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**ORIGINAL PAPER** 

# IMPACT OF A PRODUCTION SEASON ON THE CHEMICAL COMPOSITION AND PRO-HEALTH PROPERTIES OF MILK AND RENNET CHEESE FROM SHEEP\*

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#### Abstract

Owing to their health-promoting values, products obtained from sheep are the center of attention among consumers. The chemical composition of sheep's milk is largely conditioned by seasonal dietary variables, which contribute to its pro-health qualities. The aim of the study was to evaluate the impact of a production season on parameters of milk and rennet cheese produced from milk obtained from Frisian sheep during winter and summer feeding. Milk from the winter season contained more lactose, less vitamin A and less than half the vitamin E content than milk obtained in summer. Cheese from the winter season was characterized by a higher content of dry matter, proteins and minerals but less than half vitamin A content than cheese from the summer period. In cheese from the winter season, the concentration of tyramine was over fourfold higher while the concentration of histamine, while putrescine, and cadaverine were over twice as low as in cheese of the summer feeding period. The content of total biogenic amines did not vary seasonally. Milk fat from milk obtained in winter contained more medium-chained saturated fatty acids (SFA), but less long-chained SFA and polyunsaturated fatty acids (PUFA) in comparison with the summer period. Fat from cheese produced in winter had a higher content of medium-chained SFA, whereas cheese from the summer feeding season contained more long-chained SFA, monounsaturated fatty acid (MUFA), and PUFA. Milk and cheese from the summer feeding period had higher ratios of PUFA to SFA and *n*-6 to *n*-3.

Keywords: biogenic amines, chemical composition, summer, vitamin, winter, rennet cheese.

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### INTRODUCTION

Products obtained from sheep are the center of attention among consumers owing to their health-promoting values (MILEWSKI 2006). The chemical composition of sheep's milk largely depends on seasonal dietary variables, which are of key importance of its health-promoting qualities (ZABEK et al. 2014). It is also crucial to trace pesticide residues in green fodder, silage and cereal grains, as well as in meat and milk, due to the possible risk to consumers (ŁOZOWICKA et al. 2012). TALPUR et al. (2008) demonstrated significant differences between the composition of fatty acids in milk obtained from sheep during winter and summer feeding. NUDDA et al. (2005) reported that the amount of conjugated linoleic acid (CLA) and vaccenic acid in sheep cheese depends on their content in milk, and seasonal changes occur in connection with changes in the quality of pastures. The concentration of vitamin A and E in milk and cheese depends mainly on a feeding season, i.e. the content of carotenoids and tocopherols in green fodder and preserved feed. In green fodder, grass and legumes, the content of  $\beta$ -carotene may be 33-700 mg kg<sup>-1</sup> DM, but in silages it can decrease by as much as ten-fold (KALAČ 2011). According to Müller et al. (2007), grass forage supplies 10-50 mg of vitamin E in kg of fresh fodder while silages contribute the remaining 15-70% tocopherols in raw material. MAYER and FIECHTER (2012) reported that the impact of a feeding season on the concentration of cholesterol is less4er than that on the content of lactose, fat or minerals in milk sheep. TAYLOR (1985) reported that the value of total biogenic amines in cheese higher than 100 mg kg<sup>-1</sup> may be unsafe to consumers' health, while SCHIRONE et al. (2011) and POVEDA et al. (2015) observed that the content of total amine in cheese often exceeds 1000 mg kg<sup>-1</sup>. According Schirone et al. (2011), SPIZZIRRI et al. (2013) and POVEDA et al. (2015), pasteurisation of milk reduced the formation of amines, and the extension of the cheese ripening period and the presence of microflora (*Enterobacteriaceae*) favoring decarboxylation processes result in an increase of their content.

# MATERIAL AND METHODS

The research was conducted on an organic farm, where 95 Friesian milk sheep are reared. Animals are maintained indoors on deep litter. The research period was divided into two sub-periods: winter (months: December, January, February) and summer (months: June, July, August). In winter feeding, grass haylage and papilionaceous plants were fed *ad libitum* and supplemented with crushed oats grain. Summer feeding was based on grass forage *ad libitum* supplemented with oat grain. Sheep were milked twice a day with a portable milking unit. The milk is processed on site to final products such as rennet cheese. Samples (n=5) were collected from bulk tank milk of the whole herd, between the  $10^{\text{th}}$  and  $15^{\text{th}}$  day of each month. Sheep cheese is produced in a traditional manner, with the addition of rennet. After the curd has set, the coagulum is cut until the grain reached the size of 3-5 mm. About 20% of the whey is removed and water warmed to  $60^{\circ}$ C is added so that the cheese mass reaches the temp. of  $40-42^{\circ}$ C. The resulting curd is passed through a sieve into whey, which completely covers the cheese mass. After 16-20 h, cheese is transferred to brine. Batches of cheese that weigh about 3 kg are salted for 4-6 h (9.5% salt in the brine). When removed from the brine, they are left for two days to drain. Then, they are transferred for ripening. The samples of cheese (n=5) were taken randomly from the produced batches after a 30-day maturation period and just before cheese batches were sent for retail selling. All chemical measurements were made in triplicate

The basic chemical composition was determined in the samples of feed and cheese with standard methods. Cheese was analyzed for the moisture content by AOAC (1990), and the ash content was determined with the dry ash method (AOAC, 2005). The fat content in cheese samples was determined by the Gerber method and their total protein content was determined by measuring total nitrogen using the Kjeldahl method and converting it to the protein content by multiplying by 6.38 (AOAC, 2005). The nutritional value of feed was presented in IZ-INRA (1997) units (Table 1). In addition, the samples were tested for the content of  $\beta$ -carotene according to MANZ (1986) and the content of vitamin E according to PN-EN ISO 6867 (2002).

The content of dry matter, fat, protein and lactose was determined in the samples of milk with an instrumental method on a MilcoScan. Methyl derivatives were obtained from fat contained in feed (Soxhlet's method), milk (Roese-Gottlieb's method) and cheese (Schmid-Badzyński-Ratzlaff's method) and the profile of fatty acids was determined (Tables 1 and 2) with a gas chromatography (GC) method. In milk and cheese extracts obtained with the Folch's method, as described by RHEE et al. (1982), the content of cholesterol was determined in enzymatic reactions with cholesterol esterase and oxidase and with the use of Pointe Scientific tests. The content of vitamin A (retinol) and E ( $\alpha$ -tocopherol) in samples of milk and cheese was determined according to HEWAVITHARANA et al. (1996). The content of biogenic amines in the cheese samples was determined with the HPLC method on a Schimadzu apparatus with post-column derivatization involving ninhydrin (JOOSTEN, OLIEMAN 1986). The values of thrombogenic (IT) and atherogenic (IA) indices were calculated for milk and cheese fat according to the formulas proposed by Ulbricht and Southgate (1991).

The results were statistically processed in a one-factor model for the winter and summer periods. The significance of differences between the periods was verified with t-Student's test. Statistica 13.0 (StatSoft Inc. 2013) software was used for the calculations.

Specification	Haylage n=9	Oat grain n=9	Grass forage n=9		
Dry matter (g kg <sup>-1</sup> )	602.3	870.7	284.4		
Crude ash	80.3	26.5	64.4		
Crude protein	110.0	108.0	119.8		
Crude fat	16.3	46.7	18.2		
Crude fiber	334.7	114.9	301.7		
$\beta$ -carotene	290.4	0.7	546.5		
Vitamin E	16.4	5.4	40.7		
UFL	0.72	1.02	0.875		
PDIN	64.03	72.03	75.2		
PDIE	74.11	85.48	84.015		
C10:0	-	0.02	-		
C12:0	0.95	0.03	1.19		
C12:1	0.47	-	-		
C14:0	1.09	0.41	1.41		
C14:1	0.57	0.08	-		
C15:0	1.09	0.15	1.19		
C16:0	23.76	27.52	19.31		
C16:1	1.90	0.47	-		
C17:0	0.72	0.43	-		
C17:1	0.70	0.05	-		
C18:0	4.67	2.02	2.76		
C18:1	14.82	45.67	6.74		
C18:2 n-6	31.22	21.63	23.36		
C18:3 n-3	16.91	0.29	44.04		
C20:0	1.13	0.21	-		

Chemical composition (g kg <sup>-1</sup> DM), content of $\beta$ -carotene, vitamin E (mg kg <sup>-1</sup> DM),
PDIN and PDIE (g kg <sup>-1</sup> DM) of feed and composition of fatty acids (% of fatty acid sum)
in fat of feed used in winter and summer feeding of sheep

UFL – feed unit for milk production, PDIN – protein digested in the intestine subject to available nitrogen, PDIE – protein digested in the intestine subject to available energy

# **RESULTS AND DISCUSSION**

The content of nutrients in feed used for the winter and summer feeding of sheep did not diverge from the values presented by the National Research Council (2001). The content of  $\beta$ -carotene and vitamin E in fodder was over

	Milk				Cheese			
Specification	winter n=15		summer n=15		winter n=15		summer n=15	
	$\overline{x}$	SD	$\overline{x}$	SD	$\overline{x}$	SD	$\overline{x}$	SD
Dry matter (g <sup>-1</sup> kg <sup>-1</sup> )	16.04	1.02	16.07	1.70	$71.32^{x}$	3.85	$62.82^{y}$	4.18
Crude ash	-	-	-	-	$5.74^{X}$	0.28	$3.64^{Y}$	0.50
Crude protein	4.97	0.33	5.21	0.76	$27.06^{X}$	0.55	$23.66^{\circ}$	1.48
Crude fat	6.16	0.92	6.67	0.76	34.96	2.60	32.19	1.85
Lactose	$4.91^{A}$	0.35	$4.19^{B}$	0.28	-	-	-	-
Vitamin A	$0.98^{b}$	0.18	$1.44^{a}$	0.32	$1.64^{Y}$	0.86	$3.56^{X}$	0.68
Vitamin A*	$15.91^{b}$		$21.59^{a}$		$4.69^{Y}$		$11.09^{X}$	
Vitamin E	0.34 <sup>B</sup>	0.13	0.78 <sup>A</sup>	0.15	2.46	0.64	2.36	0.50
Vitamin E*	$5.52^{B}$		$11.69^{A}$		7.04		7.34	
Cholesterol	18.56	0.95	20.71	2.76	43.06	12.72	44.40	17.69
Cholesterol*	3.01		3.10		1.23		1.38	
Tyramine	-	-	-	-	$353.82^{X}$	13.09	75.63 <sup>y</sup>	15.38
Histamine	-	-	-	-	39.60 <sup>y</sup>	4.55	$102.28^{X}$	8.65
Putrescin	-	-	-	-	82.00 <sup>y</sup>	5.80	$169.85^{X}$	9.44
Cadaverine	-	-	-	-	$58.80^{\circ}$	2.81	$178.00^{X}$	9.33
Total biogenic amines	-	-	-	-	534.22	12.08	525.76	6.88

Chemical composition (%), content of vitamins: A, E (µg g <sup>-1</sup> of fat), cholesterol (mg 100<sup>-1</sup>g,) and biogenic amines (mg kg<sup>-1</sup>) in milk and cheese depending on the feeding season

A, B, X,  $Y - P \le 0.01$ ; a, b, x,  $y - P \le 0.05$ 

\* mg 100 g<sup>-1</sup> of fat

twice as high as in haylage. The fatty acid composition of haylage fat and especially in fodder was distinguished by a higher content of linoleic acid (LA) (C18:2 *n*-6) and  $\alpha$ -linolenic acid (ALA – C18:3 *n*-3), while oats had high oleic acid content (Table 1).

Milk from winter feeding contained by 15% more lactose (P<0.01) but less vitamin A (P≤0.05) and more than half the amount of vitamin E (P<0.01) determined milk from the summer period. Cheese from the winter feeding period had a higher content of dry matter by approximately 12% (P<0.05) and of minerals and protein by approximately 37 and 13%, respectively (P<0.01) almost 50% less vitamin A (P<0.05) than cheese produced during summer feeding (Table. 2).

JANDAL (1996), JELÍNEK et al. (1996), KONDYLI et al. (2012) reported various but mainly lower concentrations of vitamin A in milk, such as 8.25, 11.73, 5.07-7.40 mg g<sup>-1</sup> of fat, than the values recorded in this study (15.91 and 21.59 mg g<sup>-1</sup> fat). The higher concentration of vitamin A in milk achieved in this study (Table. 2) indicated that the fodder (Table 1) was a very good

Table 2

source of  $\beta$ -carotene, a provitamin of vitamin A. KONDYLI et al. (2007) also reported that winter milk had a lower content of vitamin A (by 14%) than summer milk, which was confirmed in our study. According to PERRETTI et al. (2004), the concentration of vitamin A (1.35-3.20 mg  $g^{-1}$ ) and vitamin E  $(0.86-6.83 \text{ mg g}^{-1})$  in Parmesan cheese was comparable to the value reported herein, and varied depending of the method of determination. The content of vitamin E in milk determined in our study was lower than reported by JELINEK et al. (1996), RAYNAL-LJUTOVAC et al. (2008) and KONDYLI et al. (2012) (average: 49.33, 16.13 and 31.35 mg vitamin E g<sup>-1</sup> fat). KONDYLI et al. (2012) reported that summer milk had a higher content of vitamin E (by 19%) than spring milk, which was confirmed by results of our research  $(11.69 \text{ vs } 5.52 \text{ µg wit. E g}^{-1} \text{ fat})$ . Grass forage was a good source of vitamin E, although the conversion of  $\alpha$ -tocopherol in milk was lower than expected. The feeding season influenced the concentration of specific biogenic amines in cheese. Cheese produced in winter had a higher concentration of tyramine (P < 0.01) than cheese produced in summer. In cheese produced in summer, the concentration of tyramine was twice as low as that of other biogenic amines (Table 2). Cheese from winter feeding contained three-fold less histamine and cadaverine ( $P \leq 0.01$ ) and over two-fold less putrescine ( $P \leq 0.01$ ) than cheese produced during summer feeding. The values of total biogenic amines in cheese from both seasons were similar (Table 2).

The content of total biogenic amines in cheese both seasons was similar and did not differ from the wide range of results provided by SCHIRONE et al. (2011), Spizzirri et al. (2013), or Poveda et al. (2015). Schirone et al. (2011) in cheese produced in winter (from December to March), SCHIRONE et al. (2013) and POVEDA et al. (2015), who reported that the content of total biogenic amines was 209-2393, 10.3-1483.1, 48-1881.7 mg kg<sup>-1</sup>, respectively. Moreover, these authors determined a high share of tyramine (over 40%). The content of this amine in cheese tested in our study was higher (66%) than the values reported by the above authors (Table 3). NOVELLA-RODRÍGUEZ et al. (2003) and GUARCELLO et al. (2015) informed about a lower content of tyramine (nd-301.4 and 5.9-69.3 mg kg<sup>-1</sup> respectively) in cheese produced from thermally processed milk. These authors suggested that the dominance of tyramine above the other amines may have been caused by numerous factors, such as the higher content of tyrosine in the amino acid composition of milk and, above all, because microorganisms favour decarboxylation of amino acids. Cheese produced in winter had twice as much histamine as cheese produced in summer, and comparably to the value reported by Schirone et al. (2011) and Spizzirri et al. (2013), Guarcello et al. (2015), POVEDA et al. (2015): 0.0-21.8; ND-19.1; 2-253, 23.2-100.1 mg kg<sup>-1</sup> respectively. Moreover, these authors determined a wide range of concentrations of putrescine: 0-668 mg kg<sup>-1</sup> and cadaverine: 1.1-80 mg kg<sup>-1</sup> in cheese. The content of putrescine and cadaverine in cheese was twice as high as the content of these amines in summer cheese tested in this experiment, and was within the ranges of values given by the aforementioned authors.

Composition of fatty acids in milk and cheese fat (%)

	Milk				Cheese			
Specification	winter		summer		winter		summer	
	n=15	SD	n=15	SD	n=15	SD	n=15	SD
1	2	3	4	5	6	7	8	9
C4:0	2.69	0.32	2.56	0.23	2.69	0.18	2.64	0.36
C6:0	2.61	0.37	2.13	0.31	$2.43^{x}$	0.21	1.99 <sup>y</sup>	0.21
C8:0	$2.59^{A}$	0.18	$1.95^{B}$	0.36	$2.45^{X}$	0.21	$1.79^{Y}$	0.20
C10:0	$8.66^{a}$	0.40	$6.45^{b}$	1.45	8.43 <sup>x</sup>	0.63	$5.85^{Y}$	0.92
C10:1	0.29	0.03	0.24	0.04	0.27	0.05	0.26	0.06
C12:0	$5.25^{a}$	0.33	$4.05^{b}$	0.77	$5.22^{X}$	0.41	$3.94^{Y}$	0.64
C12:1	0.07	0.05	0.08	0.04	0.06	0.05	0.09	0.03
C13:0	0.13	0.01	0.11	0.02	$0.13^{x}$	0.01	0.11 <sup>y</sup>	0.02
C14:0 iso	$0.13^{B}$	0.01	$0.16^{A}$	0.02	$0.13^{Y}$	0.01	$0.17^{X}$	0.01
C14:0	13.25	0.96	12.63	0.89	13.78	0.92	12.54	1.43
C14:1	0.25	0.05	0.26	0.06	0.41 <sup>y</sup>	0.25	$0.79^{x}$	0.04
C15:0	$1.22^{B}$	0.03	$1.41^{A}$	0.10	1.29	0,11	1.45	0.12
C16:0 iso	$0.27^{b}$	0.02	$0.31^{a}$	0.02	0.27	0,04	0.31	0.03
C16:0	28.66	1.30	29.33	1.37	28.66	1,23	29.87	1.28
C16:1	0.74	0.27	0.97	0.46	0.86 <sup>y</sup>	0,39	$1.45^{x}$	0.26
C17:0	0.64	0.06	0.71	0.07	0.66	0,08	0.72	0.07
C17:1	0.41	0.03	0.42	0.06	0.41	0,04	0.45	0.07
C18:0	9.39	0.86	10.70	1.79	9.21	0.87	10.56	2.14
C18:1	18.84	1.11	19.64	2.71	18.83	1.13	20.02	1.95
C18:2 <i>n</i> -6	1.93	0.11	1.71	0.20	1.91	0.14	1.58	0.28
C18:2 <i>n</i> -6 <i>cis9</i> <i>trans11</i> (CLA)	$0.58^{B}$	0.04	$1.91^{A}$	0.49	$0.57^{Y}$	0.06	$1.61^{X}$	0.14
C18:3 n-3	$0.74^{b}$	0.09	$1.22^{a}$	0.39	$0.66^{Y}$	0.14	$1.07^{X}$	0.10
C20:0	0.29	0.03	0.30	0.06	0.31	0.03	0.30	0.06
C20:1	$0.06^{b}$	0.01	$0.11^{a}$	0.04	$0.06^{Y}$	0.01	$0.09^{X}$	0.01
C20:2 <i>n</i> -6	$0.01^{B}$	0.03	$0.21^{A}$	0.06	0.00	0.00	0.00	0.00
C20:4 <i>n</i> -6	0.15	0.01	0.16	0.01	0.14	0.02	0.15	0.02
C20:5 <i>n</i> -3	$0.04^{B}$	0.01	$0.09^{A}$	0.01	0.04 <sup>y</sup>	0.01	$0.06^{x}$	0.02
C22:0	$0.08^{b}$	0.04	$0.14^{a}$	0.03	0.10 <sup>y</sup>	0.01	$0.13^{x}$	0.03
SFA	$75.87^{a}$	1.27	$72.96^{b}$	2.26	$75.76^{x}$	1.47	72.37 <sup>y</sup>	1.89
MUFA	20.68	1.24	21.73	2.28	20.92	1.29	23.15	1.89
PUFA	$3.46^{B}$	0.10	$5.30^{A}$	1.10	$3.32^{Y}$	0.22	$4.48^{X}$	0.30
UFA	$24.13^{b}$	1.27	$27.04^{a}$	2.26	24.24 <sup>y</sup>	1.47	$27.63^{x}$	1.89

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1	2	3	4	5	6	7	8	9
MUFA/SFA	0.27	0.02	0.30	0.04	$0.28^{y}$	0.02	$0.32^{x}$	0.03
PUFA/SFA	$0.05^{B}$	0.00	$0.07^{A}$	0.02	$0.04^{Y}$	0.00	$0.06^{X}$	0.00
UFA/SFA	$0.32^{b}$	0.02	$0.37^{a}$	0.04	$0.32^{y}$	0.03	$0.38^{x}$	0.04
PUFA/MUFA	$0.17^{b}$	0.01	$0.25^a$	0.06	$0.16^{Y}$	0.01	$0.19^{X}$	0.02
n-6 PUFA	$2.68^{B}$	0.11	$3.99^{A}$	0.71	$2.62^{Y}$	0.18	$3.35^{X}$	0.31
n-3 PUFA	$0.79^{b}$	0.09	$1.31^{a}$	0.40	$0.70^{Y}$	0.13	$1.13^{X}$	0.11
<i>n-</i> 6/n-3 PUFA	3.48	0.47	3.12	0.33	3.86	0.77	2.98	0.42
DFA (UFA+C18:0)	33.52	2.03	37.74	3.96	33.46 <sup>y</sup>	2.00	$38.19^{x}$	3.65
OFA (SFA–C18:0)	66.48	3.70	62.26	3.96	$66.54^{x}$	2.00	61.81 <sup>y</sup>	3.65
DFA/OFA	0.51	0.05	0.61	0.10	$0.50^{y}$	0.05	$0.62^{x}$	0.10
AI	$3.60^{A}$	0.18	$3.10^{B}$	0.24	$3.67^{X}$	0.18	$3.08^{Y}$	0.22
TI	$3.58^{A}$	0.21	$3.08^{B}$	0.28	$3.65^{X}$	0.27	$3.12^{\circ}$	0.24

cont. Table 3

A, B, X,  $Y - P \le 0.01$ ; a, b, x,  $y - P \le 0.05$ 

DFA – dietary fatty acids with a desirable (neutral or hypocholesterolemic) effect on humans, OFA – dietary fatty acids with an undesirable (hypercholesterolemic) effect on humans, AI – atherogenic index, TI – thrombogenic index

The content of putrescine and cadaverine could increase several times with a longer ripening time of cheese, mainly as a result of the increased number and activity of microflora (*Enterobacteriaceae*) (SCHIRONE et al. 2011). However, the results of a subsequent study by these authors (SCHIRONE et al. 2013) showed a lower amount of these amine in the long ripening cheese (10 months < 3 months). The increased concentration of putrescine, cadaverine and histamine during the summer feeding, although average compared with data from the literature (Table 2), could suggest an increased amount and activity of enzyme microflora in cheese. GUARCELLO et al. (2015) reported a different content of putrescine (594 and 5 mg kg<sup>-1</sup>), cadaverine (129 and 1.3 mg kg<sup>-1</sup>), histamine (253 and 2 mg kg<sup>-1</sup>) in pasteurized milk depending on the type of cheese. The content of each biogenic amine and the total content of all biogenic amines - in relation to the data reported by the above authors – implicated an inadequate extent of microbial decarboxylation of amino acids and therefore good quality of the assessed cheese.

The influence of the season of feeding on the fatty acid profile of milk fat and sheep's cheese was noted (Table 3). There were more medium-chained SFA (C8:0  $P \le 0.01$ , C10:0 and C12:0  $P \le 0.05$ ) in milk fat from winter feeding than in milk fat from the summer (Table 3). Milk fat obtained from summer milk contained more long-chained SFA than milk from winter feeding: C14:0 iso  $P \le 0.01$ , C15:0  $P \le 0.01$ , C16:0 iso  $P \le 0.05$ , C22:0  $P \le 0.05$ , and more PUFA including CLA (C18:2 *n*-6 *cis9trans*11  $P \le 0.01$ ), ALA (C18:3 *n*-3  $P \le 0.05$ ), as well as eicosadienic (C20:2 *n*-6  $P \le 0.01$ ) and EPA (C20:5 *n*-3  $P \le 0.01$ ). Fat from cheese produced in winter had a higher content of C6:0 (P < 0.05) and, similar to milk fat from this period, a higher content of medium-chained SFA (C8:0, C10:0, C12:0  $P \leq 0.01$ ) compared to cheese from summer feeding.

Cheese fat from summer feeding had a higher content of long-chained SFA - C14:0 iso ( $P \le 0.01$ ), C22:0 ( $P \le 0.05$ ), MUFA (C14:1, C16:1  $P \le 0.05$ , C20:1  $P \le 0.01$ ) and, same as milk from the summer – it contained more PUFA, including CLA (C18:2 *n*-6 *cis9trans*11  $P \le 0.01$ ), ALA (C18:3 *n*-3  $P \le 0.01$ ) and EPA (C20:5 *n*-3 0  $P \le 0.05$ ) than cheese fat from winter feeding. Milk and cheese fat from winter feeding had 4% more SFA ( $P \le 0.05$ ), and 11 and 12% less UFA ( $P \le 0.05$ ), respectively, while the content of PUFA was lower by 35% and 26%, respectively (P < 0.01), than milk and cheese fat from summer feeding. There was no seasonal change in the concentration of monounsaturated fatty acids (MUFA). During summer feeding, the proportion of UFA and PUFA in relation to SFA in milk and cheese (P < 0.05 and P < 0.01) was more beneficial than in winter. The calculated omega-6 to omega-3 ratio did not exceed 3-1 in milk or in cheese.

Cheese produced in the summer had more hypocholesterolemic acids (DFA  $P \le 0.05$ ) and less hypercholesterolemic acids (OFA  $P \le 0.05$ ). Thus, the DFA to OFA ratio was higher ( $P \le 0.05$ ) but atherogenic index (AI) was lower in cheese obtained in summer. In our study, the content of saturated fatty acids in the total fatty acid in sheep's milk fat did not differ from the values presented by the JANDAL (1996), ZERVAS and TSIPLAKOU (2011), KONDYLI et al. (2012).

The amount of medium-chained and long-chained SFA (C8:0, C10:0, C10:0 iso, C15:0, C16:0 iso, C20:0) and their seasonal variations were similar to the data reported by the cited authors. TALPUR et al. (2008) demonstrated considerable changes that occur throughout the year in the composition of fatty acids in milk obtained from sheep. These authors show that the SFA concentration (including acids for C6:0 to C14:0) was higher in winter months, and the MUFA, PUFA, CLA concentrations were higher in summer months. As viewed by the authors, fresh forage feeding in summer favours the synthesis of PUFA, including conjugated acids, which probably results from a change in the pathways of fatty acid bio-hydrogenation in the rumen. It is common that higher PUFA content, including PUFA n-3, in products obtained in the summer period results from a higher concentration of C18:3 n-3, which is the main acid in forage (Table 1). This acid is an initial substrate for trans-11 vaccenic acid (TVA) and conjugated cis-9, trans-11 C18:2 linoleic acid (CLA) in a bio-hydrogenation reaction in the rumen (LOCK, GARNSWORTHY 2003). Both metabolites generate an increase in  $\Delta 9$ -dehydrogenase activity. This enzyme catalyses the transformation of vaccenic acid into endogenous CLA in the mammary gland (Lock, GARNSWORTHY 2003). The study conducted on cows by CHION et al. (2010) showed huge influence of pastures on the increase of the concentration CLA in milk. KONDYLI et al. (2012) and SITZIA et al. (2015) determined higher concentrations of linolenic acid (by 16 and 19%) and CLA (by 4 and 42%) in the spring feeding season than in summer or autumn, and indicated a positive effect of early start of the grazing season and green forage feeding. In our study, milk obtained from the grazing season had a greater share of these acids (ALA and CLA) – Table 3. According to ELGERSMA et al. (2006), there was less CLA in winter (November to March) – about 3 mg kg<sup>-1</sup> fat - and more in summer milk fat (June to August) – about 7-8 mg kg<sup>-1</sup> fat. BIONDI et al. (2008) reported a treble increase of the levels of ALA and CLA, a more than 40% increase in PUFA but a 9% decreased share of SFA in milk fat after 23 days of sheep's grazing, which confirms the dynamics of the changes found in our study (Table 3). The content of the short (C4:0, 6:0), medium (C8:0, C10:0, C12:0) and long chain saturated fatty acids (C14:0, C16:0, C18:0) (% of total FAs) determined in cheeses (Table 3) was similar to the values shown by FERNANDEZ-GARCIA et al. (2006), by approx. 5.4, 9.3 and 23%) and by ZERVAS and TSIPLAKOU (2011), by approx. 3, 3.7 and 12.7%. ZERVAS and TSIPLAKOU (2011), MARRONE et al. (2014) and SITZIA et al. (2015) reported a lower concentration of SFA (61, 51.3, 49.4%) than the values recorded in our study (74%).

The values of MUFA and PUFA averaging 22 and 3.9% (Table 3) were lower than 32 and 7% (ZERVAS, TSIPLAKOU 2011) and 25.7 and 7% (SITZIA et al. 2015) but similar to 4.3 and 19.8% (MARRONE et al. 2014).

The content of PUFA in total fatty acids in cheese that were evaluated in this study was higher than reported in the literature, and marked the impact of a feed ration containing UFA (linseed, olive cake) on the fatty acid profile of fat sheep cheese (Branciari et al. 2012, VARGAS-BELLO-PÉREZ et al. 2013). Higher content of SFA had the impact of the UFA to SFA ratio (0.32 and 0.38) – Table 3 and was lower than 0.63, 0.47 and 0.66 reported by ZERVAS and TSIPLAKOU (2011), MARRONE et al. (2014), and SITZIA et al. (2015), respectively. Fat from cheese produced in winter had a higher content of SFA and lower PUFA including CLA and ALA ( $P \leq 0.01$ ), AA (arachidonic acid – C:20:4 n-6) and EPA (eicosapentaenoic acid – C 20:5 n-3)  $(P \leq 0.05)$  (Table 3) in comparison with cheese produced during summer feeding, which confirmed the results of FERNÁNDEZ-GARCÍA et al. (2006). Cheese produced in winter had 9-40% higher levels of medium-chain SFA and longchained SFA (12-22%), according to these authors. SITZIA et al. (2015) confirmed that the content of SFA was lower by 13-14% and PUFA higher by 16% in cheeses from the summer, which is consistent with our results (Table 3). The estimated n-6 to n-3 ratio was 3.1:1 in the winter and 2.62:1 in the summer feeding period (Table 3), being higher than that calculated by MARRONE et al. (2014) - 1.72:1, but this positive ratio, especially in summer, pointed to the pro-health quality of cheese. The values of AI (atherogenic index) and TI (thrombogenic index) – Table 3 are slightly higher than those determined by BRANCIARI et al. (2012) - 3.42 and 3.01, respectively, VARGAS-BELLO-PÉREZ et al. (2013) – 3.54 and 1.45, or MARRONE et al. (2014) – 2.75 and 1.90, but the values of both indices in cheese obtained in summer (Table 3) indicated their health-promoting quality.

# CONCLUSIONS

In conclusion, it was found that the feeding season significantly differentiated the chemical composition of both milk and cheese, but with regard to some of the examined characteristics. Significantly higher parameters in milk and cheese in favor of the summer season were noted in the case of vitamin A and E. Also in the summer season, higher values of biogenic amines in cheese, i.e. histamine, putrescin and cadaverine, were recorded. On the other hand, in the winter season, higher values were related to the chemical composition, i.e. dry matter, crude ash, crude protein and the amine called tyramine.

The positive effect of the summer season on the pro-health properties of milk and the rennet cheese made from it was found through the analysis of the fatty acid profile. In both cases, significantly higher values of unsaturated acids, i.e. MUFA, PUFA, UFA, were noted, as well as a more favourable ratio of PUFA to SFA, UFA to SFA, PUFA to MUFA and acids from the *n*-6 and *n*-3 families, with a very good mutual ratio of 3:1. Moreover, in the summer season, the ratio of DFA to OFA acids was better for human health.

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