The Journal of Animal & Plant Sciences, 25(3): 2015, Page: 633-639
ISSN: 1018-7081

THE EFFECT OF BREED, -LACTOGLOBULIN VARIANTS AND SOMATIC CELL COUNT ON YIELD, CHEMICAL COMPONENTS AND WHEY PROTEIN COMPOSITION IN MILK OF NON-DAIRY SHEEP

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ABSTRACT

The number of studies regarding the influence of genetic and environmental factors of ovine milk properties of non-dairy breeds is limited. The aim of this study was to determine the influence of breed, β-lactoglobulin variants and somatic cell count on the content of the principal milk ingredients and whey protein components. The study was carried out on 60 Polish Lowland and Polish Heath ewes, which come from the same flock and were kept under the same management and feeding practise. Both these breeds did not differ in respect of the principal components of milk. The milk of the Lowland sheep was characterized by a higher β-lactoglobulin/α-lactalbumin ratio compared to milk of Polish Heath breed. The β-lactoglobulin variants have affected the content of casein and lactose, which was higher in the milk of ewes with the BB genotype. The increase in somatic cell count decreased total protein, casein and β-lactoglobulin, and significantly increased the share of lactoferrin. The β-lactoglobulin variants and somatic cell count mainly affected the components of the protein fraction of milk.

Key words: sheep, milk ingredients, whey proteins, somatic cell count.

INTRODUCTION

Sheep milk is not only known for its high nutritional value, but also contains many biologically active ingredients. These ingredients in food products derived from ovine milk may exert beneficial health effects on consumers. Whey proteins like α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and lactoferrin (LF) are important endogenous antioxidants (Moatsu *et al*., 2005). In addition to the antibacterial and antiviral properties they may contribute to alleviation of depression, protection against cancer and stimulation of the immune system allowing rapid control of inflammation (Malaczewska and Rotkiewicz, 2007).

The content of principal ingredients as well as whey protein components of milk is determined by various genetic and environmental factors. Among them, the breed affects its quantity and quality. The research concerning the influence of breed on ovine milk composition indicated that the dairy breeds compared to non-milked produce milk with a lower fat and protein content (Bencini and Pulina, 1997; Patkowska-Sokola *et al*., 2005; Molik *et al.*, 2008). The differences in milk composition were observed also between indigenous sheep not bred specifically for milk production (Talpur *et al*., 2009). The yield, composition and technological properties of milk are also affected by β-LG polymorphism, which in sheep is not as widely recognized as in case of dairy cattle (Caroli *et al*., 2009). The polymorphism of ovine β-LG is determined by 3 alleles A, B and C. Alleles A and B are present in almost all the investigated breeds, whereas allele C is rather rare and confined to specific breeds such as Tajik, Merinoland, Lacha and Carranzana (Aliev and Koloteva, 1975; Calavia and Burgos, 1998; Erhardt, 1998). They are caused by a mutation changing the nucleotide sequence of the gene, which in turn affects the amino acid sequence of the protein. The substitution of Tyrosine by Histidine at position 20 of variant A results in variant B, while of Glutamine by Arginine at position 148 of variant A results in variant C (Moatsou *et al.*, 2005). The effect of β-LG polymorphism on milk quality was mostly described for dairy sheep from Mediterranean area (Amigo *et al.*, 2000), however not widely recognized in other sheep breeds focused on slaughter lambs production. Somatic cell count (SCC), as an indicator of the mammary gland health, has also impact on the yield and composition of milk. Many studies have shown that the increase of SCC, may lead to lowering its quality and functional values, as well as worsening technological properties of the ewe's milk. Milk with a high somatic cell count has a low rate of coagulation, thereby extending the process of cheese (Pirisi *et al.*, 2000; Nudda *et al.*, 2003; Rodriguez-Nogales *et al.*, 2007).

The number of studies on factors influencing the properties of sheep milk, particularly non-milked breeds is still limited. Therefore, the aim of this study, carried out at the same environmental and feeding conditions, was to determine the effect of breed, β-LG variants and SCC on the chemical composition of milk and content of some whey protein components of non-dairy sheep.

MATERIALS AND METHODS

The study was conducted on two breeds of sheep: Polish Heath (30 ewes) as an indigenous breed characterized by a small figure and harmonious body conformation and Polish Lowland sheep (30 ewes) with typical meat-and-wool purpose that were kept in one flock. All sampled sheep were at the age of 3-4 years and in the 4^{th} week of lactation. Length of lactation in both studied breeds lasted for a period of rearing lambs that is for three months.

The animals were fed on winter diet (October to May). The ewes of both breeds received grain mix containing: oat meal (30%), barley meal (40%), wheat meal (10%), wheat bran (20%), grass hay and the addition of oat straw and mineral mixture. The chemical composition, nutritional value and the fatty acid content of the feed are presented in Table 1. Ewes were fed twice a day and they had permanent access to water.

Table 1. The composition of feed used in the nutrition.

Feed composition (g kg^{-1})	Grain mix	Grass hay
Dry matter	892.4	908.2
Crude protein	118.0	88.0
Ether extract	17.2	13.6
Crude fiber	48.9	345.5
Ash	20.0	68.1

For two hours before milk collection, the lambs were separated from their dams, and then ewes were hand-milked and whole milk from udder was collected and mixed. Representative samples (100 mL) of milk taken for each ewe (a total of 60 samples) were placed in a sterile bottle preserved with Mlekostat CC and immediately transported to the laboratory (Milk Testing Laboratory of Warsaw University of Life Sciences) for composition analysis.

During the rearing period, milk production of ewes was estimated basing on the weight gain of lambs from 2^{nd} to 28^{th} days of age, using a conversion factor of 4.5 liter milk consumed per kg weight gain achieved (Molik *et al*., 2008). Because of breed differences (differences in animal exterior) milk yield was calculated for 1 kilogram of metabolic weight.

Each milk sample was analyzed for fat, protein, casein, lactose and total solid (TS) content with IR spectrometry using Milkoscan FT – 120 instrument (Foss Electric, Hillerod, Denmark).

SCC in milk was determined by flow cytometry using Somacount – 150 (Bentley, Warsaw, Poland).

Chemical composition of fodder was analyzed according to procedure AOAC (1990).

Whey proteins were examined using high-performance liquid chromatography (Agilent 1100 series; Agilent Technologies, Waldbronn, Germany) equipped with a UV-VIS detector with variable wave length, and column Supelcosil LC-318 Supelco and Supelguard (Supelco, Bellefonte, Pennsylvania, USA), with particle size 5 μm, pore size 300 Å according to the methodology described by Puppel *et al.* (2012). For the determination of the whey protein the following eluents were used: solutions A, 0.1% TFA trifluoroacetic acid in acetonitrile-water (5:95); and B, 0.1% TFA in solution acetonitrile-water (95:5). The conditions for linear gradient elution were: 0-32% B for 10 min, 32-52% B for 25 min, and 52-80% for 3 min. The flow rate of the mobile phase was 1.2 ml min^{-1}, and the separation was carried out at ambient temperature. Compounds in whey proteins were identified using an ultraviolet detector at 220 nm wavelength.

The data obtained were analyzed statistically by the analysis of variance and Tukey's test of the SPSS 14.0 packet software. The model used for milk composition, whey proteins content and fatty acids analysis was:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times B_j) + (A_i \times C_k) + (B_j \times C_k) + e_{ijkl}$$

where: Y_{ijkl} – dependent variable; μ - general mean; A_i – the effect of breed (i = Heath or Lowland sheep); B_j – the effect of β-LG variant (j = AA, AB, BB); C_k – the effect of SCC (k = 1- 4 in which class 1- to 100x10^3 SCC mL^{-1}, class 2- 101-200x10^3 SCC mL^{-1}, class 3- 200-500x10^3 SCC mL^{-1}, class 4- above 500x10^3 SCC mL^{-1}); (A_i x B_j) – the interaction between breed and β-LG variant; (A_i x C_k) – the interaction between breed and SCC; (B_j x C_k) – the interaction between β-LG variant and SCC; e_{ijkl} – random error.

The distribution of β-LG genotypes in studied sheep breeds was tested using χ^2 procedures.

RESULTS AND DISCUSSION

Despite the differences in morphological traits, both studied non-dairy breeds did not differ in milk production potential, especially in its performance for the first month of lactation per 1 kg^{-1} metabolic weight (Table 2).

The differences in the basic composition of milk, i.e. fat, protein, lactose and total solids content between Polish Heath and Polish Lowland ewes were not observed either (Table 2). Comparing the composition of the milk of the investigated sheep with the results obtained by other authors for different breeds the greatest differences were observed in fat content. In the experiment conducted by Radzik-Rant *et al.* (2011) the milk of Polish Merino and especially milked Polish Mountain Sheep in the 4th week of lactation contained less fat (78.2, 70.4 kg^{-1} vs. 89.1, 82.1 kg^{-1}) than the Polish Heath and Polish Lowland sheep in the present study. Considerably higher fat content of up to 126.0 kg^{-1} was reported by Wohlt *et al.* (1981) in Dorset ewes. The level of fat in the milk can be varied not only depending on breed or lactation stage but also a level of feeding and even milking time phase (Park *et al.*, 2007; Molik *et al.*, 2008). The milk from studied breeds did not differ in respect of casein content (Table 2). Its share of the total amount of protein in the milk of Polish Heath and Polish Lowland was 0.89 and 0.87 respectively. A slightly smaller value was obtained by Nudda *et al.* (2003) in the milk of dairy Sarda sheep.

Although there was no difference in the content of whey proteins like β-LG and α-LA in milk of examined breeds, the ratio β-LG/α-LA was higher (P <0.05) in Lowland sheep milk (Table 2). Moatsou *et al.* (2005) marked a greater β-LG/α-LA ratio in the milk of the milked Greek breeds: Frisarta, Karugouniko and Boutsiko, which was 5.56, 5.08, 6.95 respectively.

Only in the milk of Chios ewes the relationship between those two whey proteins was the closest (3.11) to the value for Lowland sheep. In the milk of investigated sheep breeds the share of lactoferrin was the lowest in comparison to other determined whey proteins. In studies performed by Qian *et al.* (1995) and Nudda *et al.* (2003), the content of that extremely biologically active ingredient was either similar or less.

Table 2. The milk yield, chemical composition and bioactive components content in milk

Item	Heath sheep n=30	Lowland sheep n=30	SE
	Mean	Mean	
Milk yield (kg up to 28th day of rearing period)	32.32	40.35	2.09
Milk yield (kg kg^{-1} metabolic body weight)	2.12	1.93	0.12
Fat (g kg^{-1})	89.1	82.1	2.5
Protein (g kg^{-1})	49.7	51.3	0.5
Lactose (g kg^{-1})	52.1	51.5	0.5
Total solids (g kg^{-1})	200.4	195.1	2.4
Casein (g kg^{-1})	44.6	45.1	0.4
α-LA (g L^{-1})	2.4	2.31	0.11
β-LG (g L^{-1})	7.26	7.79	0.46
β-LG/α-LA ratio	3.04[a]	3.58[b]	0.16
Lactoferrin (g L^{-1})	0.27	0.24	0.03

α-LA - α-lactalbumin; β-LG - β-lactoglobulin

[a,b] mean values in row with different superscripts were significantly different (P <0.05)

In the conducted study three β-LG genotypes (AA, AB and BB) were identified in both breeds, and the frequency of which was varied according to race (Table 3).

Table 3. The distribution of β-lactoglobulin genotypes and allele frequencies of studied sheep breeds

Breed		Genotypic frequencies			Allele frequencies		
		AA	AB	BB	¡^{2}	A	B
Heath sheep	Observed	2	16	12	1.27	0.33	0.67
(n=30)	Expected	3.3	13.2	13.5	DF=1		
Lowland sheep	Observed	8	20	2	4.54	0.60	0.40
(n=30)	Expected	10.8	14.4	4.8	DF=1		

χ^2 - chi-square value; n - number of sheep; DF - degrees of freedom

In Heath sheep more than half of the animals in the sample, and approximately 66% in the Lowland sheep had AB genotype. In Heath sheep the second large group was ewes with genotype BB, while homozygotes AA accounted for a small proportion. An inverse relationship was observed in the second breed, in which AA homozygotes were the second largest group, and the proportion of ewes with genotype BB in the sample was small. Similar results were recorded by Mroczkowski *et al.* (2004) in Polish Merino breed. Celik and Özdemir (2006) in Awassi and Morkaraman sheep also confirmed the largest share of individuals with heterozygous AB. Gouda *et al.* (2011) in a study conducted on the cow and buffalo milk have indicated that BB variant of β-LG was the most frequent. Whereas, Patel *et al.* (2007) in four buffalo breeds similarly to mention above sheep breeds, found the highest frequencies of AB genotype.

The effect of β-LG variants on the milk components is shown in Table 4. Milk with genotype BB was characterized by the highest content of casein (P <0.05) and lactose (P <0.05) compared to AB and AA genotypes. Despite the lack of statistical significance milk with β-LG BB had higher content of total protein, β-LG and α-LA. Variants of β-LG did not significantly affect the content of fat.

The data available in the literature on the impact of β-LG variants on the composition and properties of milk are different. Many studies have concluded that β-LG AA is associated with a higher content of TS, fat, protein and casein, while β-LG BB has a positive effect on milk yield (Amigo *et al.*, 2000). Schmoll *et al.* (1999) in a study conducted on the Friesian milk sheep have indicated that individuals with genotype BB produce milk with higher protein content. The similar results were obtained by Mroczkowski *et al.* (2004) in Polish Merino breed. The higher protein content in milk of ewes with BB genotype was also recorded in the present study. Generally, a higher content of ingredients in the milk of sheep with the genotype BB compared to AA and AB has been found previously by Di Stasio *et al.* (1997). In contrast, Dario *et al.* (2008) indicated that higher content of basic components in milk of sheep is related to genotype AA and AB than BB, while Yousefi *et al.* (2013) in indigenous Zel sheep found the highest amount of fat and lactose for AB genotype. Nudda *et al.* (2003) in research on milk of Sarda sheep have reported that the influence of β-LG genotypes is associated only with milk yield, and the highest daily milk production was observed in sheep with genotype AB.

Table 4. The milk yield, chemical composition and bioactive components content in milk with different variants of β-lactoglobulin

Item	AA n=10 Mean	AB n=36 Mean	BB n=14 Mean	SE
Milk yield (kg up to 28th day of rearing period)	37.93	43.44	38.57	4.24
Fat (g kg^{-1})	87.2	85.4	84.7	3.7
Protein (g kg^{-1})	49.7	49.3	53.1	1.2
Lactose (g kg^{-1})	49.8^{a}	52.1^{b}	52.4^{b}	0.6
Total solids (g kg^{-1})	201.3	196.6	197.7	3.6
Casein (g kg^{-1})	43.2^{b}	44.2^{b}	47.1^{a}	0.9
α-LA (g L^{-1})	2.21	2.39	2.41	0.2
β-LG (g L^{-1})	6.94	7.32	8.25	0.4
Lactoferrin (g L^{-1})	0.24	0.28	0.22	0.05

α-LA - α-lactalbumin; β-LG - β-lactoglobulin

^{a,b} mean values in row with different superscripts were significantly different (P <0.05)

The similar results regarding the performance of milk for the first month of lactation, although not confirmed statistically, were observed in the present study. The research on cow milk also demonstrated the impact of β-LG polymorphism on its yield and composition. Hristov *et al.* (2013) showed that BB genotype determines higher milk production. Contrary to those findings Cardak (2005) reported that cows having AB genotype produce more milk than cows of AA and BB genotypes. In cow and buffalo milk the higher protein content was associated with BB of β-LG genotype (Cardak, 2005; Gouda *et al*., 2011).

In the conducted study no influence of the β-LG variant on milk bioactive components has been found. But Dario *et al.* (2008) in research on milk of local Leccese sheep reported that β-LG AA genotype is related to higher whey protein content. The higher amount of these proteins has been found in buffalo milk with β-LG AB genotype (Gouda *et al*., 2011).

The results of the impact of SCC on the amount of principal and functional components of milk are shown in Table 5. The high SCC decreased the level of casein and total protein in milk (P <0.05), which can be attributed to the growth of proteolytic processes along with the increase in the number of somatic cells. The increase in proteolytic activity is associated with higher activity of plasmin in udders of deteriorating health (Albenzio *et al.*, 2004). Kelly and Foley (1997) also indicate the increased activity of other proteases derived from somatic cells.

The casein decline with the increase of SCC was also noted by Rodriguez-Nogales *et al.* (2007) in milk of Assaf, Churra and Castellana breeds. Nudda *et al.* (2003) in the investigations on the influence of number of somatic cells on the protein fraction in the milk of Sarda ewes found out that the increase of SCC reduces the proportion of casein, while whey protein content increases.

In the present study, together with the increase of SCC, the content of β-LG (P <0.05) decreased, while the level of α-LA slightly increased (statistically not confirmed) (Table 5). Whereas, Pirisi *et al.* (2000) and Nudda *et al.* (2003) noted that increasing SCC increased both β-LG and α-LA content.

Table 5. The milk yield, chemical composition and bioactive components content in milk with different somatic cell count

Item	Somatic cell count (SCC) (mL^{-1})				SE
	<100 x10³ n=25	101-200 x10³ n=10	201-500x10³ n=10	>500x10³ n=15	
	Mean	Mean	Mean	Mean	
Milk yield (kg up to 28^{th} day of rearing period)	47.21	37.35	33.43	33.14	5.13
Fat (g kg^{-1})	86.1	85.0	86.3	83.9	6.3
Protein (g kg^{-1})	53.3^a	48.7	48.1	44.6^b	1.4
Lactose (g kg^{-1})	53.1	51.3	50.7	51.7	1.1
Total solids (g kg^{-1})	199.2	199.0	192.7	195.9	6.0
Casein (g kg^{-1})	47.0^a	44.1	42.3^b	40.6^b	0.8
α-LA (g L^{-1})	2.19	3.03	2.3	2.42	0.23
β-LG (g L^{-1})	8.12^b	8.23^b	6.7	5.76^a	0.54
Lactoferrin (g L^{-1})	0.16^b	0.26^b	0.28^b	0.53^a	0.04

α-LA - α-lactalbumin; β-LG - β-lactoglobulin

[a,b] mean values in row with different superscripts were significantly different (P <0.05)

It should be emphasized that in the above-mentioned studies the marked increase in these proteins occurred in the milk of class more than 1 000 000 SCC. This is mainly attributed to their higher concentration due to the reduction in milk yield, rather than enhanced synthesis. The reduction of the content of β-LG and α-LA with the SCC increase in milk of cows was reported by Bernatowicz *et al*. (2004) and in the milk of sheep by Rodriguez-Nogales *et al.* (2007), but in the class of SCC above 2 500 000.

As expected, the content of lactoferrin, protein having strong antibacterial properties, belonging to the non-specific cellular immunity, increased (P <0.05) with the increase of SCC (Table 5). This is due to the fact that milk lactoferrin originated from blood neutrophil degranulation (Neville and Zhang, 2000), the number of which increases with increased SCC (Cuccuru *et al.*, 1997), and from the synthesis in the mammary gland (Schanbacher *et al.*, 1997). The increase of LF content with increasing of SCC in the sheep and cow milk was also recorded by Duranti and Casoli (1991), Nudda *et al.* (2003) and Bernatowicz *et al.* (2004).

Conclusion: The results indicated that the breed did not affect the principal milk ingredients. Differences are related only to the main whey proteins ratio. The β-LG variants and SCC mainly affected the total protein level and the components of protein fraction of milk. Although milk from non-dairy sheep is not used in processing, its composition may affect the growth rate and quality of suckling lamb carcasses.

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