# BLOOD CONCENTRATIONS OF THYROID HORMONES AND LIPIDS OF DAIRY COWS IN TRANSITIONAL PERIOD

**R. DJOKOVIĆ[1], H. ŠAMANC[2], J. BOJKOVSKI[2], N. FRATRIĆ[2]**

[1]Faculty of Agronomy Cacak, 32000, Cara Dusana No. 34, Cacak, Serbia
[2]Faculty of Veterinary Medicine, Trg Oslobođenja 18, 1100 Belgrade 9, Serbia
E-mail: djokovici@ptt.rs

**Summary**

The aim of the present investigation was to determine changes of the blood concentrations of glucose, lipids and thyroid hormones in the dairy cows in the transitional period. The animals (n=40) were divided into four groups: in the first group included late pregnant cows (n=10) from the $10^{th}$ to $4^{th}$ day before calving; the second group included late pregnant cows (n=10) from the $4^{th}$ until $1^{st}$ day before calving; the third groups included clinically puerperal healthy cows (n=10); whereas the fourth group included puerperal cows with clinical symptoms of ketosis (n=10). Samples of blood tissues were taken from all cows. Biochemical examination of blood serum showed significantly higher values ($p<0.01$) of free fatty acids- FFA in ketotic cows such as significantly lower concentrations of glucose ($p<0.01$), triacylglycerols -TAG ($p<0.01$), total cholesterol ($p<0.05$), triiodthyronine-$T_3$ ($p<0.05$) and thyroxine-$T_4$ ($p<0.05$) compared to the values obtained in the blood serum in the groups of healthy cows before and after calving. Significant increase of the concentations of free fatty acids in ketotic cows shows that during intensive lipomobilization newly synthesized triacylglycerols accumulated in the hepatocytes. In the healthy cows in puerperal period, it was determined positive correlation ($r=0.70$, $p<0.05$) between free fatty acids and triacylglycerols in the blood, which indicated that triacylglycerols do not accumulate in the hepatocytes. In ketotic cows hypothyroidal status is established and that the blood concentrations of free fatty acids, triaacylglicerols, total cholesterol and glucose served as major biochemical indicators in determining liver steatosis in dairy cows in transitional period.

**Key words**: cows, ketosis, lipids, triiodthyronine, thyroxine

Transitional period in dairy cows included 3 weeks before and 3 weeks after calving when metabolic processes were adapted to providing energy and precursors required for synthesis of milk compounds (8, 16).

In consequence in early lactation, such a state caused negative energy balance, a high mobilization of lipids from bodily fat reserves as well as hypoglycaemia (18, 23, 24).

Lipomobilization characterized by high concentrated free fatty acids in the blood, starts in a high pregnancy, reaching its maximum in the early lactation. Free fatty acids are re-esterified and accumulated in the form of triacylglycerols in the liver, primarily due to a decrease capacity of hepatocytes for transport lipids by very low density lipoproteins (VLDL). As a result, lipomobilization intense ketogenosis and lipogenesis in the liver and as consequence lower concentrations of glucose,

triacylglycerols and total cholesterol in the blood was manifested (4, 7, 12, 9, 18, 21, 23, 24). Primary homeoretic adaptation of glucose metabolism in the early lactation leads to an increased gluconeogenesis in the liver to direct glucose to the mammary gland for lactose synthesis (19). If the degree of gluconeogenesis does not satisfy increased needs in glucose of dairy cows in the early lactation, hypoglycaemia, ketonemia and ketonuria likely occurs (25)

The hormonal activity of thyroid gland has an important role in the transitional period for determining the cell metabolism intensity, metabolism of lipids and carbohydrates and lactation course itself by its thyroid hormones (14, 15). A positive correlation was established between thyroid hormones in the blood and energy balance (18) and a negative one between concentrations of triiodthyronine and thyroxine in the blood and milk production (15). Under the conditions of a negative energy balance and of high lipomobilization, the concentrations of thyroidea hormone in the blood were significantly lower in transitional period, with a markedly declined triiodthyronine in the blood shortly before and after calving (2, 5, 14, 17).

Kapp et al. (12) noticed in ketotic cows that diffuse lipid infiltration of hepatocytes, impairing most of them, occurred due to reduced mitochondria capacity to oxidize fatty acids at decreased levels of thyroid hormones in the blood.

The aim of the present investigation was to determine changes of blood concentrations of glucose, lipids and thyroid hormones of dairy cows in the transitional period.

## Materials and methods

The high pregnant and calved cows (n=40) were chosen from a Holstein dairy herd (1100 dairy cows) and divided into four groups; in the first group (group A) included late pregnant cows (n=10) from the $10^{th}$ to $4^{th}$ day before calving; the second group (group B) included late pregnant cows (n=10) from the $4^{th}$ until $1^{st}$ day before calving ; the third group (group C) included clinically puerperal healthy cows (n=10); whereas the fourth group (group D) included puerperal cows with clinical symptoms of ketosis (n=10). Samples of blood tissues were taken from all cows. The cows were averagely 4-6 years old, 645 kg by weight, with 3 lactations and 7625 l milk of average milk yield per 305 days of lactation. The diseased cows were those indicating the clinical symptoms of ketosis (inappatence, rumen atony and behavioral changes) and determined high concentrations of urinary ketone. The presence of ketone bodies in urine was examined using the Lestradet test (13) . The trial cows were kept in tie-up stalls in barn housing. Diet and feeds were in conformity with purpose and animal utilization. The meal was consisted in the way to suit the energy needs of animals over their high pregnancy and early lactation.

The blood samples for serum were collected by puncture of jugular vein (2 test tubes of blood taken per puncture, approximately 20 ml blood) from 10 h a.m. to noon or from 4 to 6 h after milking and feeding. The blood samples were allowed to clot spontaneously at room temperature. The serum was then decanted ad 1,000 g and preserved at -18$^{o}$C until analyzed.

Triiodthyronine ($T_3$) and thyroxine ($T_4$) concentrations of the blood serum samples were determined following RIA method, using commercial test packages (INEP-Zemun) and those of free fatty acids (FFA) colorimetrically according to Ducombe (1966), using colorimetric test No. 001 INEP-Zemun being used. The content of glucose in blood serum was determined through GOD-PAP phenol method Dialab (Austria) cat. No. 760312, that of triacylglycerols (TAG) in blood serum through GPO-PAP method (A 40015) and total cholesterol CHOD-PAP method (041015), reagens Serbolab (Serbia) by means of microtitar reader MULTISKAN MCC/340 (Helsinki, Finland).All biochemical parameters were assayed at the laboratory of Institute for the Application of Nuclear Energy (INEP) Zemun.

The significance of differences of thyroid hormone, lipids and glucose concentrations in the blood serum between the animal groups used in experiment was determined by ANOVA procedure. Data are expressed as means ± standard deviation ($x \pm SD$). Correlation coefficients were obtained using linear regression models. Differences with $p<0.05$ and $p<0.01$ were considered statistically significant (Microsoft STATISTICA ver.5.0, Stat. Soft. Inc.1995).

## Results and discussions

Table 1 shows the research results on the blood levels of glucose, free faty acids (FFA) triglyceride (TAG), total cholesterol, triiodthyronine ($T_3$) and thyroxine ($T_4$) in dairy cows during transitional period.

Table 1

**Selected metabolic profile parameters in the groups of the cows in transitional period (means ± standard deviation)**

| Group | High pregnancy | | Puerperium | |
|---|---|---|---|---|
| | A | B | C | D |
| n | 10 | 10 | 10 | 10 |
| Glucose (mmol·l$^{-1}$*)* | 2.94±0.32$^{A}$ | 3.12±0.42$^{B}$ | 2.71±0.35$^{bc}$ | 1.80±0.43 $^{ABCD}$ |
| FFA (mmol·l$^{-1}$) | 0.27±0.14$^{A}$ | 0.54±0.26$^{ABc}$ | 0.46±0.10$^{AC}$ | 0.74±0.12 $^{ACD}$ |
| TAG (mmol·l$^{-1}$) | 0.32±0.04$^{A}$ | 0.41±0.03$^{ABC}$ | 0.35±0.04 $^{C}$ | 0.27±0.03 $^{ABCD}$ |
| Total cholesterol (mmol·l$^{-1}$) | 1.75±0.20$^{a}$ | 1.71±0.30$^{b}$ | 1.86±0.62$^{c}$ | 1.39±0.29 $^{acd}$ |
| T3 (nmol·l$^{-1}$*)* | 2.58±0.53$^{a}$ | 2.31±0.41$^{b}$ | 2.22±0.74$^{c}$ | 1.58± 0.70$^{abcd}$ |
| T4 (nmol ·l$^{-1}$) | 50.68±9.34$^{a}$ | 45.21±4.28$^{b}$ | 45.75±14.27$^{c}$ | 35.06±12.43 $^{abcd}$ |

Legend: Values marked by letters (a,b,c,d) in one row describe significant differences ; values market by small letter differ significantly ($p<0.05$); values market by capital letter differ high-significantly ($p<0.01$).

From Table 1 can be seen that significant changes of the most parameters in the blood occurred in the group of ketotic cows.

Glucose is a blood parameter defining the energy metabolism in late pregnancy and lactating cows. In the group of puerperal healthy cows the glucose concentration in the blood is significantly lower ($p<0.05$) compared with groups of late pregnant cows, whereas hypoglycaemia was determined in ketotic cows. It was significant lower ($p<0.01$) compared to groups of healthy cows before and after calving.

The above results are in agreement with the literature data (6, 21, 23, 24) indicating that physiological glycemia in early lactation cows is at the lower physiological limit due to the sudden activity of the mammary gland and increased lactose synthesis. Furthermore, the negative energy balance, lipomobilization and increased fat accumulation in hepatocytes in ketotic cows induce a considerable reduction in glucose synthesis by gluconeogenesis in the liver (hypoglicemia).

Energy metabolism in dairy cows in transitional period is closely linked to lipid metabolism. The best indicator of negative energy balance and the degree of mobilization of lipids from bodily fat reserves in the transitional period is increased of FFA concentrations in the blood. (16, 18, 21, 23, 24). In the late pregnant cows the FFA concentrations in the blood is higher than the physiological range for the cattle (0.1-0.35 $mmol \cdot l^{-1}$ - 11). However, the FFA concentrations have been significantly increasing ($p<0.01$) (among groups A and B) as the calving day was approaching that shows that lipomobilization begins immediately before calving, respectively from four to one days before calving Similar results were obtained by Dyk et al. (3) .

Significantly higher FFA concentrations have been determined in the blood ($p<0.01$) of ketotic cows than in the groups of healthy cows before and after calving. Obtained results clearly show that significant increase of FFA concentrations in the blood causes an increase of the content of lipids in the liver cells (fatty liver) and a decrease of TAG concentrations in blood. This agrees with studies of Gröhn (6), Veenhuizen et al. (24) and Sevinc et al (21).

Opposite to that, in healthy cows in puerperal period positive correlation ($r=0.70$, $p<0.05$) has been determined among the FFA and TAG concentrations in the blood. That indicates that in the condition of lipomobilization in blood of just calved healthy cows increase of TAG concentrations in the blood and unlike the ketotic cows TAG do not accumulate in the liver, but are transported by the blood to the tissues which use them for their purposes.

In cows with fatty liver the TAG and total cholesterol concentrations in the blood decline (12, 21). At ruminants relatively low TAG concentrations in the blood serum (2.6-5.2 $mmol \cdot l^{-1}$ - 11) and further decline of their concentrations could be the consequence of lipid infiltration of liver cells.

In this experiment has been determined significantly lower TAG ($p<0.01$) and total cholesterol ($p<0.05$) concentrations in the blood in the group of ketotic cows then to the values in groups of healthy cows before and after calving. The

results clearly indicated that the blood TAG concentrations decreased and proportionally to that increases their content in the liver cells in which they accumulated. These results are in accordance with observation by Herdt et al. (9), Holtenius (10) and Sevinc et al. (21).

In the early lactation dairy cows are in the state of metabolic stress, in order to satisfy the increased energy of mammary gland and the adjustment of neuro-endocrine system of dairy cows to the new metabolic needs of the body (1, 14). One of endocrine factor is the thyroid hormones. In this study significantly lower ($p<0.05$) $T_3$ and $T_4$ concentrations in the blood had been determined in the group of ketotic cows compared to the values of these hormones in the blood of groups of healthy cows before and after calving. Also, significant positive correlation ( $r=0.73$, $p<0.05$) between the $T_3$ and $T_4$ levels in the blood. The similar results have been obtained by other authors (5, 14, 18).

Romo et al. (20) reported that as consequence of liver steatosis, FFA accumulate in the liver parenchyma and it has been demonstrated that some fatty acids inhibit type-I liver 5` –deiodinase activity

In accordance, Pezzy et al. (17) consider that in dairy cows in early lactation, the state of hypothyroism is present and it is the cause of the liver's decreased 5`- deiodinase activity or the secretion of thyroid hormones in the milk.

The intensity of oxidation in mithochondria of cells is closely linked with the functional state of thyroid gland, so it is justifiably considered that the conditions of negative energy balance and the increased lipomobilization from bodily fat reserves result in lipid infiltration of liver cells. The reason is the decreased capacity of mitochondria (with decrease the number following simultaneous size increase of mitochondria and mitochondrial damage) to oxidize fatty acids in the conditions of low concentrations of thyroid hormones in the blood (2, 12, 22).

The results confirm these opinions, because values of $T_3$ and $T_4$ in blood of ketotic have were significantly lower ($p<0.05$) then those of the healthy ones and negative correlation ($r=0.50$, $p<0.05$) between the $T_3$ and FFA concentrations in the blood of ketotic cows has established.

## Conclusions

Biochemical examination of blood serum showed significantly higher values ($p<0.01$) of free fatty acids- FFA in ketotic cows such as significantly lower concentrations of glucose ($p<0.01$), triacylglycerols -TAG ($p<0.01$), total cholesterol ($p<0.05$), triiodthyronine-$T_3$ ($p<0.05$) and thyroxine-$T_4$ ($p<0.05$) compared to the values obtained in the blood serum in the groups of healthy cows before and after calving.

Significant increase of the concentrations of free fatty acids in ketotic cows, compared with healthy ones, shows that during intensive lipomobilization newly synthetized triacylglycerols accumulated in the hepatocytes (fatty liver). In the healthy cows in puerperal period, it was determined positive correlation ($r=0.70$,

p<0.05) between free fatty acids and triacylglycerols in the blood, which indicated that triacylglycerols do not accumulate in the hepatocytes. On the basic of obtained results this investigation suggested that a hypothyroidal status was established in ketotic cows and that the blood concentrations of free fatty acids, triaacylglicerols, total cholesterol and glucose served as major biochemical indicators in determining liver steatosis in dairy cows in transitional period

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