Blood Concentrations of Thyroid Hormones and Lipids and Content of Lipids in the Liver in Dairy Cows in Transitional Period

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Abstract


The aim of the present investigation was to determine the correlation in the blood concentrations of thyroid hormones, lipids and glucose as well as the content of lipids in the liver of dairy cows in the transitional period.

The animals (n = 40) were divided into four groups: the first group included late pregnant cows (n = 10) from the 10th to 4th day before calving; the second group included late pregnant cows (n = 10) from the 4th to 1st day before calving; the third group included clinically puerperal healthy cows (n = 10), whereas the fourth group included puerperal cows with clinical symptoms of ketosis (n = 10). Samples of liver and blood tissues were taken from all cows. Pathohistological examination of liver samples showed statistically significantly higher (p < 0.01) lipid infiltration in ketotic cows compared to healthy cows in late pregnancy and puerperium. Biochemical examination of blood serum showed significantly higher values (p < 0.01) of free fatty acids in ketotic cows, such as significantly lower blood concentrations of glucose (p < 0.01), triacylglycerols (p < 0.01), total cholesterol (p < 0.05), triiodthyronine (p < 0.05) and thyroxine (p < 0.05), compared to the values obtained in the blood serum in the groups of healthy cows before and after calving. In this study, significantly positive correlations were determined between the content of lipids in the liver and blood concentration of free fatty acids (r = 0.51; p < 0.05) as well as the negative ones between the content of lipids in the liver and blood concentrations of glucose (r = -0.69; p < 0.05), triacylglycerols (r = -0.55; p < 0.05) and total cholesterol (r = -0.50; p < 0.05) in the group of ketotic cows. Our investigations suggested that a hypothyroidal status was established in ketotic cows and that the blood concentrations of free fatty acids, triacylglycerols, total cholesterol and glucose served as major biochemical indicators in determining liver steatosis in the dairy cows in the transitional period.

Liver steatosis, ketosis, free fatty acids, triacylglycerol, triiodthyronine, thyroxine

The transitional period in dairy cows included 3 weeks before and 3 weeks after calving, when metabolic processes were adapted to providing energy and nutrients required for the synthesis of milk compounds (Grummer 1995; Overton and Waldron 2004).

Early lactation in dairy cows resulted in negative energy balance, high mobilisation of lipids from bodily fat reserves as well as hypoglycaemia (Veenhuizen et al.1991; Vázquez-Añón et al.1994; Reist et al. 2002).

Lipid mobilisation characterised by highly concentrated free fatty acids in blood starts in a high degree of pregnancy, reaching its maximum in early lactation. Free fatty acids are re-esterified and accumulated in the form of triacylglycerols in the liver, primarily due to the decreasing capacity of hepatocytes for transport of lipids by very low density lipoproteins (VLDL). As a result, lipid mobilisation intense ketogenesis and lipogenesis in the liver and consequently lower concentrations of glucose, triacylglycerol and total cholesterol in blood were manifested (Herdt et al. 1983; Holtenius 1989; Veenhuizen et al. 1991; Grummer 1993; Vázquez-Añón et al. 1994; Sevinc et al. 2003). Primary homeorhetic
adaptation of glucose metabolism in early lactation leads to increased gluconeogenesis in the liver to direct glucose into the mammary gland for lactose synthesis (Reynolds et al. 2003). If the degree of gluconeogenesis does not meet the increased needs of glucose in dairy cows in early lactation, hypoglycaemia, ketonaemia and ketonuria are likely to occur (Young 1977).

The hormonal activity of the thyroid gland has an important role in the transitional period for determining cell metabolism intensity, metabolism of lipids and carbohydrates and the lactation course itself by its thyroid hormones (Nikolić et al. 1997). A positive correlation was established between thyroid hormones in blood and energy balance (Reist et al. 2002) and a negative one between concentrations of triiodothyronine and thyroxine in blood and milk production (Nixon et al. 1988). Under the conditions of negative energy balance and high lipid mobilisation, the concentrations of thyroid hormones were reduced in the blood of dairy cows in the transitional period, with a markedly declined triiodothyronine in blood shortly before and after calving (Blum et al. 1983; Gerloff et al. 1986; Nikolić et al. 1997; Reist et al. 2002; Pezzy et al. 2003).

Kapp et al. (1979) noticed 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 blood.

The aim of the present study was to determine a correlation between blood concentration of thyroid hormones, lipids and glucose as well as the content of lipids in the liver of dairy cows in the transitional period.

Materials and Methods

Late pregnant and calved cows (n = 40) were chosen from a Holstein dairy herd (1,100 dairy cows) and divided into four groups: the first group (A) included late pregnant cows (n = 10) from the 10th to 4th day before calving; the second group (B) included late pregnant cows (n = 10) from the 4th to 1st day before calving; the third group (C) included clinically healthy puerperal cows (n = 10), whereas the fourth group (D) included cows with clinical symptoms of ketosis (n = 10). The liver and blood samples were taken from all the cows. The late pregnant cows were selected during a certain period on the basis of the time of artificial insemination and after detection of conception. Calved cows were selected as single selection in calving stalls. The diagnosis of ketosis was based on the clinical symptoms (reduced appetite, rumen atony, behavioural changes) and determined high concentrations of urinary ketone. The presence of ketone bodies in urine was examined using the Lestradet test (Rosenberger et al. 1979; Kégl and Gaal 1992). Healthy cows before and after calving did not show clinical symptoms of ketosis and urinary ketone bodies were not determined in those cows.

The cows were on average 4 - 6 years old, weighing 661.3 ± 24.3 kg in groups of cows in late pregnancy and 576.1 ± 23.35 kg in groups of cows in early lactation. There were 3 lactations with a mean milk yield of 7625.2 ± 329.17 l (calculated over 305 days) in the previous lactation. The experimental cows were kept in tie-up stalls in barn housing. The meal was prepared in a manner to suit the energy needs of animals in late pregnancy and early lactation. The cows in late pregnancy were fed a diet consisting of 3 kg lucerne hay, 3 kg wheat straw, 10 kg maize silage (30% DM), 4 kg lucerne haylage, 2 kg maize ear silage (68% DM), 0.5 kg dry sugarbeet pulp, 1.5 kg concentrate (30% CP). The dietary nutrient content for dairy cows in late pregnancy is given in Table 1.

The cows in early lactation were fed a diet consisting of 4 kg lucerne hay, 15 kg maize silage (30% DM), 8 kg lucerne haylage, 4 kg maize ear silage (68% DM), 2 kg dry sugarbeet pulp, 2 kg extruded soybean grains, 4.5 kg concentrate (30% CP). The dietary nutrient content for dairy cows in early lactation is given in Table 2.

Blood samples for serum were collected from the jugular vein (2 test tubes of blood taken per puncture, approximately 20 ml blood) from 10:00 h to noon or from 4 to 6 h after milking and feeding. The blood samples were allowed to clot spontaneously (approximately 15 min) at room temperature. The serum was then decanted at 1,000 g and preserved at -18 °C until analysed.

Shortly after blood sampling the liver was sampled through liver percutaneous biopsy using a biopsy instrument (Gaal 1995), following the modified method of Gaal after Hojovcova and Kacafirek...
The biopsy was performed at the right 11th intercostal region, approximately 2 cm below the horizontal line through the tuber coxae, with 3 - 5 cm long and 3 - 4 mm wide liver specimens. Triiodothyronine (T3) and thyroxine (T4) concentrations of the blood serum samples were determined following the 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. 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 in the blood serum CHOD-PAP method (041015), reagent Serbolab (Serbia) by means of microtitre reader MULTISKAN MCC/340 (Helsinki, Finland). All biochemical variables were assayed at the laboratory of Institute for the Application of Nuclear Energy (INEP) Zemun.

Liver tissue was pathohistologically tested for the lipid content at the Pathological Department of the Faculty of Veterinary Medicine in Belgrade. The liver specimens were fixed in neutral 10% formaldehyde solution. For pathohistological determining of lipids, sections were made using a freezing microtome stained with Sudan III method. Lipid contained in the hepatocytes was determined through computer image analysis (Software Q Win) made on the appliance (Leica Q 500 MC) at the Technical Faculty in Čačak.

The significance of differences of thyroid hormones, lipids and glucose concentrations in the blood serum and the content of lipids in the liver between the animal groups used in experiment were determined by ANOVA procedure. Data were expressed as means ± standard deviation (x ± 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 Discussion

The results of pathohistological assay made on the liver specimens denoted that lipid infiltration of hepatocyte was not determined in the pregnant cows, nor was it determined in the puerperal ones (< 10% of lipids). In ketotic cows various degrees of fatty liver were estimated. Results of the content of lipids in the liver in cows in the transitional period are shown in Fig. 1.

![Fig. 1. The content of lipids in the liver (%) in the groups of the cows in transitional period (A - group of cows from 10th to 4th days before calving; B - group of cows from 4th to 1st days before calving; C - group of puerperal healthy cows; D - group of puerperal ketotic cows). **p < 0.01 (between groups A, B, C and D)](image)

The content of lipids in the liver of healthy cows before and after calving was in the physiological range (around 5%) and was 5.30 ± 1.10% (group of cows from the 10th to 4th day before calving), 6.31 ± 1.18% (group of cows since the 4th to 1st day before calving) and 8.37 ± 1.24% (puerperal healthy cows). In the group of ketotic cows the content of lipids in the liver was 32.91 ± 13.23% and it was higher (p < 0.01) compared to groups healthy cows before and after calving.
Results of single values of the content of the lipids in the liver of ketotic cows are shown in Table 3.

Table 3. The content of the lipids (%) in the liver of ketotic cows

<table>
<thead>
<tr>
<th>Ordinal number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>(\bar{x})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of lipids</td>
<td>25.44</td>
<td>23.66</td>
<td>23.98</td>
<td>61.15</td>
<td>44.82</td>
<td>42.56</td>
<td>16.04</td>
<td>31.86</td>
<td>32.98</td>
<td>26.62</td>
<td>32.91</td>
</tr>
</tbody>
</table>

Results of biochemical estimation in the blood serum of groups of cows in transitional period are shown in Table 4.

Table 4. Selected metabolic profile variables (means ± standard deviation) in the groups of the cows in the transitional period (A - group of cows from 10th to 4th days before calving, B - group of cows from 4th to 1st days before calving, C - group of puerperal healthy cows, D - group of puerperal ketotic cows)

<table>
<thead>
<tr>
<th>Group</th>
<th>Late pregnancy</th>
<th>Puerperium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Glucose (mmol·l⁻¹)</td>
<td>2.94 ± 0.32ᵃ</td>
<td>3.12 ± 0.42ᵇ</td>
</tr>
<tr>
<td>FFA (mmol·l⁻¹)</td>
<td>0.27 ± 0.14ᵃ</td>
<td>0.54 ± 0.26ᵇᶜ</td>
</tr>
<tr>
<td>TAG (mmol·l⁻¹)</td>
<td>0.32 ± 0.04ᵃ</td>
<td>0.41 ± 0.03ᵇᶜ</td>
</tr>
<tr>
<td>Total cholesterol (mmol·l⁻¹)</td>
<td>1.75 ± 0.20ᵃ</td>
<td>1.71 ± 0.30ᵇᶜ</td>
</tr>
<tr>
<td>T₃ (mmol·l⁻¹)</td>
<td>2.58 ± 0.53ᵃ</td>
<td>2.31 ± 0.41ᵇᶜ</td>
</tr>
<tr>
<td>T₄ (mmol·l⁻¹)</td>
<td>50.68 ± 9.34ᵃ</td>
<td>45.21 ± 4.28ᵇᶜ</td>
</tr>
</tbody>
</table>

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

Correlation coefficients between biochemical variables in blood and the content of lipids in the liver of ketotic cows are shown in Table 5.

Table 5. Correlation coefficients between biochemical indicators in blood and the content of lipids in the liver of ketotic cows

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>T₃</th>
<th>T₄</th>
<th>FFA</th>
<th>TAG</th>
<th>Total Cholesterol</th>
<th>% lipids in liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-</td>
<td>-0.01</td>
<td>-0.17</td>
<td>-0.38</td>
<td>0.54*</td>
<td>0.17</td>
<td>-0.69*</td>
</tr>
<tr>
<td>T₃</td>
<td>-</td>
<td>-</td>
<td>0.73*</td>
<td>-0.50*</td>
<td>-0.19</td>
<td>-0.03</td>
<td>-0.19</td>
</tr>
<tr>
<td>T₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.10</td>
<td>-0.19</td>
<td>-0.06</td>
<td>-0.04</td>
</tr>
<tr>
<td>FFA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.64*</td>
<td>-0.34</td>
<td>0.51*</td>
</tr>
<tr>
<td>TAG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.42</td>
<td>-0.55*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.50*</td>
</tr>
<tr>
<td>% lipids in liver</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*\(p < 0.05\)

From Table 4 it can be seen that significant changes of most indicators in blood occurred in the group of ketotic cows, i.e. the group of cows with fatty liver.

One of the tested indicators in blood in this research was the glucose concentration in blood. In the group of puerperal healthy cows the glucose concentration in blood is significantly lower \((p < 0.05)\) compared to the groups of late pregnant cows, whereas hypoglycaemia was determined in ketotic cows. It was significant lower \((p < 0.01)\) compared to the groups of healthy cows before and after calving. Similar results were obtained by other authors (Veenhuizen et al. 1991; Vazquez-Anon et al. 1994). In our testing we found a negative correlation \((r = -0.69; p < 0.05)\) between the glucose concentrations in blood and
the content of lipids in the liver of ketotic cows. Also a significantly positive correlation
\((r = 0.54; p < 0.05)\) among glucose and TAG levels in blood should be compared to previous
results (Veenhuizen et al. 1991; Vázquez-Añón et al. 1994; Codorniga-Valino et al. 1997)
showing that during intensive lipid mobilisation and accumulated TAG in the
liver cells, their gluconeogenetic ability is reduced and consequently hypoglycaemia is
manifested in animals.

Energy metabolism of dairy cows in the 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 the increase of FFA concentrations
in blood (Veenhuizen et al. 1991; Vázquez-Añón et al. 1994; Reist et al. 2002; Overton
and Waldron 2004). There is an increase in blood FFA concentrations, which are bound to
albumin and transported to the liver. In the liver, they may be oxidized to \(\text{CO}_2\) or ketone bodies
or they are re-esterified to TAG. TAG are then combined with phospholipids, cholesterol and
apoproteins, with the production of lipoproteins and mainly lipoproteins of very low density
(VLDH) that carry TAG to various tissues (Holtenius 1989).

Significantly higher FFA concentrations were determined in the blood \((p < 0.01)\) of
ketotic cows compared to the groups of healthy cows before and after calving. Significantly
positive correlation \((r = 0.51; p < 0.05)\) among the FFA in blood and the content of lipids
in the liver was determined in this experiment, as well as negative correlation \((r = -0.64;
\quad p < 0.05)\) among the FFA and TAG concentrations in blood of ketotic cows. These results
were in accordance with the results of other authors (Veenhuizen et al. 1991; Reist et al.
2002), clearly showing that a significant increase of FFA concentrations in blood causes
an increase of the content of lipids in liver cells and a decrease of TAG concentrations in
blood.

In late pregnant cows the FFA concentrations in blood are higher than the
physiological range for cattle \((0.1 - 0.35 \text{ mmol·l}^{-1}, \text{Jovanović 1984}). However, the
FFA concentrations were significantly increasing \((p < 0.01)\) (among the group of cows
from the 10th to 4th day before calving and the group of cows from the 4th to 1st day
before calving). As the calving day was approaching lipid mobilisation began, from
four days to one day before calving. Similar results were obtained by Veenhuizen et
al. (1991) and Dyk et al. (1995).

In cows with fatty liver the TAG and total cholesterol concentrations in the blood declined
(Sevinc et al. 2003). TAG concentrations in the blood of ketotic cows were determined
to be significantly lower \((p < 0.01)\) than the values in groups of healthy cows before and
after calving. Significant negative correlation \((r = -0.55; p < 0.05)\) between the TAG
concentrations in blood and content of lipids in the liver of ketotic cows were determined
as well. The results unambiguously indicated that the blood TAG concentrations decreased
and proportionally their amount increased in the liver cells in which they accumulated.
These results are in accordance with observations by Holtenius (1989) and Sevinc et al.
(2003).

In this study the total cholesterol concentrations in blood of the tested cows were
within the lowest physiological limit \((1.3 - 6.0 \text{ mmol·l}^{-1}, \text{Jovanović 1984}). Contents
of the total cholesterol in the blood of ketotic cows were determined to be significantly
lower \((p < 0.05)\) than the levels in the healthy groups of cows before and after calving.
These results were in accordance with the results of other authors (Herdth 1983;
Gerloff et al. 1986; Holtenius 1989; Sevinc et al. 2003), indicating that in cases of
ketosis and fatty infiltration of liver cells in dairy cows, their ability to synthesise and
transport cholesterol is decreased.

Dairy cows in early lactation are in a state of metabolic stress, in order to meet the needs
of increased energy of the mammary gland and to adjust the neuro-endocrine system to the
new metabolic needs of the body (Bauman and Currie 1980; Nikolić et al.1997). One of
the endocrine factors are the thyroid hormones. In this study significantly lower ($p < 0.05$) $T_3$ and $T_4$ concentrations in blood were determined in the group of ketotic cows compared to the values of these hormones in the blood of healthy cows before and after calving. There is also a significant positive correlation ($r = 0.73; p < 0.05$) between the $T_3$ and $T_4$ levels in blood. Similar results have been obtained by other authors (Kapp et al. 1979; Gerloff et al. 1986; Nikolić et al. 1997; Stang et al. 1998b; Reist et al. 2002).

Romo et al. (1997) reported that in 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 accordence, Pezzy et al. (2003) assume that in dairy cows in early lactation, the state of hypothyroidism is present and it is the cause of the liver’s decreased 5'-deiodinase activity or the secretion of thyroid hormones in milk.

The intensity of oxidation in mitochondria of cells is closely linked with the functional state of the thyroid gland, so it is justifiably considered that the conditions of negative energy balance and the increased lipid mobilisation 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 blood (Kapp et al. 1979; Johannsen et al. 1993; Nikolić et al. 1997; Stang et al. 1998b).

The results confirm these opinions, values of $T_3$ and $T_4$ in blood of ketotic cows were significantly lower ($p < 0.05$) than those of the healthy ones. Within all tested cows with ketosis, the fat infiltration of liver was determined, especially in cows with severe fatty liver ($> 40\%$) the lowest values of the $T_3$ (< 1.00 nmol·l$^{-1}$) and those of $T_4$ (< 15 nmol·l$^{-1}$) were established (physiological range $T_3$: around 1.5 nmol·l$^{-1}$; $T_4$: 40 - 80 nmol·l$^{-1}$; Jovanović 1984). Negative correlation ($r = -0.50; p < 0.05$) between the $T_3$ and FFA concentrations in blood of ketotic cows is confirmed, as well.

Our investigations suggested that a hypothyroidal status was established in ketotic cows and that the blood concentrations of free fatty acids, triacylglycerols, total cholesterol and glucose served as major biochemical indicators in determining liver steatosis in the dairy cows in transitional period.
p < 0,05) a celkového cholesterolu (r = -0,55; p < 0,05) v krvi. Výsledky studie naznačují, že krávy v ketóze trpí hypothyreózou. Koncentrace volných mastných kyselin, glukózy, triacylglycerolů a celkového cholesterolu v krvi slouží jako významný biochemický indikátor steatózy jater u dojnic v období stání na sucho.

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