The effect of gestation and lactation of dairy cows on lipid and lipoprotein patterns and composition in serum during winter and summer feeding

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ABSTRACT

Studies were carried out on lipid and lipoprotein fractions in the serum of cows during summer and winter feeding. The experimental group comprised 32 Black-and-White cows in the final stage of pregnancy (2-3 weeks before calving) or in the 6th week of lactation.

Total serum lipid (2.51 g/l) and cholesterol (2.51 mmol/l) concentrations were significantly lower (P < 0.01) in cows subjected to drying than in lactating animals (3.29 g/l and 3.86 mmol/l, respectively), irrespective of the feeding period. In contrast, the serum triglycerides concentration was 0.34 mmol/l in cows before calving and twice as high than during lactation. It was also shown that HDL constituted the main fraction, and that HDL in cows prior to calving represented about 74% of serum lipids, increasing to about 80-85% during lactation. The LDL concentration was lower, representing, on average, 22% in dry cows, and 13-15% in the 6th week of lactation. Prior to calving, triglycerides represented over 40% of LDL, 32% was represented by cholesterol. In the peak of lactation the TG fraction in LDL decreased to 14-19%, while that of cholesterol increased to over 45%.

KEY WORDS: dairy cow, blood serum, lipids, lipoproteins

INTRODUCTION

Lipids are transported in blood by lipoproteins, which are lipid-protein complexes. Lipoproteins are characterised by different structures, places of origin and physiological functions, depending on the share of specific proteins.

Secretion and composition of lipoproteins in ruminants are the main factors controlling lipid metabolism in tissues and organs (Bauchart, 1993). Lipoproteins

in cows play a special role in milk fat synthesis (Grummer, 1993; Yamamoto et al., 1995; Fujita et al., 1996), but they also affect steroidogenesis (Kampl et al., 1990; Hawkins et al., 1995).

Cattle lipoprotein fractions have been defined as VLDL, LDL and HDL. Very low density lipoproteins (VLDL, d=0.950-1.006 g/ml) transport mainly endogenic triglycerides synthesized in the liver. Cow liver capacity to secrete triglycerides in VLDL is regarded as very low compared to its ability to take up and esterify fatty acids (Mazur et al., 1988). Generally, less than 1 % of serum lipids circulate in the VLDL fraction of lactating cows (Schweigert, 1990). It is assumed that low density lipoproteins (LDL, d=1.006-1.068 g/ml) and high density lipoproteins (HDL, d=1.068-1,21 g/ml) constitute the major lipoprotein fractions in cows (Mazur et al., 1988; Schweigert, 1990). Their composition, distribution and metabolism are not constant and have not been fully defined as yet. These parameters can be modified by cow physiology and quality of feeds (Mazur et al., 1988; Schweigert, 1990; Van Den Top et al., 1995; Fujita et al., 1996). Mazur et al. (1988) and Özpinar et al. (1988) determined the concentration of VLDL and LDL fractions in cows 2 and 6 weeks after calving and found an invariably low level of VLDL and an increase in LDL lipoproteins. The lipid composition of the LDL fraction was also modified; the cholesterol level at the beginning of lactation was almost 3 times lower than 6 weeks after calving (Mazur et al., 1988). In other studies (Van Den Top et al., 1995) the LDL level did not change between the end of pregnancy and the initial phase of lactation, while there was an increase of HDL lipoproteins. A similarly increased level of HDL at the peak of lactation was observed by Fujita et al. (1996), and the amounts of circulating HDL as well as LDL were higher in autumn than in summer.

The small number of studies conducted on the subject reveal that low feeding levels may decrease lipoprotein concentrations (Fujita et al., 1996), while various feed additives have different effects on lipoprotein metabolism (Durand et al., 1992; Sklan et al., 1994; Burke et al., 1996). Intravenous infusions of methionine (Durand et al., 1992) to lactating cows favoured hepatic LDL secretion, but did not affect scrum cholesterol and LDL levels. Addition of cholesterol to tallow- or soyabean oil-rich diets did not modify VLDL metabolism, but increased the plasma concentration of LDL (Bauchart et al., 1996). According to some authors (Bauchart et al., 1996, Burke et al., 1996) polyunsaturated fatty acids of the diet, especially linoleic acid, not only did not lower, but increased hypercholesterolaemia in cows. On the other hand, other studies (Sklan et al., 1994) showed that an addition of protected fats in the form of fatty acid calcium soaps did not change the cholesterol concentration in the serum of cows.

The results of studies carried out to date do not give an unequivocal answer as to which factors affect lipoprotein metabolism in cows. Moreover, the majority of available data (Mazur et al., 1988; Schweigert, 1990; Durand et al., 1992; Van Den

Top et al., 1995; Fujita et al., 1996) originated from model experiments, and not from studies carried out under conditions of commercial farming.

In view of this, studies were undertaken to determine the dependence between late gestation and peak lactation and the content of some lipid classes, LDL and HDL lipoprotein fractions, in serum during summer and winter feeding.

MATERIAL AND METHODS

Animals and feeding

Studies were performed on multiparous Black-and-White cows of average body weight 518 (± 29.3) kg, 4th-6th lactation, during summer and winter feeding (4 x 8 cows). The mean milk production of the cows was 4300 kg for 305 days of lactation. The study encompassed dairy cows 14-20 days prior to calving (dry cows) and 38-42 days after calving (lactating cows).

In the summer, the cows grazed on pasture (May-July), and their diet was supplemented with ground grain mixture (1.6 kg), or they were given meadow hay (3 kg, in August-October) and a mixture of ground grain (3 kg). The amount of consumed pasture grass was estimated based on the analytical method by Różycki (intake of grass was predicted once daily from data according to control fields before and after grazing). The cows received fodder straw from oat *ad libitum* throughout the summer feeding period. In winter the animals were fed a diet containing wilted grass silage (20 kg), meadow hay (3 kg) and a mixture of ground grain (3 kg). Feed rations for the cows were balanced according to traditional Polish standards (Chomyszyn et al., 1993). When milk production exceeded 14 kg per day, the diet was supplemented with 0.5 kg of a ground grain mixture per kg of milk. The composition of the cercal mixture was (%): ground wheat 35, ground oats 35, ground barley 27, fodder phosphate 1, limestone 1, salt 0.5, and premix B 0.5.

Sampling procedures and analytical methods

Blood was examined in May/June and September/October (summer feeding), and in December/January and March/April (winter feeding). Blood samples were collected from the jugular vein before the morning feeding. Serum was separated by centrifugation at 3000 x g for 30 min. After centrifugation, 0.02 % EDTA was added to serum samples. All samples were stored at 4°C.

Lipoprotein fractions (LDL and HDL) were separated by flotation in a sodium bromide (NaBr) gradient, using ultracentrifugation at 5°C (Beckman L7-55, type 50.3 Ti-rotor), according to the method described by Mills et al. (1989). Low den-

sity lipoproteins (LDL, d=1.006-1.068 g/ml) were centrifuged at 140 000 x g for 24 h. High density lipoproteins (HDL, d=1.068-1.210 g/ml) were isolated for 48 h at 140 000 x g.

Total lipid content was determined in the scrum and in LDL and HDL fractions using a "Biochemtest" diagnostic test (Poland, Cat. No. 1782 12 144). Total cholesterol and triglycerides (TG) were determined using enzymatic methods with tests produced by Alpha Diagnostics (Poland, Cat. No. C6509-400 and T6531-400).

The chemical composition of the feeds was determined using conventional methods.

Statistical analysis

The results were analysed with the variance method in an orthogonal bifactorial system, and multiple range Duncan's test using the Statgraphics program (ver. 2.6 1985/87).

RESULTS

The diet (DM) consisted of 68-77% forages and 32-23% concentrates. The chemical composition of feeds and nutritive value of daily rations are presented in Tables 1 and 2.

Analysis of nutrient concentration in dry matter of the daily rations showed that the level of energy (MJ) was similar (Table 2). On the other hand, the crude protein content in the initial phase of summer feeding (May-July) was higher than the protein requirements of cows according to Polish standards (Chomyszyn et al., 1993).

Feeds	Dry matter (DM)	Ash	Crude protein	Ether extract	Crude fibre	NE ¹ , MJ
Pasture grass ²	21.3	2.19	3.69	0.85	4.58	1.19
Pasture grass ³	22.6	2.29	3.31	0.91	5.43	1.30
Grass silage	35.7	3.35	5.40	1.75	10.80	2.20
Meadow hay	87.5	7.61	10.06	2.12	26.13	3.20
Cereal mixture	87.9	6.91	11.50	2.35	6.22	6.20

Chemical composition of feeds, %

¹ NE – net energy (MJ) calculated from starch value

2.3 pasture grass was analysed in June² and September³

Item	Summe	r feeding	Winter feeding
	May - July	August - October	November - Apri
Intake of kg DM/day			,
pasture grass	10.7	6.8	-
grass silage	-	-	7.2
meadow hay		2.6	2.6
compound mixture	3.2	4.4	4.4
total	13.9	13.8	14.2
Nutritive value, in DM			
crude protein, %	16.2	13.5	13.8
ether extract, %	3.4	3.2	3.7
crude fibre, %	18.0	19.7	22.9
NE, MJ/kg DM	6.0	5.9	6.0
Nutrient supply:			
crude protein, g/d	2252	1865	1955
ether extract, g/d	474	448	526
crude fibre, g/d	2508	2713	3253
NE, MJ ¹	83.8	81.4	84.6

Feed intake, nutritive value and nutrient supply of daily diets during summer and winter feeding

¹ see Table 1

The concentration of total serum lipids of dry cows was highly significantly lower (by 25% on average) than in cows in the 6th week of lactation, irrespective of the feeding period (Table 3). The total cholesterol concentration was 34% lower before calving, while TG levels in dry cows were almost two times higher than during lactation.

Lipoprotein fractions separated by ultracentrifugation showed that HDL was the major fraction of serum of dairy cows, LDL was present in much lower levels (Figure 1). The concentration of lipoproteins differed during pregnancy and lactation, with more pronounced changes observed in the LDL fraction. In cows before calving, this fraction transported 22% of serum lipids on the average, while in the 6th week of lactation it transported 13-15%. The HDL fraction was lower (by 12% on average) in dry cows compared with the concentration found at the peak of lactation.

It was also shown that apart from quantitative changes, the lipid composition of lipoproteins also depended on the physiological state of the animal. In cows before calving, TG constituted over 40% of the LDL, irrespective of the feeding period (Table 4). At the peak of lactation, the TG fraction of LDL decreased to 16%, on average. In contrast, the cholesterol fraction in LDL was significantly lower in dry

TABLE 2

		Indices		Indices	ces		
Feeding system	Period of sampling, month	total lipids g/l	ids g/l	TG mmol/l	lo]/]	total cholesterol mmol/l	esterol I/I
		dry ¹	lactating ²	dry	lactating ²	dry	lactating ²
Summer	I> X	2.68 ± 0.43 ^A 2.50 ± 0.35 ^A	3.44 ± 0.35^{B} 3.74 ± 0.30^{B}	$0.37 \pm 0.04^{\text{A}}$ 0.34 + 0.05^{\text{A}}	0.18 ± 0.03^{B} 0.16 + 0.03^{B}	$2.79 \pm 0.31^{\text{A}}$	3.96 ± 0.30^{B} 3.80 ± 0.36^{B}
Winter	IX A	$2.61 \pm 0.26^{\text{A}}$	3.37 ± 0.29^{B}	$0.34 \pm 0.03^{\text{A}}$	0.15 ± 0.03^{B}	$2.53 \pm 0.14^{\text{A}}$	3.94 ± 0.51^{B}
Total	2 1	$2.51 \pm 0.34^{\text{A}}$	$3.13 \pm 0.24^{\circ}$ $3.29 \pm 0.30^{\circ}$	$0.34 \pm 0.04^{\circ}$	0.16 ± 0.03^{B}	$2.51 \pm 0.18^{\circ}$	$3.74 \pm 0.31^{\circ}$ $3.86 \pm 0.35^{\circ}$
 2-5 weeks before calving 6th week of lactation A^B – different capital letter Concentration and compose 	~ 2.5 weeks before calving $^{2.60}$ week of lactation $^{3.8}$ – different capital letters denote significant differences (P ≤ 0.01) between dry and lactating cows within a particular month and class of lipids $^{3.8}$ – different capital letters denote significant differences (P ≤ 0.01) between dry and lactating cows within a particular month and class of lipids $^{3.8}$ – different capital letters denote significant differences (P ≤ 0.01) between dry and lactating cows within a particular month and class of lipids Concentration and composition of lipids associated with serum LDL fraction, mean $\pm SD$	ifficant differences s associated with se	(P ≤ 0.01) betwee erum LDL fraction	n dry and lactating 1, mean ±SD	g cows within a par	ticular month and	l class of lipids TABLE 4
				Indices	ces		1
Feeding system	Period of sampling, month	total lipids g/l	ids g/l	TG mmol/l	io]/I	total cholesterol mmol/l	csterol
		dry	lactating ²	dry ¹	lactating ²	dry'	lactating ²
Summer	۲۱ XI	0.65 ± 0.07^{Aa} 0.55 ± 0.06^{Ab}	0.54 ± 0.06^{Bd} 0.49 ± 0.05^{Bd}	0.32 ± 0.03^{As} 0.25 ± 0.03^{Ab}	0.08 ± 0.01^{Bd} 0.08 ± 0.01^{Bd}	$0.48 \pm 0.04^{\text{A}}$ $0.46 \pm 0.02^{\text{A}}$	0.58 ± 0.05^{B} 0.59 + 0.07 ^B
Winter	IX A	0.56 ± 0.04 ^{Ab}	0.48 ± 0.03 Bd	0.27 ± 0.02 Ab	0.11 ± 0.01^{Bc}	$0.47 \pm 0.02^{\text{A}}$	0.55 ± 0.09^{B}
Totał	- 1	$0.57 \pm 0.03^{\text{A}}$	0.42 ± 0.05^{8} 0.48 ± 0.05^{8}	$0.27 \pm 0.04^{\circ}$	0.09 ± 0.01^{8}	$0.46 \pm 0.04^{\circ}$	0.55 ± 0.07^{B}
^{1,2} see Table 3 ^{A,B} – different capital le ^{a,b,e,d} – different small l	upital letters denote sign small letters denote sign	etters denote significant differences ($P \le 0.01$) between dry and lactating cows within a particular month and class of lipids etters denote significant differences ($P \le 0.05$) between months within particular class of lipids	$(P \le 0.01)$ betwee $(P \le 0.05)$ betwee	n dry and lactating n months within p	g cows within a par articular class of li	ticular month anc pids	l class of lipids

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LIPIDS AND LIPOPROTEIN IN COW'S BLOOD SERUM

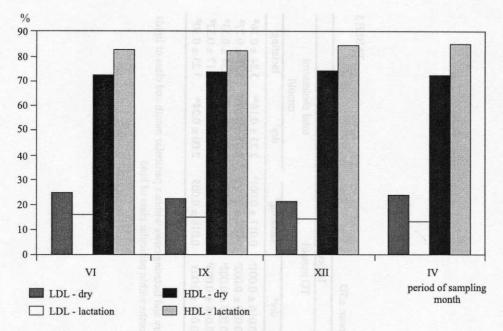


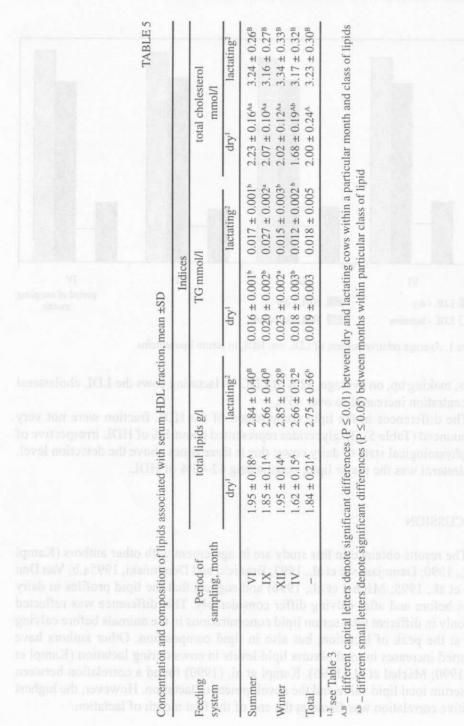
Figure 1. Average relative percent of LDL and HDL in serum lipoproteins

cows, making up, on average, 32% of LDL. In lactating cows the LDL cholesterol concentration increased to over 45%.

The differences in the lipid composition of the HDL fraction were not very pronounced (Table 5). Triglycerides represented about 1% of HDL irrespective of the physiological state of dairy cows; this is three times above the detection level. Cholesterol was the major lipid, constituting 42-45% of HDL.

DISCUSSION

The results obtained in this study are in agreement with other authors (Kampl et al., 1990; Damnjanovič et al., 1993; Bronicki and Dembiński, 1995a,b; Van Den Top et al., 1995; Máchal et al., 1996) and suggest that the lipid profiles in dairy cows before and after calving differ considerably. This difference was reflected not only in different total serum lipid concentrations in the animals before calving and at the peak of lactation, but also in lipid composition. Other authors have reported increases in total plasma lipid levels in cows during lactation (Kampl et al., 1990; Máchal et al., 1996). Kampl et al. (1990) found a correlation between the serum total lipid value and the development of lactation. However, the highest positive correlation was found at the end of the first month of lactation.



LIPIDS AND LIPOPROTEIN IN COW'S BLOOD SERUM

The high TG concentration observed 2-3 weeks before calving and the steep fall afterwards suggests that TG play an important role in the synthesis of milk fat (Table 3). Other authors have also observed a decrease of serum TG levels after calving (Schweigert, 1990; Grummer, 1993; Bronicki and Dembiński, 1994; Yamamoto et al., 1995). The high requirements of the mammary gland for lipids may be met by an increase of free fatty acids, which is associated with a decrease in TG levels (Vazquez-Anon et al., 1994). Many authors assume that the ratio between free fatty acids and TG can be used as an index of lipid retention in the liver, reflecting disturbances in fat metabolism (Holtenius, 1989; Bronicki and Dembiński, 1995a) and causing irreversible fatty degradation of the liver (Andrews et al., 1991; Bronicki and Dembiński, 1998). Given that fat metabolism is strictly related to hormone activity (Bronicki and Dembiński, 1995b; Hawkins et al., 1995), these changes lead to disturbances in cholesterol biosynthesis by the liver and in steroid transformations, negatively affecting a cow's fertility and health after calving.

It can be assumed that the observed significant increase of cholesterol levels in lactating cows compared with the period before calving was related to increased requirements for cholesterol of the glands producing steroid hormones. This suggestion is confirmed by the studies of Mazur et al. (1988), Kampl et al. (1990), Schweigert (1990), Bronicki and Dembiński (1995b), and Van Den Top et al. (1996) who observed the lowest cholesterol levels in the calving period, followed by a systematic increase of its concentration, up to a peak value 6-8 weeks after calving. These changes of cholesterol concentration in the blood serum of cows were observed in animals with no clear symptoms of lipid metabolism disturbances (Doležal et al., 1991; Máchal et al., 1996). In cows suffering from impaired liver function, the concentration of total lipids and total cholesterol in serum was lower (Kampl et al., 1990; Máchal et al., 1996).

It is interesting that there were no noticeable differences in lipid levels between the summer and winter feeding periods. This might have resulted from the diets having been based on either green forages (pasture) or conserved feedstuffs (grass silage and hay). The daily ration (DM) consisted of 68-77% forages. In addition to this, a feed mixture of the same composition was used throughout the entire experiment. Although the share of mixture I during basic feeding (May-July) represented 23% of the dry feed dose, and 32% in the other periods, rations were characterised by a similar content of crude fat. Its concentration in DM of the diet was from 3.2 to 3.7%. It seems that this factor could not have significantly differentiated lipid levels in the serum of cows. This is confirmed by a variable effect of added fat in cow diets on the level of some lipids in serum. Sklan et al. (1994) did not find any effect of added fat on cholesterol levels. In other studies (Marty and Block, 1992; Burke et al., 1996), addition of fat increased the cholesterol concentration. The reasons for these discrepancies are unknown. On the other hand, adding fat usually increases the serum TG level (Marty and Block, 1992; Sklan et al., 1994). Given that in out study feed rations were not modified with any additives, lipid metabolism was affected more by late pregnancy and peak of lactation. This suggestion is supported by the pattern of lipoprotein changes in the serum. Irrespective of the feeding period, the LDL and HDL fractions were noticeably different in the dry period and at peak of lactation. LDL decreased significantly in lactating cows. Schweigert (1990) also examined lipoproteins in dairy cows and observed a rapid decrease of the LDL fraction from over 20% before delivery to only 4% at calving. LDL during lactation increased, however; by 6 weeks after calving the LDL level was 25% lower than during pregnancy. Because lipid and lipoprotein levels were determined in each cow only two times (2-3 weeks before calving and 6 weeks after calving), it was not possible to observe this rapid decrease of LDL during calving. This, however, does not change the fact that the LDL fraction changes noticeably in cows in the calving period (Özpinar et al., 1988; Kampl et al., 1995). The decrease in LDL levels may be compared with the changes in the Ig G1 fraction in the serum of cows during calving (Schweigert, 1990).

In contrast with the LDL level, the HDL concentration in lactating cows was significantly higher than in dry animals. According to Van Den Top et al. (1995) an increase in HDL concentration should reflect changes taking place in the levels of VLDL. Their catabolism is associated with the transfer of surface material (free cholesterol, phospho-lipids and Apo-C) to HDL. Although VLDL were not determined in this study, it was roughly assessed that their percentage was low and in cows before calving VLDL may comprise about 4% of serum lipids, while their percentage during lactation only about 1%. Studies by Schweigert (1990) suggest that the VLDL concentration in cows 3-4 weeks before calving represented some 3% of serum lipids, and increased to 5% a week before delivery, to decrease again to less than 1%, remaining on this low level throughout lactation. Mazur et al. (1988), Özpinar et al. (1988) and Van Den Top et al. (1995) observed similar changes in VLDL levels during pregnancy and lactation in cows. The lack of differences in lipoprotein levels in summer and winter observed in this study does not confirm the conclusions of Fujita et al. (1996) on higher amounts of circulating LDL and HDL during lactation in autumn than in summer. According to these authors, these differences resulted from decreased metabolic activity in summer, caused by higher temperatures.

Data on the lipid composition of lipoproteins seem interesting. HDL composition was only slightly modified by the physiological state of cows or feeding system. The high percentage of cholesterol in this fraction confirms the results of Van Den Top et al. (1995) and Herdt and Smith (1996) that 80-90 % of serum cholesterol was transported in dairy cows by HDL molecules. The LDL fraction showed more pronounced changes during gestation and lactation. Different concentrations of TG and cholesterol in dry and lactating cows seem to be reflected in their levels

in the LDL fraction. TG accounted for over 40% of the LDL fraction in dry cows. Similarly, a higher level of TG in the LDL fraction in the antepartum period and a lower level during lactation were found by Bobowicc et al. (1997), while in peak of lactation the main lipid in the LDL fraction was cholesterol (over 45 %). In view of these data it may be suggested (Schweigert, 1990) that the LDL fraction plays a key role in cholesterol metabolism. Cholesterol bound to LDL molecules is an important source of this lipid for all cells of the body. Studies *in vitro* as well as *in vivo* showed that inhibition of cholesterol supply to the steroid-producing glands, caused by a decrease in LDL, resulted in lower synthesis of steroid hormones. Hawkins et al. (1995) suggested that due to progesterone deficiency, abortions may reach levels as high as 20-45% in ruminants.

The quantitative and qualitative changes in the LDL fraction observed in our experiment confirm the concept (Mazur et al., 1988; Schweigert, 1990) that metabolic disturbances after calving and fertility problems in cows may be related to the time required by the animal for its LDL concentration to increase to its normal level.

CONCLUSIONS

The obtained results revealed that in the calving period of cows, the pattern of lipid changes was subject to considerable variations independent of the feeding system. This was reflected not only in the serum lipid concentration, but also in the profile of the lipoprotein fraction.

The proportions and composition of the lipoprotein fraction of LDL and HDL depended most of all on the physiological state of the cows, with pregnancy and lactation having a more pronounced effect on quantitative and qualitative changes of LDL lipoproteins.

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STRESZCZENIE

Wpływ okresu ciąży i laktacji na skład lipidów i lipoprotein w surowicy krwi krów w okresie żywienia letniego i zimowego

W surowicy krwi 32 krów cb w końcowym okresie ciąży (2-3 tyg. przed porodem) oraz w 6 tygodniu laktacji, podczas żywienia letniego i zimowego, oznaczano stężenie niektórych frakcji lipidowych i lipoprotein. Niczależnie od okresu żywienia stężenia całkowitych lipidów (2,51 g/l) i cholesterolu (2,51 mmol/l) było istotnie niższe (P<0,01) w surowicy krów zasuszonych niż krów produkujących mleko (3,29 g/l i 3,86 mmol/ł, odpowiednio). Stężenie trójglicerydów w surowicy krów przed porodem wynosiło 0,34 mmol/l i było ponad dwukrotnie wyższe niż w okresie laktacji. Stwierdzono także, że u krów frakcje lipoprotein o wysokiej gęstości (HDL) stanowią główną frakcję lipidów surowicy, u krów przed porodem około 74% i 80-85% w okresie laktacji. Stężenie frakcji lipoprotein o niskiej gęstości (LDL) było niższe; u krów zasuszonych wynosiło średnio 22%, w 6-tym tygodniu laktacji 13-15%. U krów przed porodem ponad 40% lipidów LDL stanowiły trójglicerydy (TG) i około 32% cholesterol. W szczycie laktacji udział TG w LDL obniżył się do 14-19%, a choresterolu wzrósł do ponad 45%.