al.* 1991, Tucker,1994, Butler *et al.*2003, Balogh *et al*.2008).

The blood concentrations of thyroid hormones in this study were lower, T3 ($P < 0.05$) and T4 ($P > 0.05$), in puerperal cows than in late pregnant and mid lactation cows. These results are in agreement with those of other authors (Acaves *et al.*1985, Tiirats, 1997, Eppinga *et al.*1999, Huszenicza *et al*.2002, Djoković *et al.*2007, Kasagić *et al.*2011) suggesting that under conditions of marked NEB characterized by increased mobilization of NEFA from body reserves in puerperal cows, blood levels of thyroid hormones are decreased, particularly in cows with metabolic disorders.

Fat infiltration into the liver may affect the concentration of some blood components. Glucose concentrations as well as concentrations of TP, albumin and urea may be diminished (Veenhuizen *et al*. 1991, Sevinc *et al.*2003, Djoković *et al.*2007, Djoković *et al.*2011). In the present work, the serum concentrations of glucose, TG, albumin and urea in early lactation cows were significantly lower ($P < 0.05$) than those of late pregnant and mid lactation cows. The serum concentrations of glucose, TG, TP, albumin and urea are indicators of hepatic functionality (Sevinc *et al.* 2003, Bobe *et al.*2004, Djoković *et al.*2011,) and decreases in their concentration may indicate fat infiltration in the liver. Actually, significant positive correlations were observed between serum glucose and albumin concentrations ($r=0.46$, $P < 0.01$), glucose and TG concentrations ($r=0.65$, $P < 0.01$), glucose and urea concentrations ($r=0.45$, $P < 0.01$) and urea and albumin concentrations ($r=0.46$, $P < 0.01$). Additionally, a negative correlation was determined between plasma glucose and serum NEFA concentrations ($r=-0.35$, $P < 0.05$) and between glucose and BHB concentrations ($r=-0.47$, $P < 0.01$). Possible alterations in the liver function may have deleterious effects on the metabolism of these animals, and may impact milk production or reproduction.

Conclusions

These results suggest that early lactation cows undergo homeorhetic adaptation of the regulation of organic nutrient metabolism being manifested through significant changes in the blood levels of the test hormones, resulting in increased lipomobilization, hypoglycemia, hypoproteinemia and intensive ketogenesis and lipogenesis in liver cells. These endocrine and metabolic changes can serve as useful indicators in evaluating the endocrine and metabolic status of dairy cows during lactation.

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