*Scientific Annals of Polish Society of Animal Production - Vol. 10 (2014), No 4, 151-158*

# The effect of β-lactoglobulin variants on the chemical composition and fatty acid profile of the milk of Wrzosówka ewes

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**The aim of this study was to determine the relationship between β-lactoglobulin (β-LG) polymorphism and the composition and fatty acid profile of milk. The study was carried out on 30 Wrzosówka ewes aged 3-4 years. Milk samples were collected during the 4th week of lactation and analysed for basic composition, whey protein content and fatty acid composition. Detailed analysis of milk proteins enabled β-LG variants to be determined. Three β-LG genotypes (AA, AB and BB) were identified in the Wrzosówka ewes. The frequency of allele variant B was twice as high as that of allele variant A. Milk with genotype BB was characterized by higher content of casein (P≤0.05) and lactose (P≤0.01). Milk with β-LG BB and AB had higher (P≤0.05) content of MUFA. The highest content of polyunsaturated fatty acids and *n*-3 PUFA in the milk was associated with the AB genotype, although the highest LCFA content, including C18:0 and C18:1 *cis*9, was found in the BB genotype. No definitive relationship between β-lactoglobulin variant and fatty acid content was determined.**

**KEY WORDS: milk / sheep / β-lactoglobulin / fatty acid**

Beta-lactoglobulin (β-LG) is the main whey protein in the milk of ruminants. It is also present in the milk of many mammalian species, but not in human milk [9]. Studies on various livestock species have shown that it is a polymorphic protein. Beta-lactoglobulin polymorphism in sheep is determined by three alleles, A, B and C. The first two are common, while the last is found only in a few breeds. Their presence is due to a mutation that alters the nucleotide sequence of the gene, which in turn affects the amino acid sequence in the protein. Substitution of Tyr by His in position 20 in

variant A results in variant B, while substitution of Gln by Arg in position 148 results in variant C [10].

Beta-lactoglobulin plays an important role in the transfer of passive immunity to the neonate and regulates phosphorus metabolism in the mammary gland. It is also a source of cysteine, which is involved in the formation of glutathione [5]. In addition, the protein may be a carrier of retinol, as well as triglycerides, fatty acids, vitamin D, and cholesterol [8]. By binding free fatty acids, β-LG also facilitates the digestion of milk fat.

In ruminants used for dairy purposes, many studies have examined the link between genetic variants of β-LG and the composition and technological properties of milk. The results of studies on the effect of variants of this protein on the characteristics of sheep milk, which remain in the minority as compared to cow milk, are conflicting. Some authors confirm the effect of β-LG variants on milk composition [1, 4, 11], while others report that they are associated only with the level of production [12, 14]. While numerous studies have examined the association between β-lactoglobulin variants and milk yield or its chemical composition, including the protein fraction, the influence of this protein on fatty acid content has been the subject of little research.

The aim of the study was to evaluate the relationship of β-lactoglobulin polymorphism with the composition of milk and the fatty acid profile of its fat fraction.

## Material and methods

The study was carried out on 30 ewes of the Polish Heath Sheep breed from the Agricultural Experimental Station in Żelazna. Ewes were fed according to standards for lactating ewes. The basic feed was a concentrate mixture and grass hay. In addition, the ewes received straw and a mineral and vitamin mixture. The chemical composition and nutritional value of the basic feeds are given in Table 1.

**Table 1**
Chemical composition and nutritional value of fodder

| Specification | Cereal meal | Grass hay |
|---|---|---|
| Dry matter (%) | 89.24 | 90.82 |
| Crude protein (%) | 11.8 | 8.8 |
| Ether extract (%) | 1.72 | 1.36 |
| Crude fibre (%) | 4.89 | 34.55 |
| UFL/kg DM | 1.06 | 0.6 |
| PDI/kg DM | 69 | 68 |

UFL – feed unit for milk production; PDI – protein digestible in the small intestine

Milk samples were collected in the 4th week of lactation from ewes aged 3-4 years. The ewes were milked manually after being separated from lambs for 2 hours. A 100 ml sample of the milk was taken to determine its chemical composition and the content of casein, whey proteins and fatty acids.

The percentage content of basic milk constituents, i.e. protein, fat, lactose, dry matter and casein, was determined in a Foss Electric Milkoscan FT analyser by infrared spectrophotometry.

Whey proteins were determined using an Agilent 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a variable wavelength UV-VIS detector and a Supelcosil LC-318 column (Sigma-Aldrich), according to the method described by Puppel et al. [16]. The following eluents were used to determine the main whey proteins: solution A – mixture of 0.1% TFA (Merck) in acetonitrile with water (5:95); solution B – mixture of acetonitrile with water (95:5). Phase flow: 1.0 ml/min, UV detection at 220 nm.

Milk fat was extracted by the Röse-Gotlieb method according to AOAC [2]. Separation and quantitative analysis of fatty acid methyl esters were performed by gas-liquid chromatography using a Hewlett Packard 5890 gas chromatograph with a flame ionization detector and a DD 23 column (60 m length, 0.25 mm inner diameter and 0.25 μm film thickness). The following conditions were used for separation of fatty acids: carrier gas (helium) 20 cm/s; detector temperature 240ºC; injector temperature 220ºC; split 1:40.

The separation was conducted at a programmed temperature: initial temperature of 130ºC/min; temperature increase from 130 to 210ºC at 10ºC/min; isotherm 210°C for 25 min; temperature increase from 210 to 230ºC at 2.5ºC/min; isotherm 230°C for 18 min. Identification and quantitative analysis of fatty acids were carried out using Sigma and Supelco standards.

The effect of the β-lactoglobulin variant on milk constituents and the content of fatty acids was assessed by one-way analysis of variance in SPSS, 2003. Statistical evaluation of differences between β-LG variants was performed using Duncan's test. The frequency of β-lactoglobulin genotypes in the ewes was tested by the $\chi^2$ test.

## Results and discussion

The detailed analysis of the milk proteins made it possible to determine the genetic variants of β-lactoglobulin. Three β-LG genotypes (AA, AB and BB) were found in the Polish Heath ewes. More than half of the individuals in the sample had the AB genotype, 40% had the BB genotype, and the fewest had the AA genotype. The frequency of the B allele was twice as high as that of the A allele. The value of the $\chi^2$ test was statistically insignificant (Table 2).

The AB genotype of β-lactoglobulin has been found dominant in Polish Merino crossbreds from rams of prolific breeds – Piwczyński et al. (15); in Polish Merinos – Mroczkowski et al. [11]; and in Awassi and Morkaraman sheep – Celik and Ozdemir [3]. In the case of the Awassi and Morkaraman sheep, there was also a high proportion of individuals

**Table 2**
The distribution of β-lactoglobulin genotypes and allele frequencies in the ewes

| | Genotype frequency | | | Allele frequency | | |
|---|---|---|---|---|---|---|
| | AA | AB | BB | $\chi^2$ | A | B |
| Number observed | 2 | 16 | 12 | 1.27 | 0.33 | 0.67 |
| Number expected | 3.3 | 13.2 | 13.5 | | | |

with the AA genotype, and the frequency of the A allele was much higher than that of the B allele, in contrast to the Polish Heath Sheep. In a study by Kawęcka and Radko [7] on genetic polymorphism of β-LG in Polish Mountain and Friesian sheep, heterozygous individuals with genotype AB were predominant. In the Polish Mountain sheep population, the AA and BB genotypes were present with equal frequency, while in the Friesian sheep more individuals had genotype AA than BB.

Analysis of the effect of β-lactoglobulin genetic variants on milk constituents revealed that ewes with the BB genotype produced milk with higher content of casein (P≤0.05), lactose (P≤0.01) and total protein, although in the case of protein the differences were not statistically significant (Table 3). This was not confirmed by Dario et al. [4] in their study on the Altamurana breed, which indicated that the AB variant of β-LG had a beneficial

**Table 3**
The effect of β-lactoglobulin variant on milk composition

| Milk composition | AA | | AB | | BB | |
|---|---|---|---|---|---|---|
| | LSM | SE | LSM | SE | LSM | SE |
| Fat (%) | 9.58 | 0.96 | 8.74 | 0.34 | 9.1 | 0.39 |
| Protein (%) | 4.98 | 0.25 | 4.82 | 0.09 | 5.11 | 0.1 |
| Casein (%) | 4.44 | 0.14 | $4.36^{a}$ | 0.05 | $4.58^{a}$ | 0.06 |
| Lactose (%) | $4.81^{AB}$ | 0.09 | $5.12^{B}$ | 0.03 | $5.24^{A}$ | 0.04 |
| Dry matter (%) | 20.45 | 0.85 | 19.82 | 0.3 | 20.32 | 0.35 |
| Lactofferin (g/l) | 0.21 | 0.14 | 0.3 | 0.05 | 0.26 | 0.06 |
| α-lactoalbumin (g/l) | 2.36 | 0.39 | 2.33 | 0.14 | 2.5 | 0.16 |
| β-lactoglobulin (g/l) | 7.21 | 1.05 | 6.75 | 0.37 | 7.81 | 0.43 |

Values in the same row designated with the same letter differ significantly at: capital letters P≤0.01; lower-case letters P≤0.05

effect on protein, casein and lactose content. As in the present study, Mroczkowski et al. [11] obtained higher protein content in the milk of Polish Merino sheep with the BB genotype. Research on various breeds of dairy sheep in the Mediterranean Basin concerning the effects of β-lactoglobulin variants on milk constituents did not provide conclusive results, as indicated in a review article by Amigo et al. [1].

Analysis of the effects of β-LG variants on the fatty acid profile, which was the primary subject of our study, revealed that ewes with the BB and AB genotypes produced milk with higher content (P≤0.05) of monounsaturated fatty acids (MUFA) than ewes with the AA genotype. The AB genotype was associated with the highest proportion of polyunsaturated fatty acids (PUFAs) and *n-3* PUFAs, although statistical differences were noted only in comparison with the AA genotype. The milk of ewes with the AA or AB genotype had higher content of (P≤0.05) long-chain fatty acids (LCFA) than that of ewes with the AA genotype (Table 4).

**Table 4**
The effect of β-lactoglobulin variant on the content of fatty acid groups (g/100 g fat)

| Specification | AA | | AB | | BB | |
|---|---|---|---|---|---|---|
| | LSM | SE | LSM | SE | LSM | SE |
| SFA | 52.44 | 5.21 | 55.79 | 2.61 | 58.97 | 3.30 |
| MUFA | 28.53$^{ab}$ | 3.38 | 36.99$^{a}$ | 1.69 | 39.52$^{b}$ | 2.14 |
| PUFA | 3.54$^{a}$ | 0.78 | 5.35$^{a}$ | 0.39 | 4.51 | 0.49 |
| PUFA *n-3* | 0.79$^{a}$ | 0.13 | 1.12$^{a}$ | 0.,06 | 0.96 | 0.08 |
| PUFA *n-6* | 2.34 | 0.66 | 3.70 | 0.33 | 3.03 | 0.42 |
| LCFA | 39.48$^{ab}$ | 4.36 | 49.95$^{a}$ | 2.18 | 53.82$^{b}$ | 2.76 |

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; LCFA – long chain fatty acids
Values in the same row designated with the same letter differ significantly at: capital letters P≤0.01; lower-case letters P≤0.05

Among this group of acids, the highest content of stearic acid (C18:0) and oleic acid (*cis*9 C18:1) was found in the milk of ewes with the BB genotype, while the content of linoleic acid isomer *cis*9, *trans*11 C18:2 was positively associated with both the AB and BB variants of β-lactoglobulin (Table 5).

A study by Mele et al. [9] on the relationship between β-LG variants and the fatty acid profile in the milk of the Italian breed Massese found the highest proportion of MUFA, LCFA and *trans* C18:1 in individuals with the AB genotype, while β-lactoglobulin polymorphism had no effect on PUFA content. The same study found a higher proportion of C18:0, C18:2 and *cis*9, *trans*11 C18:2 in the milk of sheep with the AB genotype of β-lactoglobulin. In our study, the BB genotype showed the highest correlation with the content of LFCA in milk fat and with fatty acids of this group.

**Table 5**
The effect of β-lactoglobulin variant on the content of long chain fatty acids (g/100 g fat)

| Fatty acids | AA | | AB | | BB | |
|---|---|---|---|---|---|---|
| | LSM | SE | LSM | SE | LSM | SE |
| C18:0 | 9.92[a] | 1.30 | 10.82[b] | 0.65 | 13.20[ab] | 0.82 |
| C18:1 *trans*11 | 1.84 | 0.25 | 2.31 | 0.13 | 2.30 | 0.16 |
| C18:1 *cis*9 | 23.42[ab] | 2.83 | 30.50[ac] | 1.41 | 32.87[bc] | 1.79 |
| C18:2 | 1.95 | 0.61 | 3.16 | 0.30 | 2.57 | 0.39 |
| C18:3 *n-6* | 0.17 | 0.02 | 0.20 | 0.01 | 0.21 | 0.01 |
| C18:3 *n-3* | 0.49 | 0.08 | 0.60 | 0.04 | 0.56 | 0.05 |
| CLA | 0.41[a] | 0.05 | 0.54[a] | 0.03 | 0.52 | 0.03 |
| C20:1 | 0.23 | 0.03 | 0.22 | 0.01 | 0.24 | 0.02 |
| C20:4 *n-6* | 0.22 | 0.06 | 0.33 | 0.03 | 0.25 | 0.04 |
| C20:3 *n-3* | 0.10 | 0.03 | 0.14 | 0.02 | 0.17 | 0.02 |
| C20:5 | 0.03 | 0.01 | 0.03 | 0.00 | 0.03 | 0.00 |
| C22:5 | 0.11 | 0.10 | 0.30 | 0.05 | 0.16 | 0.06 |
| C22:6 | 0.06 | 0.01 | 0.05 | 0.01 | 0.04 | 0.01 |

Values in the same row designated with the same letter differ significantly at: capital letters P≤0.01; lower-case letters P≤0.05

The ability of β-LG to bind fatty acids is due to the calyx structure of this protein, consisting of an 8-stranded anti-parallel β-sheet surrounded by four flexible, movable loops regulating access to the β-barrel interior (calyx) [13]. The interior of the β-barrel and the groove between the α-helix and the barrel are potential binding sites for hydrophobic compounds, which include fatty acids [6].

Apart from the study on the milk of the Italian breed Massese, cited above, we found no studies on the association between β-LG polymorphism with fatty acid content. It is therefore difficult to confirm the ability of specific β-LG variants to bind particular fatty acids. Differences in fatty acid content depending on the β-LG variant also cannot be associated with the amount of β-LG in milk. The polymorphism of this protein did not affect its content in the milk of the ewes. Although sheep with the BB genotype had the most β-LG, the differences between genotypes were statistically insignificant (Table 2). Mele et al. [9] also observed no significant link between β-LG variants and its content in the milk of dairy sheep of the Italian breed Massese, and the amount of this protein was almost identical for the analogous genotypes in the Polish Heath Sheep.

To sum up, the results of our study are consistent with some earlier studies indicating the influence of β-LG polymorphism on the content of certain constituents of milk. The β-LG variants were shown to be associated with the content of MUFA, PUFA, LCFA and individual fatty acids. However, we found no clear association between the

lipids tested and the β-lactoglobulin genetic variant, suggesting the need for further research to attain a better understanding of the biological role of this protein in fatty acid metabolism.

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