FATTY ACIDS CONTENT AND CONJUGATED DIENES OF LINOLEIC ACID cis-9, trans-11, IN THE MILK OF RUMINANTS

SADRŽAJ MASNIH KISELINA I DVOJNIH DIENA LINOLNE KISELINE cis-9, trans 11 U MLIJEKU PREŽIVAČA

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SUMMARY

In our research the fatty acids profile in milk from ruminants (cow, sheep, goat) kept under similar environmental conditions (summer feeding) was evaluated. Also the changeability of fatty acids content in milk was evaluated. The most profitable composition of fatty acids (lowest saturated fatty acids (SFA) content and the highest unsaturated (UFA)) content was found in cow milk, then in sheep and the least in goat milk. However sheep milk was characterized by the highest concentration of poly-unsaturated fatty acids (PUFA) and conjugated dienes of linoleic acid cis-9, trans-11 (CLA). Most (g/100 g milk) of both unsaturated (mono (MUFA)- and polyunsaturated) and saturated fatty acids was found in sheep milk. Most of the conjugated dienes of linoleic acids (c9,t11) was also established there.

Key words: dienes, fatty acids, milk, linoleic acid, ruminants, cow, sheep, goat

INTRODUCTION

An interest in fatty acids as a constituent of human diets began when scientists observed that the Eskimos, who fed a diet very rich in fat, had a very low rate of heart disease and a low incidence of other diseases.

Over the last few years, there is has been an increased interest in chemical composition of edible fats, mainly in their fatty acids profile. It obviously follows from the medical studies, proving that many civilization diseases are strictly associated with wrong nutrilion, i,e. with incresed consumption of fats rich in saturated fatty acids.

Nutritionists, dieticians, medical consultants, biologists and food technologists have also been interested, in heterogeneous group of isomers of conjugated linoleic acid (CLA) discovered at the end of XX century. In the case of these isomers, contrary to linoleic acid C18:2, from which they are formed, double bonds in both forms - cis and trans - are isolated only with one single bond, which results in new functional properties.

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Conjugated dienes of linoleic acid are converted mainly by enzymes of ruminal bacteria *Butyrivibrio fibrisolvens*. After subsequent phases of transformations, a fraction of these isomers is absorbed from digestive tract and is incorporated into the blood lipids, tissues and organs, milk fat and meat (Parodi,1977; Hughes et al.,1982, Kemp and Lander 1984; Lin et al.,1995). The ability of these bacteria to convert the linoleic acid into its coupled form has been confirmed by many researchers. In contrast to ruminants, monogastric animals have limited possibilities of isomerization of conjugated dienes of linoleic acid (Rafalski,1998).

Amongst a large group of isomers of conjugated dienes of linoleic acid the highest biological activity was proved for the isomer in configuration of cis-9, trans-11. This isomer shows health promoting properties i.e. it inhibits carcinogenesis, arteriosclerosis and osteoporosis, prevents obesity and stimulates immune system.

It has been found that the most important source of conjugated dienes of linoleic acid for human is ruminant' fat (Bartnikowska et al.,1999). Thus the purpose of research was to evaluate the differences in the FA content in milk from ruminant females.

MATERIAL AND METHODS

Experiment I

The studies were carried out on the samples of milk taken from different females - sheep of Frisian breed (15 samples), goats of white graded breed (15 samples) and cows of black and white breed (15 samples). All animals were in the fourth month of third lactation. Milk samples were taken from grazing animals (pasture feeding, additionally concentrate, hay or mineral additive were given). Observations were performed at the Ecological Farm "Ekoland" in Poręby-Łaziska.

In the analytical part, fatty acids were determined along with the linoleic acid isomer c9,t11 content. Samples of milk were extracted twice using Folch mixture (chloroform in methanol in the ratio 1:1). The isolated lipid fraction of fat after addition of water, centrifugation and filtration through anhydrous sodium sulphate, was hydrolyzed with 0.5 M KOH in methanol and next estrified with 14 % BF_3 in methanol. The obtained ethyl esters of fatty acids in the amount of 1 µl were analyzed in chromatograph PU 4410 Philips with flame-ionization detector in order to determine the profile of fatty acids and the amount of dienes of linoleic acid having configuration cis-9. trans-11. In order to perform separation, capillary column, Rtx-2330 was used, covered with bound stationary phase (10 % cyanopropylphenyl, 90 % biscyanopropyl polysiloxane) of width 20 µm, length 105 m and diameter 0.25 mm. The separation was conducted under programmed temperature: initial isotherm 160 °C (30 min.) 3°C/min to 180°C - 17 min. at temperature 180°C for 5 min. to 210°C - 20 min. at temperature 210 °C. Other separation parameters: detector 230°C, injection chamber 220°C, column temperature 160°C, carrier gas - Helium 80 PSI. The identification of fatty acids and conjugated form of linoleic acid cis-9, trans-11 was done by comparison with retention time of Sigma standards. These analyses were carried out in Bromatology Department, Medical Academy in Wrocław.

In order to estimate the significance of differences between species the one-way variance analysis and Duncan multiple range test were performed.

Experiment II

Experimental part of the research was conducted at the ecological farm "Folwark Polski" in Łaziska Poręby on 60 lactating (third lactation) ewes of the Frisian breed. The animals were divided into three groups (20 in each group) and fed diets consisting of the following feeds: group I - concentrate and meadow hay; group 2 - concentrate, meadow hay and linseed; group 3 – pasture green fodder

The average food ration given to ewes from group I contained 1.59 UPM[1], 165 g of PDIN[2] and 175 g of PDIE[3]; group II - 1.57 UPM, 165 g of

[1] UPM – milk production units

[2] amount of ileal digested protein depending on nitrogen availability

[3] amount of ileal digested protein depending on energy availability

PDIN and 167 g of PDIE. Group III fed diets containing - 1.47 UPM, 166g of PDIN and 155g PDIE (diets were prepared according to the INRA system).

In the experiment chemical composition and fatty acids profile in fat were determined three times in green fodder and twice in concentrate, meadow hay and linseed. Samples of milk were taken at the beginning and on 60th day of the experiment in order to determine the fatty acids content and composition along with the level of conjugated dienes of linoleic acid c9,t11 (Table 1).

The analytical part of the research was done in the laboratory of the Department of Food Products and Food Technology of the Agricultural University of Wrocław. The extraction of fat from milk was conducted according to Folch's method. The same procedures as in Exp. I (see above) were applied.

In order to determine the significance of the differences in fatty acids at different levels of saturation and among particular fatty acids (including the isomer of linoleic acid c9t11) a one-factor analysis of variance and Duncan's multiple range test were performed.

Table 1. Chemical composition and the profile of fatty acids in feeds (%)
Tablica 1. Kemijski sastav i profil masnih kiselina u krmivima (%)

Chemical composition Kemijski sastav	Concentrate Koncentrat	Pasture green fodder Zelena krma s pašnjaka	Meadow hay Livadno sijeno	Linseed Laneno sjeme
Dry matter - Suha tvar	85.26	13.50	94.37	93.25
Protein - Bjelančevina	16.90	3.85	12.37	25.26
Fat -Masnoća	3.19	0.86	3.71	39.11
C16:0	29.82	15.64	26.96	8.42
C 16:1	0.35	3.26	2.38	1.20
C 17:0	1.33	0.11	0.32	0.11
C 18:0	5.56	2.05	4.26	2.71
C 18:1	34.60	6.52	11.67	19.24
C 18:2	23.11	14.82	17.74	17.89
C 18:2 izomer c9t11	0.03	-	-	-
C 18:3	1.50	50.83	24.20	46.85
C 20:0	0.85	0.60	0.95	0.17
C 20:1	0.70	0.07	0.18	0.29
C 20:2	0.05	0.08	0.06	0.04
C 20:4	0.02	0.42	1.11	0.36
C 20:5	0.08	0.06	0.56	0.31
C 22:0	0.53	0.60	1.24	0.16
C 22:1	0.02	-	-	0.13
C 22:5	0.16	0.35	0.65	0.45
C 22:6	0.14	0.08	1.05	0.61
C 23:0	0.08	0.47	0.35	0.13
C 24:0	0.42	0.42	1.02	0.25
C 24:1	0.02	0.04	0.10	0.02
other - ostale	0.03	0.91	0.62	0.14

RESULTS AND DISCUSSION

Experiment I

The lowest saturated fatty acids (SFA) content was observed in the fat of cow milk. It was by 4 % and 46 % lower than in goats and sheep milk, respectively (P<0.01) (Table 2). The fat of sheep milk was characterized by 33-45 % higher fatty acids content with one triple band than in milk of other species (P<0.01) (Table 2). Sheep milk contained the highest amount of poly-unsaturated fatty acids. The obtained values were higher by 16 or 25 % than in cow or goat milk, respectively (P<0.01). The most favourable ratio of poly-unsaturated vs. saturated fatty acids was found in sheep milk (0.68) followed by in cow milk (0.61) and the least in goat milk (0.51).

The highest fat content was found in sheep milk (6.6 %) then in cow milk (3.82 %) and finally in goat milk (3.67 %).

The highest share of saturated fatty acids was found in sheep milk and the lowest in cow milk (Table 3). The highest share of monounsaturated fatty acids was estimated in cow milk. The highest amount of linoleic C18:2 and linolenic C18:3 acids and conjugated dienes of linoleic acid cis-9. trans-11 were found in sheep milk (Table 3). In the present study, isomer of linoleic acid in configuration cis-9. trans-11 was prevalent, its amount reaching almost 80-90 % of the sum of dienes of this acid. Differences among species were also observed in the important unsaturated fatty acids content: linoleic C18:2 and linolenic C18:3 in milk fat (Table 3). The amount of linoleic acid C18:2 was on a similar level in the fat of sheep and cow milk and lower in the fat of goat milk (P<0.05). The highest linolenic acid C18:3 content was found in sheep milk, which was by 45 % higher than in goats milk and by 35 % than in cows milk fat (P<0.0l) (Table 2). Significant differences (P<0.01) in the level of conjugated dienes of linoleic acid c9,t11 (CLA) were also found between sheep and cow and goat milk. Sheep milk fat contained more isomer of linoleic acid c9,t11 than cow and goat milk (P<0.05).

Table 2. The fatty acids content in milk of ruminants (g/100 g milk) (means, SD) Experiment I

Tablica 2. Sadržaj masnih kiselina u mlijeku preživača (g/100g mlijeka) (prosjek, SD) Pokus I

Fatty acids Masne kiseline	Sheep Ovce	Goats Koze	Cows Krave
SFA	4.542A ±0071	2.553Ba ±11.140	2.443Bb ±0.122
UFA	2.126A ±0.069	1.116B ±0.136	1.377C ±0.121
In them: U čemu:			
MUFA	1.815A ±0.051	0.987B ±0.122	1.227C ±0.110
PUFA	0.312A ±0.023	0.130B ±0.023	0.150B ±0.018
C 18;2	0.134 ±0.110	0.065 ±0.012	0.078 ±0.015
CLA c9t11	0.082A ±0.009	0.024Ba ±0.004	0.031Bb ±0.005
C18:3	0.077A ±0.013	0.023B ±0.006	0.029B ±0.004

Means marked with different small letters differ significantly at P≤0.05: marked with different capital letters differ significantly at P≤0.01

Prosjeci označeni različitim malim slovima značajno se razlikuju P≤0.05 označeni različitim velikim slovima značajno se razlikuju P≤0.01

Analysis of the data concerned indicate that the share of fatty acids groups in total FA in milk of ruminants was similar to its percentage content. Differences in the range of these parameters were particularly visible due to significant differences in milk fat content of the studied animal species.

Based on the performed analysis, it was found that its content was 1.24 % of the sum of fatty acids present in milk fat. This could be due to the fact that in sheep, transestrification occurring in the rumen, that resu1ts in the formation of conjugated dienes of poly-unsaturated fatty acids - mainly CLA, is

much more efficient than in other species studied (cows, goats). Moreover, the obtained results could suggest that microorganisms living in the rumen of sheep are to a greater extent able to convert linoleic acid into conjugated diene c9,t11.

Table 3. The share of unsaturated fatty acids in milk fat of ruminants (% of the sum of fatty acids), (means, SD) Experiment I

Tablica 3. Udio nezasićenih masnih kiselina u masnoći mlijeka preživača (% zbroja masnih kiselina) (prosjek, SD) Pokus I

Fatty acids Masne kiseline	Sheep Ovce	Goats Koze	Cows Krave
SFA	68,10A	69,56A	63,94B
	±1.07	±3.82	±3 19
UFA	31,90A	30,42A	36,04B
	±0.68	±2.13	±1.76
In them: U čemu:			
MUFA	27,22A	26.89A	32,11B
	±0.76	±3.32	±2.89
PUFA	4.68A	3,53B	3,94B
	±0.354	±0.63	±0.48
C18:2	2,015	1,781	2.036
	±0,159	±0327	±0408
CLA c9t11	1.238A	0.670Ba	0,827Bb
	±0,135	±0.112	±0.137
C18:3	1,149A	0,628B	0,753B
	±0107	±0.170	±0.106

Means marked with different small letters differ significantly at P≤0.05: marked with different capital letters differ significantly at P≤0.01

Prosjeci označeni različitim malim slovima značajno se razlikuju P≤0.05 označeni različitim velikim slovima značajno se razlikuju P≤0.01

The obtained results for the fatty acids content in ruminants milk with different saturation ratio are similar to the data found in the literature, which indicates that saturated fatty acids constitute in ruminants milk ca. 62-71 %, mono-unsaturated about 29-34 % and poly-unsaturated 3-4 % (Pisulewski et al.1999, 2000; Popiołek, 1999; Rafalski, 1998). The cited authors also found that the particular fatty acids content and conjugated dienes of linoleic acid in milk was characteristic of each species. Also Jahreis et al. (1998) and Jahreis (1999) found that the CLA content in sheep milk was 1.5-times higher than in cow milk.

Summarizing the results of the performed research it could be said, that sheep milk contained most fat and the most profitable, from the nutritional point of view fatty acids composition (lowest saturated fatty acids and the highest unsaturated content) was established in cow milk; The highest amount of poly-unsaturated fatty acids and CLA c9,t11 was found in sheep milk.

Experiment II

The highest fat content determined chemically, was observed in linseed (39.11 %), while the lowest in green fodder (0.86 %). In the concentrate and in meadow hay the fat content was similar and varied between 3.19 and 3.71 % (Table 1). Similarly, most of some fatty acids, such as oleinic C18:1 linoleic C18:2, linolenic C18:2, that are the main source of CLA generated in the rumen during the biodegradation processes, was observed in linseed and green fodder.

The effect of feeding sheep on dry matter content in milk was not observed (on average 17.4 %, protein about 5.20 % and lactose about 4.5 %). Significant statistical differences were noted only in the milk fat content (Table 4). Milk from sheep fed concentrate with linseed was characterized by about 5 % higher (P≤0.05) fat content than in sheep fed concentrate only and about 7 % (P≤0.05) higher than in sheep fed green fodder.

The lowest saturated fatty acids content was noted in the milk fat of sheep fed concentrate with linseed, while the highest – in milk from sheep fed concentrate only (Table 5). The milk fat of sheep fed concentrate supplemented with linseed was characterized by the highest unsaturated fatty acids content (P<0.01). The addition of linseed also positively affects the polyunsaturated fatty acids amount in milk fat. Its increase in comparison to the group fed concentrate only amounted to 24.0 %-units (P<0.05), while compared to the group fed green fodder was higher by 17.8 %- units (P<0.05).

Table 4. Chemical composition of sheep milk (%) (means, SD)
Tablica 4. Kemijski sastav ovčjeg mlijeka (%) (prosjek, SD)

Chemical composition Kemijski sastav	Content (%) - Sadržaj (%)					
	Concentrate Koncentrat		Concentrate + linseed Koncentrat + laneno sjeme		Green fodder Zelena krma	
Dry matter - Suha tvar	17.54	±2.09	17.78	±1.75	17.20	±1.35
Crude protein - Sirova bjelančevina	5.19	±0.31	5.20	±0.26	5.25	±0.39
Crude fat – Sirova mast	6.51ab	±1.15	6.83a	±1.35	6.40b	±0.78
Lactose - Laktoza	4.49	±0.30	4.43	±0.35	4.52	±0.29

Means marked with different small letters differ significantly at P≤0.05.
Prosjeci označeni različitim malim slovima značajno se razlikuju P≤0.05

Table 5. Profile of fatty acids in milk fat (%) (means, SD)
Tablica 5 Profil masnih kiselina u masnoći mlijeka (% /prosjek, SD)

Fatty acids Masne kiseline	Feeding groups - Hranidbene skupine					
	Concentrate - Koncentrat		Concentrate + linseed Koncentrat + laneno sjeme		Green fodder - Zelena krma	
SFA	69.47A	±1.67	66.54B	±2.88	67.83A	±2.84
UFA	30.39A	±1.078	33.08B	±0.88	31.92	±1.17
In them: U čemu:						
MUFA	25.84	±2.09	27.39	±2.67	27.10	±1.95
PUFA	4.56Aa	±0.41	5.68B	±0.59	4.82Ab	±0.55
C 16:0	28.840a	±1.141	26.618b	±2.321	28.703a	±1.087
C 16:1	0.631A	±0.098	0.790B	±0.111	0.599A	±0.126
C 17:0	0.641 Aa	±0.130	0.530B	±0.076	0.704Ab	±0.104
C 18:0	12.786	±1.238	11.200	±0.867	11.866	±1.104
C 18:1	22.474a	±1.127	23.910b	±1.542	23.195	±2.217
C 18:2	2.389a	±0.245	2.915b	±0.192	2.650	±0.371
C 18:2 (c9t11)	0.524A	±0.076	0.920B	±0.180	0.720C	±0.093
C 18:3	1.294A	±0.123	1.650B	±0.213	1.422	±0.146
C 20:0	0.280	±0.046	0.250	±0.063	0.293	±0.058
C 20:1	1.930	±0.136	2.016	±0.235	2.275	±0.171
C 22:0	0.116A	±0.039	0.193B	±0.045	0.162B	±0.023
C 22:5	0.286Aa	±0.067	0.146B	±0.037	0169b	±0.054
C 22:6	0.064a	±0.017	0.050b	±0.009	0.053	±0.011
C 24:0	0.036a	±0.012	0.095b	±0.055	0.062	±0.027
other - ostale	0.136	±0.037	0.383	±0.176	0.256	±0.103

Means marked with different small letters differ significantly at P≤0.05: marked with different capital letters differ significantly at P≤0.01
Prosjeci označeni različitim malim slovima značajno se razlikuju P≤0.05 označeni različitim velikim slovima značajno se razlikuju P≤0.01

Milk fat of sheep fed concentrate with linseed was characterized by the highest share of fatty acids such as linoleic C18:2 and linolenic C18:3 important for humans and categorized as the essential unsaturated fatty acids. It also contained the highest quantity of conjugated dienes of linoleic acid of cis-9 trans-11 configuration (biologically active antiatherosclerotic isomer). In comparison to the group fed green fodder the isomer content was higher by 27.8 %-units ($P<0.01$), whereas in relation to the group fed concentrate - by 75.6 percent units ($P<0.01$).

A significant effect of feeding on the characteristics of milk fat features, such as the profile of fatty acids and the CLA content was observed. Milk of sheep fed diets enriched with linseed was characterized by the highest CLA content. In terms of the assessed parameters milk of sheep fed green fodder also seems to be of desirable dietetic quality. Least desirable composition of fatty acids and the lowest CLA content was observed in milk of sheep fed on concentrate.

The obtained results, concerning the basic components content, fatty acids profile in milk fat and the CLA content are within the wide range of data presented by other authors (Marques and Belo,1994; Patkowska-Sokoła et al.,2001; Pisulewski et al.,1999) but publications on this subject are scarce. A wide span of results are mainly due to the conditions of feeding (Borys et al., 2000). Understanding the effect of feeding and the physiological mechanisms could be a good way of modification of fatty acids composition in milk fat and its effective enhancement with CLA.

As a final thought it could be stated that feeding based on green fodder and application of linseed in the diets significantly increased the amount of unsaturated fatty acids (including C18:1, C18:2 and C18:3 as well as conjugated dienes of linoleic acid CLA) in milk fat of sheep.

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SAŽETAK

U našem istraživanju ocijenjen je profil masnih kiselina u mlijeku preživača (krave, ovce, koze) držanih u sličnim okolišnim uvjetima (ljetno hranjene). Praćena je i promjenjivost sadržaja masnih kiselina. Najkorisniji sastav masnih kiselina (najniži sadržaj zasićenih masnih kiselina (SFA) i najveći sadržaj nezasićenih (UFA) nađen je u kravljem mlijeku zatim u mlijeku ovaca i najmanji u mlijeku koza. Međutim, mlijeko ovaca obilježila je najviša koncentracija mnogostrukonezasićenih masnih kiselina (PUFA) i dvojnih diena linolne kiseline cis-9, trans-11 (CLA). Najviše (g/100 g mlijeka) nezasićenih (mono /MUFA)- i mnogostrukonezasićenih i zasićenih masnih kiselina nađeno je u ovćem mlijeku. Tamo je ustanovljeno i najviše dvojnih diena linolne kiseline (c9,t11).

Ključne riječi: dieni, masne kiseline, mlijeko, linolna kiselina, preživači, krava, ovca, koza