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The dynamics of changeability of fatty acid profile in woman, cow and sow milk during lactation

Dynamika zmienności kwasów tłuszczowych w mleku kobiet,
krów i loch podczas laktacji

Summary. Changes in fatty acid profile of woman, cow and sow colostrum and milk fat during lactation were investigated. Colostrum samples were taken 2 times: a few hours postpartum and then 2–3 days postpartum. Also, milk samples were collected 2 times. The first collection from each 3 investigated species took place in 2nd week of lactation and the second collection – in 4th, 6th and 3rd month of lactation from women, cows and sows, respectively. The level of linoleic acid in the colostrum and milk of women gradually decreased during lactation, whereas linolenic acid gradually increased until 2nd week of lactation, after which its level slighty decreased. In cow milk fat, the linoleic acid was stable during the two first weeks of lactation, then its content decreased. The level of linolenic acid increased (from 0.74 to 1.14%) within the investigated 6 months of lactation. The percentage of arachidonic acid also slightly increased (from 0.41% to about 0.50%). High linoleic content in sow colostrum (2.6–3.1%) decreased considerably in milk fat (1.8%). The lowest (0.3%) linolenic acid content in colostrum increased gradually with time of lactation up to 0.8% in 3rd month postpartum.

Key words: fatty acids, colostrum, milk, women, cows, sows

INTRODUCTION

The phenomenon of lactation is continuously examined from the angle of numerous biologically active substances present in milk, which are still being discovered and recognised, such as immune factors and new mechanisms of their activity [Atwood and Hartmann 1993, Verstegen *et al*. 1998, Hamosh 2001].

Among the numerous substances found in colostrum and milk, there are fatty acids, with essential unsaturated fatty acids (UFA) playing a significant role among them.

The purpose of the research work was to determine the dynamics of changes in UFAs in fatty acid profile in colostrum and milk sampled from women, cows and sows during lactation period.

The mixture of exogenous unsaturated fatty acids essential for the organism: linoleic (C_{18} Δ 9–10, 12–13), linolenic (C_{18} Δ 9–10, 12–13, 15–16) and arachidonic (C_{20} Δ 5–6, 8–9, 11–12, 14–15) acids, is sometimes called vitamin F. These acids can be considered as factors bearing certain characteristics of vitamins, although they are not vitamins in the full sense of the word – they are a building material and source of energy for the organism, which is against the definition of vitamins, which, in their active form, are coenzymes and as a rule do not act as substrates in metabolic changes. The most active components of vitamin F are arachidonic and linoleic acids. Linolenic acid shows lower activity.

The amount and the kind of fat in food affect immunity as well [Barej 1996, Lipiński 1999, Księżyk 2002]. It applies especially to essential unsaturated fatty acids (UFA) – linoleic, linolenic and arachidonic acids. They are precursors of prostaglandins, prostacyclins, thromboxanes, lipoxins and leucotrienes. Essential unsaturated fatty acids and their derivatives have a lot of important biological functions, including their significance for immunity. Essential unsaturated fatty acids deficiency decreases the proliferation of lymphocytes and production of interleukin 2 (IL2), among other things. The effects of particular fatty acids and their derivates on animals' immunity are often contradictory. The results of some investigation [Lipiński 1999] show that diets rich in UFA can result in higher susceptibility of animals to some diseases. Other components of fat can also affect animals' immunity. For example, a high content of saturated fatty acids and cholesterol decreases humoral immunity and increases susceptibility to infections. A possible effect of fat on immunity is very diverse. It shows by affecting the composition of cell membranes, cell receptors, chemical transmitters (e.g. cytokines) and the composition of blood lipoproteins among other things. For this reason the effect of fat on immunity is comprehensive. A lot of mechanisms within this field have not been precisely recognised yet.

The participation of essential unsaturated fatty acids, as well as of lower unsaturated fatty acids, is also significant for working against free radicals, which are reported to be a probable cause of many carcinogenic diseases.

The precursors from which particular unsaturated acids are derived can be recognised on the basis of the number of methylene groups between carbon ω (end group CH3) and the nearest double bond in the molecule.

In organisms of mammals there are no enzymes capable of forming double bonds beyond the c-9 carbon in the chains of fatty acids. That is why mammals are not able to synthesize linoleic or linolenic acid. Linoleic and linolenic acids are the starting point of synthesis of many other unsaturated fatty acids.

Arachidonic acid, a 20-carbon fatty acid with four double bonds (20:4), is the main precursor of several classes of signalling molecules, such as prostaglandins, prostacyclins, thromboxanes and leucotrienes.

Prostaglandins, prostacyclins, thromboxanes and leucotrienes are called icosanoids, because they are composed of 20 carbon atoms. Prostaglandins and other icosanoids are local hormones, because they have short halflife and that is why they change the activity of the cells in which they are synthesised and the adjacent cells only. The effect of icosanoids on the cell differs depending on the type of cell, as opposed to more uniform action of general hormones, such as insulin or glucagon. Prostaglandins stimulate inflammatory states, regulate the blood flow to particular organs, control the transport of ions through intracellular membranes, modulate transmitting nerve impulses by synapses and induce sleep.

MATERIAL AND METHODS

Collecting of colostrum and milk samples

Colostrum samples were taken 2 times: a few hours postpartum and then 2–3 days postpartum. Also milk samples were collected 2 times. First collection from each 3 investigated species – in 2nd week of lactation and the second collection – in 4th , 6th and 3rd month of lactation from women, cows and sows, respectively.

Colostrum and milk were collected from women and animals to sterile containers and they were carried in appropriate vacuum flasks to the laboratory where the analyses were carried out. Milk was collected from cows according to the PN-90/A-86003 norm. The samples were used for fatty acid determination.

Determination of fatty acids

Fatty acids were determined using gas chromatography technique.

This method is based on fat hydrolysis using KOH to produce glycerols and free fatty acids (FFAs), and then to form methyl esters from fatty acids with BF_3 as a catalyst. Methyl esters of free fatty acids are then determined using gas chromatography technique with the ester of margaric acid as a reference standard.

An internal standard was prepared by weighing out 500 mg of margaric acid and dissolving it in 100 cm^3 of chloroform.

The process of determining fatty acids according to Perkin – Elmer instruction. 100–200 mg of fat obtained from hydrolysis was weighed out. Then internal standard was added (10 mg/2 cm^3 of margaric acid). After evaporating chloroform on a waterbath at 80°C, 0.5 m of KOH in methanol was added and evaporation was carried out again. Next, 14% BF_3 in methanol was added and evaporated at 80^{o}C. The esters were subjected to hot salting out with saturated KCl. The samples were placed on the column of Varian CP-3800 gas chromatograph. Capillary column CPWAX 52-CB 60 m in length was used. Injector temperature was 260^{o}C, column temperature 120^{o}C, rising to 210^{o}C at 2^{o}C/min and FID detector temperature was 260^{o}C. Helium was used as the carrier gas.

On the basis of measuring the size of fields defined by curves corresponding to particular fatty acids, the quantity of the acids was calculated. The content of vitamin F and other fatty acids was determined. The results were expressed in the percent of total fatty acids.

RESULTS AND DISCUSSION

Fatty acid composition of woman milk

Table 1 show the profile of both saturated and unsaturated fatty acids in the colostrum and breast milk of women (n = 10) during lactation, expressed in percent in total determined fatty acids, whereas Figure 1 shows the content of essential unsaturated fatty acids in fat of the investigated samples.

Table 1. Fatty acid profile of woman (n = 10) colostrum and milk (% of total fatty acids) in different time from parturition
Tabela 1. Profil kwasów tłuszczowych w siarze i mleku kobiet (n = 10) (% sumy kwasów tłuszczowych) w zależności od czasu od porodu

Acid Kwas	Colostrum Siara		Milk Mleko	
	few hours kilka godzin	day 2–3 2–3 dzień	week 2 2 tydzień	month 4 4 miesiąc
C 8:0 caprylic C 8:0 kaprylowy	–	0.55	0.80	0.65
C 12:0 capric C 12:0 kaprynowy	2.75	4.50	5.48	3.41
C 14:0 myristic C 14:0 mirystynowy	5.44	7.22	9.23	6.25
C 16:0 palmitic C 16:0 palmitynowy	25.23	25.10	25.46	26.32
C 16:1 palmitoleic C 16:1 palmitoleinowy	2.95	2.22	2.52	3.83
C 18:0 stearic C 18:0 stearynowy	7.50	7.13	7.13	7.01
C 18:1 oleic C 18:1 oleinowy	42.02	40.21	37.84	40.55
C 18:2 linoleic C 18:2 linolowy	13.14	11.98	10.28	10.20
C 18:3 linolenic C 18:3 linolenowy	1.03	1.41	1.96	1.62
C 20:4 arachidonic C 20:4 arachidonowy	0.11	0.12	0.14	0.13
Linoleic/linolenic Linolowy/linolenowy	12.7	8.5	5.2	6.3
Linoleic/arachidonic Linolenowy/arachidonowy	119.4	99.8	73.4	78.4
Linolenic/arachidonic Linolenowy/arachidonowy	9.3	11.7	14.0	12.4

The level of linoleic acid decreases from 13.1% in colostrum, through 12.0% on the 2^{nd}–3^{rd} day, to 10.3 in 2^{nd} week and to 10.2% in the 4^{th} month. The changeability of linolenic acid level during lactation goes inversely. Its mean content in colostrum (1.0%), increases to 1.4% on 2^{nd}–3^{rd} day and to 2,0% in milk in 2^{nd} week. In the next period, i.e. in the 4th month, it slightly decreases to 1.6%. The mean values of arachidonic acid do not show any major changes during the whole period of lactation, varying from 0.11 to 0.14%.

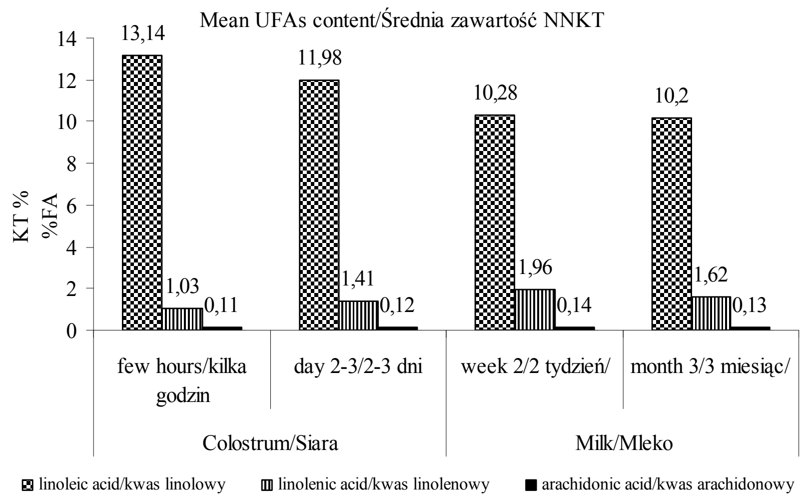

Fig. 1. Mean essential unsaturated fatty acids (UFAs) content in colostrum and breast milk of women, in diffrent time from parturition expressed in the percent of total fatty acids
Ryc. 1. Średnia zawartość niezbędnych nienasyconych kwasów tłuszczowych (NNKT) w siarze i mleku kobiet w różnym czasie od porodu wyrażona w % ogółu kwasów tłuszczowych

Fatty acid composition of cow milk

The fatty acid profile in colostrum and milk of cows during lactation expressed in percent is shown in Table 3 and contents of essential unsaturated fatly acids (linoleic, linolenic, arachidonic) illustrates Fig. 2.

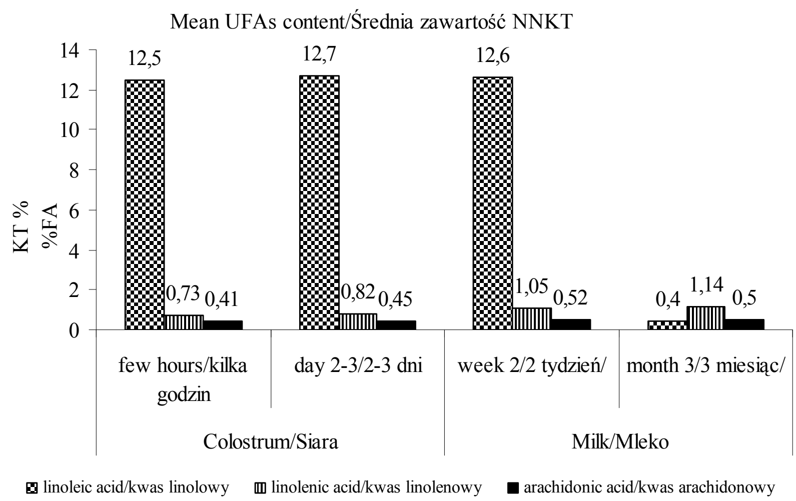

Fig. 2. Mean essential unsaturated fatty acids (UFAs) content in colostrum and breast milk of cows in different time from parturition expressed in the percent of total fatty acids
Ryc. 2. Średnia zawartość niezbędnych nienasyconych kwasów tłuszczowych (NNKT) w siarze i mleku krów w różnym czasie od porodu wyrażona w % ogółu kwasów tłuszczowych

Table 2. Fatty acid profile of cow (n = 10) colostrum and milk (% of total fatty acids) in different time from parturition
Tabela 2. Profil kwasów tłuszczowych w siarze i mleku krów (n = 10) (% sumy kwasów tłuszczowych) w zależności od czasu od porodu

Acid Kwas	Colostrum Siara		Milk Mleko	
	few hours kilka godzin	day 2 2–3 dzień	week 2 2 tydzień	month 6 6 miesiąc
C 8:0 caprylic C 8:0 kaprylowy	0	0	0.20	0.14
C 12:0 capric C 12:0 kaprynowy	0	0	0.04	0.04
C 14:0 myristic C 14:0 mirystynowy	3.10	3.19	3.62	4.85
C 16:0 palmitic C 16:0 palmitynowy	34.2	32.20	37.0	36.8
C 16:1 palmitoleic C 16:1 palmitoleinowy	5.45	5.58	8.90	10.53
C 18:0 stearic C 18:0 stearynowy	6.30	6.36	6.08	5.92
C 18:1 oleic C 18:1 oleinowy	37.3	38.6	32.9	32.3
C 18:2 linoleic C 18:2 linolowy	12.5	12.7	12.6	8.40
C 18:3 linolenic C 18:3 linolenowy	0.74	0.82	1.05	1.14
C 20:4 arachidonic C 20:4 arachidonowy	0.41	0.45	0.52	0.50
Linoleic/linolenic Linolowy/linolenowy	17.1	15.5	12.0	7.4
Linoleic/arachidonic Linolowy/arachidonowy	30.5	28.2	24.2	16.6
Linolenic/arachidonic Linolenowy/arachidonowy	1.8	1.8	2.0	2.3

The absence of C8:0 and C12:0 in colostrum samples was noted. These acids appear in milk in the further course of lactation. The level of linoleic acid is constant at the beginning of lactation: 12.5–12.7% (until the 2^{nd} week postpartum). After this stage, however, its decrease is observed (8.4% in 6^{th} month of lactation). The level of linolenic acid increases permanently with lactation period (from 0.73% in colostrum fat to 1.14% in 6^{th} month milk fat). The percent of arachidonic acid also slightly increases (from 0.41% to about 0.50%).

Fatty acid composition of sow milk

The fatty acid profile of colostrum and milk of sows during lactation, expressed in percent of total fatty acids, is shown in Table 3. Short carbon fatty acids (C6:0) are not present in sows' colostrum or milk, therefore they are not included in Table 3.

Table 3. Fatty acid profile of sow (n = 10) colostrum and milk (% of total fatty acids) in different time from parturition
Tabela 3. Profil kwasów tłuszczowych w siarze i mleku loch (n = 10) (% sumy kwasów tłuszczowych) w zależności od czasu od porodu

Acid Kwas	Colostrum Siara		Milk Mleko	
	few hours kilka godzin	day 2–3 2–3 dni	week 2 2 tydzień	month 3 3 miesiąc
C 8:0 caprylic C 8:0 kaprylowy	0	1.3	1.3	1.3
C 12:0 capric C 12:0 kaprynowy	0	2.1	2.3	3.4
C 14:0 myristic C 14:0 mirystynowy	7.8	10.2	10.8	12.1
C 14:1 myristic C 14:1 mirystoleinowy	0.7	1.3	1.9	1.9
C 16:0 palmitic C 16:0 palmitynowy	34.3	35.9	54.9	42.4
C 16:1 palmitoleic C 16:1 palmitoleinowy	2.5	2.1	3.1	1.6
C 18:0 stearic C 18:0 stearynowy	16.5	13.6	6.4	11.6
C 18:1 oleic C 18:1 oleinowy	32.2	30.1	17.0	23.0
C 18:2 linoleic C 18:2 linolowy	2.6	3.1	1.8	1.9
C 18:3 linolenic C 18:3 linolenowy	0.6	0.3	0.5	0.8
C 20:4 arachidonic C 20:4 arachidonowy	0.11	0.14	0.12	0.13
Linoleic/linolenic Linolowy/linolenowy	4.3	10.3	3.6	2.4
Linoleic/arachidonic Linolowy/arachidonowy	23.6	22.1	15.0	14.6
Linolenic/arachidonic Linolenowy/arachidonowy	5.4	2.1	4.2	6.1

During lactation the values of linoleic and arachidonic acids show a rising tendency on the 2^{nd} – 3^{rd} day after parturition and they stabilize in the further course of lactation (Fig. 3). The linoleic acid content is increasing from 2.6% in colostrum to 3.1% on the 2^{nd}–3^{rd} day, and then it stabilizes (1.8–1.9% in the 2^{nd} week and 3^{rd} month). A greater stabilization is observed for arachidonic acid both in colostrum and in milk. This is proved by the values obtained for this acid at subsequent research stages: colostrum – 0.11%, 2^{nd}–3^{rd} day – 0.14%, 2^{nd} week – 0.12% and 3^{rd} month – 0.13%. Linolenic acid undergoes only small changes in values (0.6–0.3%) and it only slightly increases to 0.8% at the end of lactation.

The results of the study show that tendencies in proportion values of means of essential unsaturated fatty acids in woman, cow and sow milk have similar changeability during lactation.

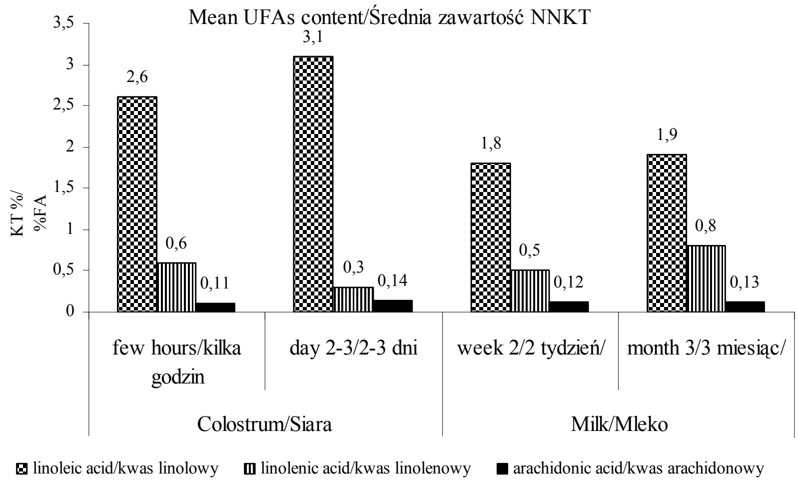

Fig. 3. – Mean essential unsaturated fatty acids (UFAs) content in colostrum and breast milk of sows in diffrent time from parturition expressed in the percent of total fatty acids
Ryc. 3. Średnia zawartość niezbędnych nienasyconych kwasów tłuszczowych (NNKT) w siarze i mleku loch w różnym czasie od porodu podczas laktacji wyrażona w % ogółu KT

The proportion of mean values of linoleic acid to averages of linolenic acid shows from the beginning of lactation a falling tendency both in woman (Tab. 1) as well as in cow (Tab. 2) and sow (Tab. 3) milk. The relation of mean values of linoleic acid to mean values of arachidonic acid in women, cows and sows also shows a falling tendency. The relation of mean values of linolenic acid to mean values of arachidonic acid in women, however, shows a rising tendency.

A special role of linolenic acid should be emphasised. Its values show a rising tendency during the whole lactation period in all three species researched. It indicates the increase in the suckling's need for this acid. The values of arachidonic acid, remaining at the same level in women, cows and sows, prove a stable need for this acid during lactation, although it is a very important precursor of transmitter hormones, icosanoids, which should suggest a rising tendency during this period.

The changeability of unsaturated acids values during lactation in women is peculiar to a given acid. The decrease in linoleic acid, the changeability of linolenic acid as well as the stable level of arachidonic acid during lactation are determined by both the mother's ability to produce and the genetic conditions concerning the suckling's needs. Obviously, other environmental factors and the kind of food consumed by the mother are also involved.

The greatest changeability in cow milk is observed for linoleic acid. Its level in colostrum on the 2^{nd}–3^{rd} day and in 2^{nd} week is stable (12.5–12.7%), whereas in 6^{th} month it dropps to 8.4% (Tab. 2). Both linolenic and arachidonic acid, increase their values during the course of lactation, reaching the highest values in sixth month postpartum.

Also in sow colostrum and milk the greatest changeability is observed for linoleic acid, ranging between 2.6% in colostrum, 1.8% in 2^{nd} week and 1.9% in 3^{rd} month (Tab. 3). Linolenic acid, however, decreases its level in colostrum in 2^{nd}–3^{rd} day, afterwards it has a tendency for increase and it reaches its highest concentration in 3rd month postpartum. Arachidonic acid increases its values on 2nd–3rd day in comparison with its content in colostrum. In the 2nd week and 3rd month of lactation its slight decrease is observed.

Human milk is considered to possess the optimal form of nutrition for infants in the first six months of their life. In terms of macronutrients, the lipid fraction is crucial in fulfilling a newborn's nutritional needs because almost 50% of calories are supplied as fat. Numerous studies have demonstrated that lipids are also involved in some of the structural and physiologic functions of the organism [Sala-Vila *et al.* 2005].

The main components of milk fat are fatty acids [Malcarne *et al.* 2002, Genzel-Boroviczeny *et al.* 1997]. They are esterified mainly as triglycerides (TGs), which account for 98% of milk fat. A smaller portion of fatty acids is esterified as phospholipids (PL), which are included in the membrane surrounding and stabilising the lipid core of the fat globule of milk. Phospholipids (PLs) also perform a nutritional function, supplying long-chain polyunsaturated fatty acids (LC-PUFAs), nervonic acid (NA; C24:1ω-9) and choline. These nutrients are necessary to achieve optimal development and function in the newborn [Pawlus *et al.* 2001].

The most important LC-PUFA (arachidonic acid AA C20:4 ω-6 and docosahexaenoic acid DHA C22:6 ω-3) are incorporated in phospholipid membranes of the retina and brain in the third trimester of pregnancy and they continue to accumulate during the first two years of life. In mature human milk about 85% of LC-PUFA is in the form of PLs. As a result of the differences in their chemical structures, LC-PUFA delivered by PL or TG follow different metabolic pathways, mainly due to the processes of enzymatic hydrolysis, absorption and incorporation in lipoproteins. Recently several authors have investigated if this is the reason for the increase in LC-PUFA in plasma, and hence the uptake of LC-PUFA into developing tissues [Sala-Vila *et. al.* 2005].

Human milk is a dynamic system, in which fat composition is affected by factors such as the mother's diet, duration of pregnancy or the stage of lactation. There are three phases of milk production: colostrum (1–5 days postpartum), transitional milk (6–15 days postpartum) and mature milk (after 15 days). Recent studies have reported the differences between total fatty acids composition of human breast milk and TG composition at different stages of lactation.

It has been noted that the main PL classes (phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine) and the composition of fatty acids and TG show changeability in colostrum and in fully established mature milk in mothers who gave birth to full-term infants. This provides information on the changes that occur in the composition of human milk fat during the course of lactation. This knowledge should contribute to a better design of infant milk replacer formulas.

CONCLUSIONS

1. Contents of linoleic acid in colostrum and milk fat of all three species investigated females (woman, cow and sow) decreased gradually with time of lactation.

2. Opposite tendency was observed regarding linolenic acid, whose contents was increasing towards the end of lactation.

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Streszczenie. Zbadano zmiany składu kwasów tłuszczowych w siarze i mleku kobiet, krów i świń w okresie laktacji. Próby siary pobrano dwukrotnie: kilka godzin po porodzie, a następnie w 2–3 dobie po porodzie. Próby mleka również pobrano dwukrotnie. Pierwsza próba pobrana została od każdego z badanych gatunków w drugim tygodniu laktacji, a druga próba w czwartym (kobiety), szóstym (krowy) i trzecim (świnie) miesiącu. Poziom kwasu linolowego w siarze i mleku kobiet stopniowo spadał w okresie laktacji, natomiast poziom kwasu linolenowego stopniowo wzrastał aż do drugiego tygodnia laktacji, a następnie nieznacznie zmalał. W mleku krów poziom kwasu linolowego był stabilny podczas dwóch pierwszych tygodni laktacji, a później nastąpił jego spadek. Poziom kwasu linolenowego wzrastał od chwili porodu do końca laktacji (od 0,73% do 1,14% w szóstym miesiącu). Nieznacznie wzrosła również zawartość procentowa kwasu arachidonowego (od 0,41% do ok. 0,50%). Zawartość kwasu linolowego była duża w siarze świń (2,6–3,1%), a znacznie zmalała w mleku (1,8%). Mała zawartość kwasu linolenowego w siarze tych zwierząt (0,3% w 2–3 dniu po porodzie) stopniowo wzrastała w okresie laktacji aż do 0,8% w trzecim miesiącu po porodzie.

Słowa kluczowe: kwasy tłuszczowe, siara, mleko, kobiety, krowy, lochy