Original Article

Endocrine and Metabolic Status of Dairy Cows during Transition Period

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Abstract

The objective of this study was to determine blood levels of cortisol, insulin, triiodothyronine (T_3), thyroxine (T_4), glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), triglycerides (TG) and total cholesterol in dairy Holstein cows (n = 40) during the transition period. The test cows were classified into four groups: Group A (n = 10), clinically healthy late-pregnant cows from day 20 to day 10 before calving. Group B (n = 10), clinically healthy late-pregnant cows from day 10 to day 1 before calving. Group C (n = 10), clinically healthy puerperal cows. Group D (n = 10), puerperal ketotic cows. The blood serum of ketotic cows was found to have lower levels of T_3 ($p < 0.01$), T_4 ($p < 0.05$), insulin ($p > 0.05$), cortisol ($p > 0.05$), glucose ($p < 0.01$), TG ($p < 0.01$) and total cholesterol ($p < 0.05$), and higher ($p < 0.01$) levels of NEFA and BHB, as compared to the blood levels in healthy pregnant and healthy puerperal cows. Results suggest that ketotic cows undergo homeorhetic adaptation of the regulation of organic nutrient metabolism being manifested through a decrease in the blood levels of the test hormones, resulting in increased lipomobilization, hypoglycemia, and intensive ketogenesis and lipogenesis in liver cells.

Keywords: dairy cows, hormones, ketosis, lipids, transition period

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Introduction

Major changes in hormonal regulation of metabolic functions in high-yielding cows occur during the transition period. At the onset of lactation, the body of high-yielding dairy cows enters the critical metabolic load stage. Negative energy balance (NEB) during early lactation leads to meeting part of energy demands from body reserves, possibly resulting in deterioration of organic matter metabolism and metabolic diseases (Grummer, 1993; Overton and Waldron, 2004; Dann et al., 2005). This involves changes in the activity of almost all cells in the organ in order to satisfy the needs of the mammary gland through nutrient redistribution. Hence, the large importance of mechanisms involves the regulation of metabolism during its load. Adaptation of the endocrine system during the transition period, primarily the thyroid gland, endocrine pancreas and adrenal gland cortex, is the key factor in maintaining metabolic balance (Bauman and Currie, 1980; Aceves et al., 1985). Thyroid hormones, primarily triiodothyronine (T_3), play an important role in regulating energy metabolism. A decrease in thyroid hormone levels (hypothyroidism) occurs in the blood of peripartal cows, particularly during early lactation (Bonczek et al., 1988; Nikolić et al., 1997; Tiirats, 1997; Stojić et al., 2001; Huszenicza et al., 2002; Kasagić et al., 2011), when body reserves are mobilized for the production of high amounts of milk. NEB, lipomobilization and hypothyroidism at the onset of lactation in dairy cows are accompanied by a serious risk of carbohydrate and lipid metabolism disorders, such as ketosis and fatty liver due to a decline in energy metabolism and oxidation processes, particularly in liver cells. T_3 and T_4 levels are considered to be indicators of adaptation (homeorhetic adaptation) to NEB until energy balance is achieved (Aceves et al., 1985; Huszenicza et al., 2002). Homeorhesis is orchestrated change in metabolism necessary to support a physiological state, in this case lactation (Bauman and Currie, 1980; Aceves et al., 1985). Glucocorticosteroid (cortisol) levels in the circulation during pregnancy remain relatively low until just before parturition when they markedly increase due to stress. After calving, blood cortisol levels decrease, particularly in acetonemic cows. Namely, under low blood cortisol levels in puerperal cows, under intense lipomobilization, the ability of liver cells to synthesize glucose through gluconeogenesis decreases, while ketogenesis and lipogenesis in the liver become intensified (Bertoni et al., 2005; Šamanc and Kirovski, 2008; Forslund et al., 2010).

Insulin plays a role in the adaptation of organic matter metabolism in dairy cows during the transition period, particularly in terms of nutrient redistribution and partitioning towards the mammary gland during early lactation (insulin resistance) (Balogh et al., 2008). Insulin levels in the blood plasma of cows are high before parturition but they gradually decrease from 10 to 5 days before parturition, and show high variations from 5 days before parturition until calving. Thereafter, plasma insulin levels 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). It is believed that under NEB and hypoinsulinemia during early lactation catabolic processes dominate and lipomobilization increases, likely leading to ketosis and fatty liver. Main blood indicators of ketosis and lipomobilization in ruminants are BHB, the most important and abundant ketone body, and NEFA (Oetzel, 2004; González et al., 2011). Lipomobilization from body reserves starts to increase during late pregnancy, reaching maximum intensity during early lactation (Veenhuizen et al., 1991; Drackley et al., 2001; Oetzel, 2004; González et al., 2011). NEFA mostly accumulates as triglycerides (TG) in liver cells as the result of decreased synthesis of very low density lipoproteins (VLDL) in hepatocytes. 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, total cholesterol and triglycerides (Veenhuizen et al., 1991; Drackley et al., 2001; Sevinc et al., 2003, Dann et al., 2005; González et al., 2011; Djoković et al., 2013).

The objective of this study was to determine blood levels of T_3, T_4, insulin, cortisol, glucose, NEFA, BHB, TG and total cholesterol in Holstein cows during the transition period.

Materials and Methods

Animals: Late pregnant and calved cows (n = 40) were chosen from a Holstein dairy herd (PIK Belgrade, Serbia) and assigned to four groups: Group A, late pregnant cows (n = 10) from day 20 to day 10 before calving; Group B, late pregnant cows (n = 10) from day 10 to day 1 before calving; Group C, clinically puerperal healthy cows (n=10) and Group D, clinically ketotic puerperal cows (n = 10). Late pregnant cows were selected during a certain period on the basis of the time of the artificial insemination and after detection of conception. Calved cows were selected as single selection in calving stalls. Diagnosis of ketosis was based on clinical symptoms (decreased appetite, rumen atony, behavior changes), including high concentrations of β-hydroxybutyrate in the blood > 2.6 mmol/l (Oetzel, 2004) and ketone bodies in the urine. Lestradet test was used to examine the presence of ketone bodies in the urine. The average age of cows was 4-6 years. Late pregnant cows weighed 685.28±55.52 kg and early lactating cows weighed 595.21±48.27 kg (body score condition, BSC, 3.5-4.0). The average milk yield was 7695±655 kg (calculated over 305 days) in previous lactation. The experimental cows were housed in tie-stalls. Both diet and feed were used in accordance with the intended use of the test animals. Late pregnant cows received a diet composed of 1.5 kg grass hay, 0.60 kg wheat straw, 10 kg corn silage (33% DM), 2.50 kg alfalfa haylage (51.79% DM), 0.98 kg corn grain, 0.50 kg barley grain, 0.30 kg soybean grits, 1.10 kg soybean meal (44% N) and 0.50 kg wheat flour. Diet for early lactation cows consisted of 3.43 kg alfalfa hay, 9.50 kg corn silage (44% DM), 9.0 kg corn silage (33.94% DM), 5.0 kg alfalfa haylage (47.40% DM), 5.0 kg brewers grain (21.0% DM), 2.50 kg corn grain, 1.50 kg barley grain, 1.30 kg soybean grits, 1.13 kg soybean meal (44% N), 1.30 kg wheat flour and 1.82 kg sugar beet pulp. Table 1 shows the diet composition of daily rations for late pregnant and early lactating dairy cows.

Biochemical analysis: Blood sampling was conducted in the morning 4 to 6 h after milking and feeding by jugular puncture into sterile disposable test tubes without anticoagulant. Blood samples were allowed to clot for 3 h at 4°C and, then, they were centrifuged (1500g, 10 min), following which the serum was carefully harvested and stored at -20°C until analysis. Blood samples collected into fluoride-containing tubes were immediately centrifuged in the same manner, and plasma glucose levels were determined. Cconcentrations of cortisol, insulin, T_3 and T_4 in the blood serum were determined by ELISA methods (Endocrine Technologies Inc. CA, USA) using Humareader S. Different colorimetric techniques and spectrophotometers (Cobas Mira and Gilford Stasar) were used to measure the following biochemical blood components: BHB and NEFA levels were measured by Randox (United Kingdom) kit, total cholesterol and glucose by Human (Germany) kit, and TG by Elitech (France) kit (Biochemical Laboratory, INEP, Zemun, Serbia).

***Statistical analysis*:** Data obtained were statistically analyzed by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va, Virginia, USA). Probability of the significance of statistical differences between mean parameter values in each group was evaluated by analysis of variance and LSD test. Pearson's test was used to assess significant correlations. *P* values below 0.05 or 0.01 indicated significant differences.Ethical approval for the experiment was obtained from the Ethics Committee of the Veterinary Chamber of Serbia, Bulevar oslobodjenja 18, 11129, Belgrade.

Results

Table 2 presents the test results on the endocrine and metabolic parameters in transition cows (Group A, from day 20 to day 10 before calving; Group B, from day 10 to day 1 before calving; Group C, puerperal healthy cows; Group D, puerperal ketotic cows).

Table 2 shows significant changes in most blood parameters in ketotic cows. Mean serum insulin concentrations for all animals were not significantly different ($p > 0.05$) throughout the experimental period due to high individual variability, with the highest insulin level found in puerperal healthy cows. Serum cortisol concentrations insignificantly increased ($p > 0.05$) to a peak before calving (Group B), then dropped again to an approximately steady level in puerperal healthy and ketotic cows. Blood serum T_3 ($p < 0.01$) and T_4 ($p < 0.05$) were significantly lower in ketotic cows than in healthy cows. Blood glucose was significantly lower in ketotic cows compared to healthy cows ($p < 0.01$). Biochemical analysis of lipids and ketone bodies in the blood serum showed significantly higher values ($p < 0.01$) of NEFA and BHB in ketotic cows such as significantly lower levels of TG ($p < 0.01$) and total cholesterol ($p < 0.05$), compared to respective values in healthy cows.

Correlations between blood endocrine and biochemical parameters in puerperal ketotic cows are shown in Table 3. Furthermore, as reported in Table 3, blood serum T_3 levels significantly positively correlated with T_4 ($r = 0.730$, $p<0.05$). In contrast, blood serum T_3 levels correlated significantly and negatively with BHB ($r = -0.580$, $p<0.05$) concentrations. A significantly negative correlation was obtained in cortisol and insulin values ($r = -0.550$, $p<0.05$). NEFA concentrations were significantly and negatively correlated with insulin ($r = -0.550$, $p<0.05$), T_3 ($r = -0.500$, $p<0.05$), TG (r = -0.640, $p<0.05$) and significantly positively with BHB ($r = 0.680$, $p<0.05$) concentrations in blood serum. Serum cholesterol was significantly and positively associated with insulin ($r = 0.660$, $p<0.05$) and significantly and negatively with glucose (r = 0.540, $p< 0.05$). Serum glucose was significantly and positively correlated with TG ($r = 0.560$, $p<0.05$). Additionally, a significantly negative correlation was evidenced between cholesterolemia and cortisolemia ($r = -0.750$, $p< 0.05$).

Table 1 Nutrient composition of daily rations for dairy cows in late pregnancy and puerperium

	late pregnancy	puerperium
Dry matter (DM), kg	9.82	23.63
Net energy of lactation (NEL), MJ	65.50	163.03
Crude protein (CP), % DM	15.03	16.05
Rumen undegradable protein (RUP), %	5.09	5.06
Fat, % DM	3.82	4.78
Fiber, % DM	25.12	17.2
Acid detergent fiber (ADF), % DM	25.31	22.08
Neutral detergent fiber (NDF), % DM	40.59	35.48

Table 2 Selected blood endocrine and metabolic parameters in dairy cows during the transition period (means ± standard deviation). (Group A, cows from day 20 to day 10 before calving; Group B, from day 10 to day 1 before calving; Group C, puerperal healthy cows; Group D, puerperal ketotic cows

	Late pregnancy		Puerperium	
Group	**A**	**B**	**C**	**D**
n	10	10	10	10
Insulin (mU/l)	15.46±4.89 [a]	18.41±6.96[a]	32.77±19.70[a]	19.92±9.07[a]
Cortisol (nmol/l)	29 40±16.13[a]	108.58±69.55[a]	34.59±31.50[a]	24.17±17.42[a]
T_3 (nmol/l)	2.63±0.63[a]	2.31±0.96[a]	2.28±0.55[a]	1.54±0.84[b]
T_4 (nmol/l)	52.77±11.78[a]	42.35±16.34[a]	45.72±15.27[a]	33.55±14.57[b]
Glucose(mmol/l)	3.05±0.47[a]	2.92±0.39[a]	2.56±0.35[a]	1.84±0.35[b]
NEFA (mmol/l)	0.30±0.10[a]	0.51±0.17[b]	0.42±0.15 [c]	0.84±0.22[d]
BHB (mmol/l)	0.78±0.42 [a]	0.84±47 [a]	0.98±0.35[a]	3.07±0.35 [b]
TG(mmol/l)	0.21±0.05[a]	0.26±0.05[a]	0.19±0.04[a]	0.14±0.04[b]
Cholest. (mmol/l)	1.89±0.24[a]	1.78±0.30[a]	1.92±0.44[a]	1.52±0.33[b]

Legend: Mean values within a row with no common superscript differ significantly.

Discussion

During the peripartal period, dairy cows undergo major endocrine and metabolic changes that can cause considerable metabolic disorders, and experience a decline in productive and reproductive performance (Grummer, 1993; Overton and Waldron, 2004; Dann et al., 2005). Immediately before and particularly after parturition, and during the first stage of lactation, increased mammary gland activity results in energy deficiency, increased lipomobilization from body reserves and intensive ketogenesis and lipogenesis in the liver. Lipomobilization from body reserves begins during late pregnancy, reaching maximum values during the puerperal period. Increased blood levels of NEFA and BHB are the best indicators of lipomobilization in dairy cows during early lactation (Veenhuizen et al., 1991; Dann et al., 2005; González et al., 2011). Significantly higher blood NEFA and BHB levels were found in ketotic cows than in healthy cows during the peripartal period.

Moreover, a significantly positive correlation was observed between serum NEFA and BHB levels in ketotic cows. Blood insulin values during the same period were lower in late-pregnant cows than in healthy puerperal cows. Moreover, blood insulin levels were lower in ketotic cows as compared to healthy newly calved cows, but no statistical significance was observed. A decrease in blood insulin levels in ketotic cows is directly propotional to increasing blood NEFA levels, clearly suggesting that the reduced anabolic effect of insulin on lipid metabolism results in sudden uncontrolled mobilization of NEFA from body reserves (Bonczek et al., 1988, Veenhuizen et al., 1991; Butler et al., 2003; Balogh et al., 2008). This finding is supported by the significantly negative correlation observed in this study between serum insulin levels and NEFA levels in ketotic cows.

Cortisol is known as one of the most important hormones involved in the regulation of gluconeogenesis as well as in the regulation of carbohydrate and lipid metabolism. This fact is of great importance in high-yielding cows during high metabolic load periods such as late pregnancy and lactation. Lower blood cortisol levels were determined in puerperal cows than in pregnant cows, particularly in high-producing cows. Namely, there are literature data on adrenal cortex insufficiency, *i.e.* dysfunction of the hypothalamus-adenohypophysis-adrenal cortex axis in peripartal dairy cows. Most cases involve only relative insufficiency based on an increased exhaustive load of the adrenal gland cortex due to increased gluconeogenesis in the liver from its own sources (Bertoni et al., 2005; Šamanc and Kirovski, 2008;

Table 3 Correlations between blood endocrine and biochemical parameters in puerperal ketotic cows. Significant correlations (p<0.05) are indicated in bold letters

	Insulin	**Cortisol**	**T_3**	**T_4**	**NEFA**	**BHB**	**TG**	**Choles.**
Glucose	*r* =-0.030	*r* =-0.010	*r*=-0.010	*r*=-0.170	*r*=-0.380	*r*=-0.250	***r*= 0.560**	***r*=0.540**
Insulin		***r* =-0.550**	*r*=0.390	*r*=0.070	***r*=-0.550**	*r*=-0.320	*r*=0.470	***r*=0.660**
Cortisol			*r*=0.070	*r*=0.140	*r*=0.050	*r*=0.110	*r*=-0.440	***r*=-0.750**
T_3				***r*=0.730**	***r*=-0.500**	***r*=-0.580**	*r*=-0.190	*r*=-0.030
T_4					*r*=0.100	*r*=-0.250	*r*=-0.190	*r*=-0.060
NEFA						***r*= 0.680**	***r*=-0.640**	*r*=-0.340
BHB							*r*=-0.350	*r*=-0.180
TG								*r*=0.420

Forslund et al., 2010). The highest blood cortisol levels were found in cows immediately before calving (Group B), and they were insignificantly higher than in the other 3 groups of cows. These results comply with those of other authors (Bertoni et al., 2005; Šamanc and Kirovski, 2008; Forslund et al., 2010) and suggest that dairy cows undergo severe stress immediately before and during calving, which leads to hormonal changes manifested as significant increases in glucocorticosteroid and catecholamine levels in the circulation. In ketotic cows, blood cortisol levels were lower as compared to healthy puerperal cows, but no statistical significance was observed. Similar results were reported by other authors (Nikolić et al., 1997; Bertoni et al., 2005) who found decreased blood cortisol levels during early lactation, particularly in ketotic cows. Namely, under low blood cortisol levels in puerperal cows undergoing a negative energy balance and increased lipomobilization from body reserves, the ability of liver cells to synthesize glucose from gluconeooplastic precursors is substantially decreased. The ketogenic and lipogenic processes in the liver are intensified, blood levels of NEFA and ketonic bodies increase, and hypoglycemia develops (Veenhuizen et al., 1991 Drackley et al., 2001; González et al., 2011; Djoković et al., 2013).

In this experiment, blood glucose levels were significantly lower in ketotic cows (hypoglycemia) than in healthy cows during the peripartal period. The decrease in blood glucose levels in puerperal dairy cows is attributed to an increased mammary gland activity in lactose synthesis as well as to a reduced hepatocyte activity to synthesize glucose through gluconeogenesis under lipomobilization and lipogenesis in the liver, during reduced adrenal cortex activity (Veenhuizen et al., 1991; Drackley et al., 2001; Overton and Waldron, 2004; Dann et al., 2005).

Thyroid hormones, particularly T_3 which is about 4 times more active than T_4, are considered to play an important role in the etiopathogenesis of ketosis, and fatty liver, due to the fact that their very low levels in the blood of transition cows bring about a decrease in energy metabolism, mobilization of body fat reserves and their partitioning toward high milk production (Nikolić et al., 1997; Tiirats, 1997; Huszenicza et al., 2002; Kasagić et al., 2011). This also involves disorders of metabolic balance and uncontrolled mobilization of lipids which, apart from being used for milk synthesis, very often remain within parenchymatous organs, liver in particular. The blood levels of the thyroid hormones T_3 and T_4 in this study were significantly lower in ketotic cows than in healthy cows during the peripartal period. These results are in agreement with those of other authors (Aceves et al., 1985; Nikolić et al., 1997; Tiirats, 1997; Stojić et al., 2001; 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 significantly decreased, particularly in ketotic cows, i.e. in animals diagnosed with hepatic lipidosis. This finding has been confirmed in this study by the significantly negative correlation between the blood levels of T_3 and NEFA, and T_3 and BHB in ketotic cows.

Furthermore, significantly lower blood TG and total cholesterol levels were found in ketotic cows than in healthy cows during the transition period. Moreover, a significantly negative correlation was observed between blood NEFA and TG levels in ketotic cows and a significantly positive correlation between TG, total cholesterol and glucose. These results confirm the fact that under NEB and high lipomobilization, increased ketogenesis, TG and cholesterol accumulation in the liver cells, in the condition of low blood serum values of cortisol, insulin, T_3 and T_4 (Veenhuizen et al., 1991; Drackley et al., 2001; Sevinc et al., 2003; Dann et al., 2005; González et al., 2011; Djoković et al., 2013).

These results suggest that ketotic cows undergo homeorhetic adaptation of the regulation of organic nutrient metabolism being manifested through a decrease in the blood levels of the test hormones, resulting in increased lipomobilization, hypoglycemia, and intensive ketogenesis and lipogenesis in liver cells.

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บทคัดย่อ

ปริมาณของสารจากต่อมไร้ท่อและเมแทบอลิซึมของโคนมในช่วงการเปลี่ยนแปลง ก่อนและหลังการคลอด

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การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจวัดปริมาณ คอร์ติซอล อินซูลิน ไตรไอโอโดไธโรนีน (T_3) ไธรอกซิน (T_4) กลูโคส กรดไขมันอิสระชนิด non-esterified (NEFA) เบตาไฮดร็อกซีบิวไทเรท (BHB) ไตรกลีเซอไรด์ (TG) และโคเลสเตอรอลในเลือดของโคนมพันธุ์โฮสไตน์ ฟรีเชี่ยน จำนวน 40 ตัว ในช่วงการเปลี่ยนแปลงก่อนและหลังการคลอด (transition period) ทำการแบ่งโคนมเป็น 4 กลุ่ม ดังนี้ โคนมกลุ่ม A จำนวน 10 ตัว เป็นโคนมตั้งท้องที่มีสุขภาพสมบูรณ์อยู่ในระยะก่อนกำหนดคลอด 20 ถึง 10 วัน โคนม กลุ่ม B จำนวน 10 ตัว เป็นโคนมตั้งท้องสุขภาพสมบูรณ์อยู่ในระยะก่อนกำหนดคลอด 10 วัน ถึง 1 วัน โคนมกลุ่ม C จำนวน 10 ตัว เป็นโคช่วงระยะการคลอด และกลุ่ม D จำนวน 10 ตัว เป็นโคที่มีภาวะคีโตซีสในช่วงคลอด ผลการศึกษาพบว่ากลุ่มโคที่มีภาวะคีโตซีสมีปริมาณ T_3 (p<0.01) T4 (p<0.05) อินซูลิน (p>0.05) คอร์ติซอล (p>0.05) กลูโคส (p<0.01) TG (p<0.01) และโคเลสเตอรอล (p<0.05) ในเลือดน้อยกว่าโคสุขภาพสมบูรณ์ที่ตั้งท้องและอยู่ในระยะการคลอด อย่างไรก็ตามโคที่มีภาวะคีโตซีสนี้มีค่า NEFA และ BHB ของสูงกว่าโคที่มีสุขภาพสมบูรณ์ (p<0.01) จากผลการศึกษานี้แสดงให้เห็นว่าสภาวะคีโตซีสมีผลต่อการการปรับสมดุลของการควบคุมกระบวนการสันดาปของสารอาหารอินทรีย์ผ่านทางการลดระดับของสารจากต่อมไร้ท่อในกระ-แสโลหิต และจะมีผลต่อการเพิ่มการนำไขมันสะสมมาใช้ การเกิดภาวะน้ำตาลในเลือดต่ำร่วมกับการเกิดคีโตซีสและการสร้างไขมันเพิ่มขึ้นในตับ

คำสำคัญ: โคนม ฮอร์โมน ภาวะคีโตซีส ไขมัน การเปลี่ยนแปลงก่อนและหลังการคลอด

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