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Changes in Lipid Content of the Blood and Liver of Cows in Peripartal Period

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Abstract: Free fat acids, total lipids, triglycerides contained in the blood and, hystological changes in the liver of the healthy and ketotic cows in their peripartal period, were studied. Considerably higher content of the free fat acids in the blood serum of ketotic cows indicates a far more expressed tendency of mobilizing fats from the body depos in these cows than it happens with healthy ones. Thus, such a high level of lipo-mobilization may be one of the crucial mechanisms responsible for the occurrence of fat infiltration and liver cell degeneration. This is also manifested by a significantly positive correlation existing between the concentrations of free fat acids in the blood and those of fats in the liver (r=0.51).

Fairly reduced concentrations of triglycerides in the blood of ketotic cows suggest their accumulation in the liver cells. Thus, the existence of the negative correlation between the content of triglycerides in the blood and that of fats in the liver (r=0.55). Namely, investigations indicated that ketotic cows had a differing degree of fat liver, whereas no sign of liver fatness could be seen in the healthy cows neither before nor after calving

Key words: free fat acids, lipids, triglycerides, liver, ketotic cows, healthy cows

Introduction

In the peripartal period, significant changes occur in the metabolism of milking cows so that nutrients and energy needed for fetus growth and milk glands during lactation may be provided in sufficient amounts. High-pregnancy is indicated by a positive balance of energy, since the cows obtain much more energy with food than required. Part of this energy is utilized for fetus growth and, the rest is depoed in the form of fat tissue, which causes fatness in some cows. Initially, lactation of the highly milking cows represents an extremely stressful state. Namely, a sudden and enhanced activity of the milk gland inevitably leads to energy dis-equilibrium so that organism is forced to utilize its own reserves, in the first place, fats from body depos (Reid, 1983; Veenhuizen et al., 1991; Gaal, 1993).

On the last days of pregnancy and, particularly in the first weeks of lactation, free fat acids are mobilized from body reserves. Thus, their content in the blood is the basic indicator of the degree of lipomobilization as well as of that of the negative energy balance (Henrichion et al., 1977; Reid et al. 1979; Staufenbiel et al. 1993).

At a considerably high degree of fat mobilization, high amounts of free fat acids enter into the liver and impair its energy equilibrium. Then, esterified fat acids are retained in the form of triglycerides and hapatocytines, ketogenasis processes become more intensive so that fat liver and ketosis take place consequently (Grohn and Laimberg, 1985; Veernhuizen et al., 1991; Johnson et al. 1991).

It is in this period that total lipids increase in the blood, particularly due to a considerably higher fraction of the free fat acids, which reflects more intensive lipolitic processes (Husveth et al., 1982; Shamac et al. 1993).

In contrast to the concentration of total lipids in the blood that increases during peripartal period of cow growth, that of triglycerides however decreases in the period after calving. In other words, in the cows with fat liver, triglycerides are less transported from the liver to the blood. It is because they remain in the liver for a longer time so that the content of lipoproteins, rich in triglycerides in the blood serum, decreases to a great extent (Herdi et al., 1983; Gaai, 1993; Shamanac et al., 1991).

Sandra Bertics et al. (1992) considered fat liver in milking cows to be a meatabolic disease. The disease takes place when the free fat acids and esterification intensify to such an extent that liver cells are no more able to oxidize or transport fat acids in the form of triglycerides, as the constituent of low density.

Material and Method

Randomly chosen high pregnant and newly calved cows (n=40) from the Holstein breed were split up into 2 groups. Clinically healthy and pregnant cows (m=20) were in the first group. The specimens of the liver tissue and blood were taken in high pregnancy from each cow. The animals from this group were

subdivided into 2 groups. Tissue and blood specimens were taken from the 10th to the 4th day before calving in the first group of cows (n=13) and from the 4th to the 1st day before calving in the second group of cows, consisting of 20 newly calved cows from the 1st to 4th day after calving. In view of health condition, the animals selected for the experiment,were divided into 2 groups, with the first consisting of the clinically healthy cows (n=10) and the second consisting of those with clinical symptoms of ketosis. The specimens of blood tissue and liver were taken from all the cows.

The blood of all test cows was taken by puncturing jugular vein at about the same time, from 10 to12 pa.m., ie. 4-6 hours after milking and feeding. After spontaneous coagulation at room temperature, the blood serum was separated by means of centrifugation with 3000 revolutions per minute. The concentration of the free fat acids in the blood serum was determined colorimetrically after Ducombeu (1966). Concentration of total lipids in the blood serum was determined photometrically using vanillin, whereas that of triglycerides contained in the blood serum was determined enzymatically (Merieux ref. 6.123.6). The specimens of liver tissue were taken from each cow by means of percutaneous biopsy of liver, then, using a modification of Gaal's method after Hajovcova-Kacifirex (1967). Liver specimens were taken by means of a biopsy instrument (Davieset and Jebbert, 1981; Gaal, 1995) immediately after blood sampling.

In all the cases, biopsy was performed in 10th inter-rib portion. The amount of fats in the liver were determined stereometrically, computing volume density. Statistical analysis of the data was made at the Computer Centre at the Institute of Statistics, using the method of repeated measurement in SAS, GLM and REG.

Results and Discussion

The results of concentrations of the free fat acids in the blood serum of the healthy cows before calving and, those of the healthy and ketotic ones after calving, are given in tab.1.

Tab.1 Concentrations of free fat acids in the blood serum (mmol/l) of cow groups before and after calving

The time of testing		Group	n	$\bar{x}$	SD	CV	Sx	VŠ
High pregnancy	1-10 days before calving	A	20	0.40	0.25	64.41	0.05	0.10-0.95
	5-10 days before calving	A1	13	0.27	0.14	53.09	0.04	0.10-0.50
	1-4 days before calving	A2	7	0.54	0.26	41.11	0.09	0.25-0.95
Puerperium	Healthy cows	B	10	0.46	0.10	22.63	0.03	0.32-0.64
	Ketotic cows	C	10	0.74	0.12	16.82	0.03	0.55-0.90

$p< 0.01$, A:A_2; A_1:A_2; A_1:B; A:C; A_2:C; C:B $p< 0.05$, A:A_1; A_2:B

From Tab.1, it can be seen that the average concentration of the free fat acids was the highest in ketotic cows amounting to 0.74 mmol/l. The obtained value was statistically significantly higher ($P<0.01$) than the average one for free fat acids in the blood of the healthy cows before and after calving.

The results are in full agreement with those obtained by the other authors, pointing to significantly higher concentrations of free fat acids in the blood of ketotic cows compared to those in the healthy ones. The main cause of this was enhanced esterification in the liver cells, hence the occurrence of "fat" liver, subsequently (Kauppinen, 1984; Gerloff, 1986; Damnjanovic, 1993; Veenhuizen et al., 1991; Gaal, 1993). This is also confirmed by a positive correlation between concentrations of free fat acids and triglycerides in the liver cells (Gaal, 1993). Such a positive correlation was also stated in the present experiment and amounted to $r=0.51$. Therefore, it is beyond doubt that higher concentrations of the fat free acids in the blood lead to higher amounts of triglycerides in the liver cells. Newly synthetisized triglycerides are depoed in the liver which becomes characteristically "fat" in the cows affected by ketosis.

Investigation results of the concentrations of total lipids in the blood serum of the healthy cows before and after calving, as well as those regarding ketotic ones, are given in tab.2.

Tab.2. Concentration of total lipids in the blood serum (g/l) of the cows before and after calving

The time of testing		Group	n	$\bar{x}$	SD	CV	Sx	VŠ
High Pregnancy	1-10 days before calving	A	20	3.51	0.47	13.60	0.10	2.52-4.30
	5-10 days before calving	A1	13	3.44	0.54	15.74	0.15	2.52-4.30
	1-4 days before calving	A2	7	3.64	0.32	8.92	0.12	3.20-4.00
Puerperium	Healthy cows	B	10	4.26	0.96	22.57	0.30	2.30-6.35
	Ketotic cows	C	10	3.84	1.08	28.34	0.34	2.12-5.60

$p<0.01$, A:B; A_1:B

From tab.2, it can be seen that statistically significantly higher values ($p<0.01$) of the total lipids existed in the blood of healthy cows before calving, indicating the highest degree of lipomobilization from the body depos at the very start of lactation. These results are well substantiated by numerous authors, focusing on the highest degree of lipomobilization from the body depos during the first two weeks of lactation, just at the time of increased milk production (Baird et al., 1974; Reid et al., 1981; Sandra Bertics et al., 1992; Shamanac et al., 1991).

Lipids indicated slightly higher values of blood ($x=3.84$ g/l) in ketotic cows than in those before calving and, relatively lower ones compared to the values of total lipids in healthy cows after calving.

These data clearly show that fractions of the total lipids and triglycerides remain in the liver cells of ketotic cows.

Concentrations of the total lipids in the blood serum of the healthy cows before calving and those regarding healthy and ketotic ones after calving are presented in tab.3.

Tab.3. Concentrations of triglycerides in the blood serum (mmol/l) of the cows before and after calving

The time of testing		Group	N	$\bar{x}$	SD	CV	Sx	VŠ
High pregnancy	1-10 days before calving	A	20	0.35	0.05	15.92	0.01	0.27-0.48
	5-10 days before calving	A1	13	0.32	0.04	12.42	0.01	0.27-0.39
	1-4 days before calving	A2	7	0.41	0.03	9.55	0.01	0.37-0.48
Puerperium	Healthy cows	B	10	0.35	0.04	11.51	0.01	0.30-0.44
	Ketotic cows	C	10	0.27	0.03	12.15	0.01	0.21-0.32

$p<0.01$, A:A_1; A:A_2: A_1:A_2; A_2:B; A:C; A_1:C; A_2:C: B:C $p<0.05$, A_1:B

From $_{tab}$.3, it is clear that average concentration of triglycerides in the blood serum of ketotic cows amounted to x=0.27 mmol/l and, as such, is statistically markedly lower ($p<0.01$) than that of the healthy cows after calving (x=0.35 mmol/l). It should also be emphasized that the content of triglycerides in the blood of ketotic cows is much lower ($p<0.01$) than that in the highly pregnant cows. This finding is in full agreement with results obtained by other authors (Baird et al. 1979; Reid et al. 1979, 1981; Gaal, 1983).

Nearly all the authors beleive that, in the case of a relatively low content of triglycerides in the blood serum, further decrease of triglycerides is most likely to be the consequence of a fat infiltration and degeneration of liver cells. This is substantiated by the experimentally established statistically significant negative correlation (r=-0.55) between concentration of triglycerides in the blood and that of lipids in the parenchyma of liver in the ketotic cows. Therefore, the lower concentration of triglycerides in the blood, the higher their amount in the liver cells, in which triglycerides may accumulate to such an extent that the cell functional and morphological integrity may severely be degraded.

The results of parahystological surveys of the samples of liver tissue taken through biopsy have shown that a varying degree of fat infiltration and liver cell degeneration exists only in ketotic cows, whereas fat accumulation in the hepatocytes either before or after calving is insignificant in the healthy ones.

The results of fat content in the liver cells in ketotic cows are given in tab.4.

Tab. 4. Content of Fats in the Liver of Cows Affected by Ketosis ($\mu m^3/100\mu m^3$) in per cent

No.	1	2	3	4	5	6	7	8	9	10	$\bar{x}$
% fats in the liver	25.44	23.66	23.98	61.15	44.82	42.56	16.04	31.86	32.98	26.62	32.91

According to the data given above, the average amount of lipids in the liver of ketotic cows amounted to 32.91% ranging from 16.04 % up to maximal 61.15%.

These data fully comply with those obtained by other authors who also established "fat" liver of a differing intensity (Reid et al. 1979; Gaal, 1993).

Based on everything done, it may be assumed with certainty that lipomobilization from body depos causes fat infiltration and liver cell degradation. Moreover, lower content of triglycerides in the blood indicate that they are retained in the liver cells and, thus, less transported from the liver.

Conclusion

On the basis of everything done, the following conclusions may be drawn:

- fairly higher concentration of free fat acids in the blood serum of ketotic cows shows that fat mobilization from body depos is much more expressed in these cows than in the healthy ones, suggesting rather a high degree of lipomobilization, as a key mechanism responsible for the occurrence of fat infiltration and liver cell degeneration.
- considerably lower concentrations of triglycerides in the blood of ketotic cows in relation to the healthy ones unanimously show their being accumulated in the liver cells.

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PROMENE KONCETRACIJA LIPIDA U KRVI I JETRI KOD KRAVA U PERIPARTALNOM PERIODU

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Rezime

Ispitivane su koncetracije slobodnih kiselina, ukupnih lipida, triglicerida, u krvi I histološke promene u jetri kod zdravih I ketoznih krva u peripartalnom periodu. Rezultati ovih ispitivanja su ukazala da značajno veća koncetracija slobodnih masnih kiselina u krvnom serumu ketoznih krava pokazuje da je kod njih daleko izraženija mobilizacija masti iz telesnih depoa nego kod zdravih životinja, što sugeriše da visok stepen lipomobilizacije može da bude jedan od ključnih mehanizama odgovornih za nastajanje masne infiltracije i degeneracije ćelije jetre. U prilog tome je i značajno pozitivna korelacija između koncetracija slobodnih masnih kiselina u krvi I sadržaju masti u jetri (r=0,51).

Značajno niže koncetracije triglicerida u krvi ketoznih krava u odnosu na zdrave, ne dvosmisleno ukazuje na njihovo nakupljanje u ćelijama jetre. U skladu s tim je utvrđena negativna korelacija između koncetracije triglicerida u krvi I sadržaja masti u jetri (r=-0,55). Naime, patohistološkim ispitivanjem je utvrđena masna jetra različitog stepena kod ketoznih krava, dok kod ni jedne zdrave krave pre I posle telenja nije utvrđeno značajnije nakupljanje masti u jetri.