

## **The association of genetic variants of $\beta$ -lactoglobulin and $\kappa$ -casein with yield and chemical composition of milk obtained from four breeds of cow\***

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The aim of the study was to determine  $\kappa$ -casein and  $\beta$ -lactoglobulin polymorphism in cows of four breeds, including two pure dairy breeds (Polish Black-and-White Holstein-Friesian and Jersey) and two local breeds (Polish Red and White-backed) and to evaluate the relationships between the genetic variants of these fractions, yield and chemical milk composition. Two  $\beta$ -lactoglobulin alleles (A and B) were identified in the breeds studied. Allele B was clearly dominant in the Polish Red cows (0.73), while in the Jersey breed a higher frequency of allele A was observed (0.62). The proportion of alleles A and B in the Polish Holstein-Friesian and White-backed cows was close to 1:1. Two alleles (A and B) were also identified for  $\kappa$ -casein. The frequency of allele B was highest in the Jersey cows (0.74), and relatively high in the local breeds, i.e. Polish Red and White-backed (above 0.4). The frequency of this allele was considerably lower in the Holstein-Friesians (0.28). The presence of the allele A BLG was associated with significantly higher milk yield ( $p \leq 0.01$ ), protein content ( $p \leq 0.01$ ) and dry matter ( $p \leq 0.05$ ) content. Cows with the AA BLG genotype produced 4.21 kg more milk with higher content of dry matter (by 0.62 p.p.), including protein (by 0.28 p.p.), compared with the BB BLG homozygotes. Cows with allele A of the CSN3 gene also produced significantly more milk ( $p \leq 0.05$ ), but with lower concentration of dry matter ( $p \leq 0.05$ ,  $p \leq 0.01$ ). The BB homozygotes produced 2.9-4.32 kg less milk, but with higher dry matter content (by 0.29-1.33 p.p.), including fat (by 0.12-0.52 p.p.) and protein (by 0.13-0.51 p.p.), compared with the AA CSN3 homozygotes. Allele B CSN3 was also linked to higher casein content in the milk.

**KEY WORDS:** cow breed / polymorphism of  $\beta$ -lactoglobulin (BLG) and  $\kappa$ -casein (CSN3) / milk composition

Among milk proteins displaying genetic polymorphism, four casein fractions ( $\alpha$ S1,  $\alpha$ S2,  $\beta$  and  $\kappa$ ) and two whey fractions ( $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin) can be distin-

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\*Abridged doctoral thesis

guished. In cattle the casein loci are located on chromosome 6 (6/BTA 6q31-33) and together occupy a DNA fragment 200 kb in length [26]. These genes are arranged in the order CSN1S1, CSN2, CSN1S2, CSN3 and encode  $\alpha$ s1-casein,  $\beta$ -casein,  $\alpha$ s2-casein and  $\kappa$ -casein, respectively. They are closely associated with one another, forming a cluster [10]. Beta-lactoglobulin is encoded by the gene BLG located on chromosome 11 (11q28), which consists of 7 exons and is about 6,700 bp in length [29]. The LALBA gene encoding  $\alpha$ -lactalbumin is located on chromosome 5 (BTA5q12-13), consists of four exons, and is 3,061 bp in length [6]. Of all milk protein fractions only the polymorphic forms of two, i.e. kappa-casein and beta-lactoglobulin, are considered genetic markers of quantitative traits [25, 37]. The greatest importance in dairy technology is ascribed to  $\kappa$ -casein (CSN3), where two alleles generally occur—A and B. The differences between them are caused by two point mutations involving the substitution of isoleucine for threonine at position 136 of the polypeptide chain and of alanine for aspartic acid at position 148 [14, 31]. Many authors [4, 6, 7] report that cows with the B allele of the gene CSN3 produce milk with higher protein content, including casein; it has a faster rennet clotting time and produces a more compact curd. In terms of polymorphic forms, however, the most varied protein is  $\beta$ -lactoglobulin, and the most frequently identified alleles are A and B [25]. The difference between variants A and B is that the A allele at position 64 of the amino acid sequence contains aspartic acid, while B contains glycine. At position 118, valine is present in the A variant and alanine in the B variant [19]. This fraction, alongside  $\kappa$ -casein, arouses the most interest among researchers.

There are currently 12 cow breeds used for dairy purposes in Poland, with Polish Black-and-White Holstein-Friesians comprising about 90% of the active population. In a few herds cows of the Jersey breed are raised as well. As compared to large dairy breeds, Jersey cows produce the same amount of milk per 100 kg of body weight, but 30-50% more fat and 20-30% more protein [33]. Due to its high concentration of dry matter components, the milk of these cows is a valuable material for the dairy industry. In addition to highly productive breeds, local breeds of cattle are raised as well, including Polish Red and White-backed. These cows are less productive, but are exceptionally well suited to local climate conditions and traditional farming systems, displaying numerous advantages, such as resistance to disease, longevity and high fertility [20].

The aim of the study was to determine the polymorphism of  $\beta$ -lactoglobulin and  $\kappa$ -casein in four breeds of cow, including two pure dairy breeds (Polish Black-and-White Holstein-Friesian and Jersey) and two local breeds (Polish Red and White-backed) and to evaluate the relationships between the genetic variants of these protein fractions and both daily yield and the chemical composition of the milk.

### Material and methods

The material for the study consisted of blood and milk samples. In order to determine  $\beta$ -lactoglobulin and  $\kappa$ -casein polymorphism, peripheral blood samples were collected once from the external jugular vein of 213 cows, including 63 Polish Holstein-Friesians (PHF HO) and 50 each of the Jersey (JE), Polish Red (RP) and White-backed (BG)

breeds. The blood samples were collected into vacuum tubes containing  $K_3$ EDTA as an anticoagulant. DNA was isolated from the blood using a QIAamp DNA Blood Mini Kit by QIAGEN.

Beta-lactoglobulin (BLG) genotypes were determined by PCR-RFLP according to Medrano and Aquilar-Cordova [21], and kappa-casein (CSN3) genotypes according to Medrano and Aquilar-Cordova [22].

The results obtained were used to determine the numbers of  $\beta$ -lactoglobulin and  $\kappa$ -casein genotypes and the frequency of alleles and genotypes according to Hardy-Weinberg. To establish whether the population was in a state of genetic equilibrium, the results were compared with the expected value by the chi-squared test [35].

From each cow for which  $\beta$ -lactoglobulin and  $\kappa$ -casein genotypes were determined, milk samples from a complete milking procedure were collected as well. They were collected during test-day milking (AT4 method) in two production seasons: spring/summer (May-July) and autumn/winter (November-March). The somatic cell count (SCC) in the milk was determined with a Bentley Somacount 150 apparatus in order to eliminate milk samples with SCC over 400,000/ml. The following were determined in the milk: content of fat, protein, lactose and dry matter, with a Bentley Infrared Milk Analyzer, and casein content according to the AOAC method [1]. The relationships between genetic variants of  $\beta$ -lactoglobulin and chemical composition were evaluated using 675 milk samples, including 210 from Polish Black-and-White Holstein-Friesian cows, 144 from Jersey cows, 181 from Polish Red and 140 from White-backed. For genetic variants of  $\kappa$ -casein, 737 milk samples were analysed, including 206 from Polish Black-and-White Holstein-Friesians, 188 from Jersey cows, 181 from Polish Red and 162 from White-backed.

Data pertaining to the daily milk yield of the cows in the months when the samples were collected were obtained from records (RW-2 reports on milk performance evaluations) maintained by the Polish Federation of Cattle Breeders and Dairy Farmers.

Statistical computations were performed in StatSoft Inc. STATISTICA software ver. 6. The analyses were based on a General Linear Model (GLM)—an ANOVA procedure for factorial designs with interaction. Significance of differences between means was determined by Tukey's test at  $p \leq 0.05$  and  $p \leq 0.01$ .

## **Results and discussion**

Analysis of polymorphism of the  $\beta$ -lactoglobulin and  $\kappa$ -casein genes revealed substantial genetic variation between the breeds evaluated (Tab. 1 and 2). In the case of  $\beta$ -lactoglobulin, the presence of three genotypes was observed, AA, AB and BB, determined by the two alleles A and B (Tab. 1). In the breeds evaluated (except for Polish Red) AB heterozygotes predominated, with a share ranging from 44.44% in the Polish Black-and-White Holstein-Friesians to 50% in the White-backed breed. In the Polish Red breed homozygous BB animals were dominant (52%), with AA homozygotes accounting for only 6%. In the White-backed and Polish Black-and-White Holstein-Friesian cows the ratio of A and B alleles was close to 1:1 (0.55; 0.45 and 0.48; 0.52, respectively), while the A allele was clearly dominant in the Jersey breed (0.62), and the B allele in Polish Red

**Table 1**

Frequencies of  $\beta$ -lactoglobulin alleles and observed and expected distribution of genotypes in the analysed cow breeds

| Breed  | Genotype | Number of cows observed (head (%)) | Allele frequency | Expected genotype frequencies | Theoretical number of cows | $\chi^2$ |
|--------|----------|------------------------------------|------------------|-------------------------------|----------------------------|----------|
| PHF HO | AA       | 16 (25.4)                          | A=0.48           | AA=0.2268                     | 14.29                      | 0.5690   |
|        | AB       | 28 (44.4)                          | B=0.52           | AB=0.4989                     | 31.00                      |          |
|        | BB       | 19 (30.2)                          |                  | BB=0.2744                     | 17.85                      |          |
| JE     | AA       | 19 (38)                            | A=0.62           | AA=0.3844                     | 19.22                      | 0.0174   |
|        | AB       | 24 (48)                            | B=0.38           | AB=0.4712                     | 23.56                      |          |
|        | BB       | 7 (14)                             |                  | BB=0.1444                     | 7.22                       |          |
| RP     | AA       | 3 (6)                              | A=0.27           | AA=0.0729                     | 3.65                       | 0.0000   |
|        | AB       | 21 (42)                            | B=0.73           | AB=0.3942                     | 19.71                      |          |
|        | BB       | 26 (52)                            |                  | BB=0.5329                     | 26.65                      |          |
| BG     | AA       | 15 (30)                            | A=0.55           | AA=0.3025                     | 15.13                      | 0.0053   |
|        | AB       | 25 (50)                            | B=0.45           | AB=0.4950                     | 24.75                      |          |
|        | BB       | 10 (20)                            |                  | BB=0.2025                     | 10.13                      |          |

PHF HO – Polish Black-and-White Holstein-Friesian

JE – Jersey

RP – Polish Red

BG – White-backed

(0.73)—Table 1. Oner and Elmaci [28], Heidari et al. [17] and Tsiaras [36] confirm the predominance of the AB heterozygote in the cow populations analysed. Heidari et al. [17] report that the frequency of the AB  $\beta$ -lactoglobulin genotype in a herd of Holstein-Friesian cows was 0.54; the frequency of the A allele was 0.53 and that of the B allele was 0.47. Zatoń-Dobrowolska et al. [38], in a study on the polymorphism of the BLG gene in Polish, German and Czech Red cattle, found a higher frequency of the B allele in these breeds: 0.81, 0.68 and 0.56, respectively. Barłowska et al. [5] also noted a high frequency of the B allele of BLG in Polish Red cows (0.72), whereas the frequency of the A and B alleles in the White-backed breed was identical (0.50).

The chi-squared test revealed that genetic equilibrium was maintained within BLG in all breeds analysed (Tab. 1).

In the case of  $\kappa$ -casein three genotypes were detected, AA, AB and BB, determined by the two alleles A and B (Tab. 2). In most of the breeds (except for Jersey) there was a higher frequency of the A allele (from 0.51 in Polish Red to 0.72 in Polish Holstein-Friesian). In the population of Jersey cows, however, the frequency of the B allele was markedly higher than that of the A allele (0.74 and 0.26, respectively). In this breed BB homozygotes were dominant (54%), while the fewest such animals were identified in the Polish Holstein-Friesian breed (under 5%)—Table 2.

**Table 2**

Frequencies of  $\kappa$ -casein alleles and observed and expected distribution of genotypes in the analysed cow breeds

| Breed  | Genotype | Number of cows observed (head (%)) | Allele frequency | Expected genotype frequencies | Theoretical number of cows | $\chi^2$ |
|--------|----------|------------------------------------|------------------|-------------------------------|----------------------------|----------|
| PHF HO | AA       | 31 (49.2)                          | A=0.72           | AA=0.5184                     | 35.44                      | 3.5822   |
|        | AB       | 29 (46.0)                          | B=0.28           | AB=0.4032                     | 23.63                      |          |
|        | BB       | 3 (4.8)                            |                  | BB=0.0784                     | 3.94                       |          |
| JE     | AA       | 3 (6)                              | A=0.26           | AA=0.0680                     | 3.40                       | 0.0824   |
|        | AB       | 20 (40)                            | B=0.74           | AB=0.3848                     | 19.24                      |          |
|        | BB       | 27 (54)                            |                  | BB=0.5476                     | 27.38                      |          |
| RP     | AA       | 13 (26)                            | A=0.51           | AA=0.2601                     | 13.01                      | 0.0000   |
|        | AB       | 25 (50)                            | B=0.49           | AB=0.4998                     | 24.99                      |          |
|        | BB       | 12 (24)                            |                  | BB=0.2401                     | 12.01                      |          |
| BG     | AA       | 17 (34)                            | A=0.57           | AA=0.3249                     | 16.25                      | 0.1884   |
|        | AB       | 23 (46)                            | B=0.43           | AB=0.4902                     | 24.51                      |          |
|        | BB       | 10 (20)                            |                  | BB=0.1849                     | 9.25                       |          |

PHF HO – Polish Black-and-White Holstein-Friesian

JE – Jersey

RP – Polish Red

BG – White-backed

The chi-squared test showed genetic equilibrium in CSN3 in all breeds analysed (Tab. 2).

The results of the present study are confirmed in both the domestic and foreign literature. Botaro et al. [8] showed that the share of AA, AB and BB kappa-casein genotypes was 66.83%, 31.84% and 1.33%, respectively in Holstein cattle and 71.38%, 27.90% and 0.72% in the Girolando breed. The A allele occurred more frequently than the B allele in both the Holstein (0.83) and Girolando (0.85) breeds. Molee et al. [26], in an analysis of cow populations with varying contributions of Holstein-Friesian genes (G1 up to 87.5% and G2 over 87.5%), observed higher frequency of the A allele in both groups. Anggraeni et al. [3], in a study on Holstein-Friesian cows in West Java, confirmed the higher frequency of the A allele (0.64). The results of the present study on polymorphism of the CSN3 gene in the Jersey breed correspond with those obtained by Ren et al. [32]. They identified only two genotypes (AB and BB) in this breed, due to the low frequency of the A allele (0.11). Poulsen et al. [30] also report low frequency of the AA genotype in this breed (0.04). The CSN3 AB genotype was present with a frequency of 0.33, and the BB genotype with a frequency of 0.63. Similar results as in the present study (for Polish Red cows) were obtained by Zatoń-Dobrowolska et al. [38], who showed that in cows of the Czech Red breed the frequency of the A allele was 0.56. Jann et al. [18] reported even higher frequency of the

**Table 3**  
Relationships between genetic variants of  $\beta$ -lactoglobulin and daily milk yield and chemical composition of milk from the analysed cow breeds

| Breed                   | Genotype         | n   | Daily milk yield (kg) |      | Fat (%)           | Protein (%) |                   | Casein (%) | Lactose (%)        |      | Dry matter (%) |      |                     |      |
|-------------------------|------------------|-----|-----------------------|------|-------------------|-------------|-------------------|------------|--------------------|------|----------------|------|---------------------|------|
|                         |                  |     | $\bar{x}$             | SD   |                   | $\bar{x}$   | SD                |            | $\bar{x}$          | SD   | $\bar{x}$      | SD   | $\bar{x}$           | SD   |
| PHF HO                  | AA               | 62  | 25.61 <sup>bb</sup>   | 5.38 | 4.35              | 0.81        | 3.50              | 0.52       | 2.56               | 0.35 | 4.79           | 0.20 | 13.21               | 1.17 |
|                         | AB               | 78  | 23.39 <sup>aa</sup>   | 7.18 | 4.28              | 0.74        | 3.41              | 0.46       | 2.57               | 0.42 | 4.73           | 0.21 | 13.14               | 1.14 |
|                         | BB               | 70  | 21.62 <sup>a</sup>    | 5.37 | 4.20              | 0.57        | 3.26              | 0.33       | 2.64               | 0.36 | 4.77           | 0.21 | 13.03               | 0.80 |
| JE                      | AA               | 52  | 21.93                 | 4.94 | 5.54 <sup>b</sup> | 0.82        | 4.18              | 0.44       | 3.05 <sup>ab</sup> | 0.51 | 4.81           | 0.24 | 15.41 <sup>b</sup>  | 0.93 |
|                         | AB               | 68  | 21.47                 | 4.26 | 5.03 <sup>a</sup> | 0.81        | 4.07              | 0.37       | 3.00 <sup>a</sup>  | 0.36 | 4.80           | 0.21 | 14.61 <sup>a</sup>  | 1.03 |
|                         | BB               | 24  | 19.65                 | 6.14 | 4.91 <sup>a</sup> | 0.90        | 4.02              | 0.35       | 3.19 <sup>b</sup>  | 0.44 | 4.79           | 0.25 | 14.42 <sup>a</sup>  | 1.08 |
| RP                      | AA               | 8   | 13.67                 | 6.02 | 4.45              | 0.49        | 3.46              | 0.31       | 2.54               | 0.39 | 4.86           | 0.18 | 13.45               | 0.57 |
|                         | AB               | 72  | 13.58                 | 5.15 | 4.41              | 0.66        | 3.42              | 0.44       | 2.57               | 0.42 | 4.83           | 0.29 | 13.39               | 0.89 |
|                         | BB               | 101 | 12.28                 | 4.76 | 4.32              | 0.72        | 3.43              | 0.39       | 2.62               | 0.38 | 4.77           | 0.30 | 13.30               | 0.96 |
| BG                      | AA               | 42  | 14.32                 | 3.20 | 4.17              | 0.93        | 3.49              | 0.55       | 2.49 <sup>a</sup>  | 0.43 | 4.70           | 0.35 | 13.05               | 1.21 |
|                         | AB               | 64  | 13.29                 | 3.11 | 4.13              | 0.91        | 3.43              | 0.45       | 2.58 <sup>ab</sup> | 0.41 | 4.61           | 0.39 | 12.87               | 1.33 |
|                         | BB               | 34  | 12.07                 | 2.44 | 3.91              | 0.93        | 3.36              | 0.57       | 2.65 <sup>b</sup>  | 0.40 | 4.59           | 0.43 | 12.58               | 1.38 |
| Total                   | AA               | 164 | 20.97 <sup>b</sup>    | 7.81 | 4.67              | 0.88        | 3.72 <sup>b</sup> | 0.51       | 2.71 <sup>a</sup>  | 0.44 | 4.77           | 0.26 | 13.87 <sup>b</sup>  | 1.30 |
|                         | AB               | 282 | 17.39 <sup>ab</sup>   | 7.00 | 4.46              | 0.84        | 3.58 <sup>b</sup> | 0.52       | 2.69 <sup>a</sup>  | 0.44 | 4.75           | 0.27 | 13.53 <sup>ab</sup> | 1.18 |
|                         | BB               | 229 | 16.76 <sup>a</sup>    | 7.77 | 4.28              | 0.83        | 3.44 <sup>a</sup> | 0.45       | 2.75 <sup>b</sup>  | 0.41 | 4.74           | 0.29 | 13.25 <sup>a</sup>  | 1.17 |
| Influence of the factor | breed            |     | ***                   |      | ***               |             | ***               |            | ***                |      | ***            |      | ***                 |      |
|                         | genotype         |     | *                     |      | *                 |             | *                 |            | *                  |      | ns             |      | *                   |      |
|                         | breed x genotype |     | ns                    |      | **                |             | ns                |            | ns                 |      | ns             |      | *                   |      |

A, B – differences significant at  $p \leq 0.01$ ; a, b – differences significant at  $p \leq 0.05$ . Influence of the factor: \* – at  $p \leq 0.05$ ; \*\* – at  $p \leq 0.01$ ; \*\*\* – at  $p \leq 0.001$ ; ns – not found  
PHF HO – Polish Black-and-White Holstein-Friesian; JE – Jersey; RP – Polish Red; BG – White-backed

**Table 4**  
Relationships between genetic variants of  $\kappa$ -casein and daily milk yield and chemical composition of milk from the analysed cow breeds

| Breed                   | Genotype         | n   | Daily milk yield (kg) |      |                    | Fat (%)   |                    |      | Protein (%)        |      |                    | Casein (%) |                     |      | Lactose (%) |    |  | Dry matter (%) |    |  |
|-------------------------|------------------|-----|-----------------------|------|--------------------|-----------|--------------------|------|--------------------|------|--------------------|------------|---------------------|------|-------------|----|--|----------------|----|--|
|                         |                  |     | $\bar{x}$             | SD   |                    | $\bar{x}$ | SD                 |      | $\bar{x}$          | SD   |                    | $\bar{x}$  | SD                  |      | $\bar{x}$   | SD |  | $\bar{x}$      | SD |  |
| PHF HO                  | AA               | 104 | 24.13 <sup>B</sup>    | 6.21 | 4.26 <sup>a</sup>  | 0.80      | 3.37 <sup>a</sup>  | 0.38 | 2.63 <sup>a</sup>  | 0.45 | 4.80               | 0.20       | 12.91 <sup>a</sup>  | 1.16 |             |    |  |                |    |  |
|                         | AB               | 90  | 22.51 <sup>AB</sup>   | 6.98 | 4.31 <sup>a</sup>  | 0.88      | 3.50 <sup>ab</sup> | 0.44 | 2.70 <sup>ab</sup> | 0.42 | 4.73               | 0.22       | 13.21 <sup>ab</sup> | 0.88 |             |    |  |                |    |  |
|                         | BB               | 12  | 19.81 <sup>A</sup>    | 4.54 | 4.40 <sup>b</sup>  | 0.60      | 3.74 <sup>b</sup>  | 0.41 | 2.82 <sup>b</sup>  | 0.34 | 4.73               | 0.13       | 13.35 <sup>b</sup>  | 1.37 |             |    |  |                |    |  |
| JE                      | AA               | 12  | 22.45                 | 5.06 | 5.09 <sup>a</sup>  | 0.68      | 4.03               | 0.39 | 3.01               | 0.38 | 4.77               | 0.30       | 14.61               | 1.18 |             |    |  |                |    |  |
|                         | AB               | 80  | 21.12                 | 4.34 | 5.06 <sup>a</sup>  | 0.91      | 4.09               | 0.38 | 3.07               | 0.44 | 4.79               | 0.24       | 14.75               | 0.89 |             |    |  |                |    |  |
|                         | BB               | 96  | 19.91                 | 4.59 | 5.21 <sup>b</sup>  | 0.77      | 4.16               | 0.35 | 3.11               | 0.42 | 4.83               | 0.21       | 14.90               | 0.94 |             |    |  |                |    |  |
| RP                      | AA               | 42  | 14.86 <sup>B</sup>    | 5.64 | 4.24 <sup>a</sup>  | 0.67      | 3.38 <sup>b</sup>  | 0.37 | 2.47               | 0.41 | 4.89 <sup>b</sup>  | 0.27       | 13.13               | 0.93 |             |    |  |                |    |  |
|                         | AB               | 91  | 13.20 <sup>AB</sup>   | 5.08 | 4.44 <sup>ab</sup> | 0.75      | 3.47 <sup>a</sup>  | 0.42 | 2.58               | 0.39 | 4.76 <sup>a</sup>  | 0.31       | 13.46               | 0.95 |             |    |  |                |    |  |
|                         | BB               | 48  | 11.96 <sup>A</sup>    | 4.53 | 4.52 <sup>b</sup>  | 0.64      | 3.59 <sup>a</sup>  | 0.39 | 2.69               | 0.39 | 4.76 <sup>a</sup>  | 0.26       | 13.52               | 0.77 |             |    |  |                |    |  |
| BG                      | AA               | 52  | 14.20 <sup>C</sup>    | 4.35 | 3.79 <sup>a</sup>  | 0.78      | 3.21 <sup>A</sup>  | 0.43 | 2.35 <sup>A</sup>  | 0.38 | 4.63 <sup>A</sup>  | 0.33       | 12.52 <sup>A</sup>  | 1.01 |             |    |  |                |    |  |
|                         | AB               | 75  | 12.45 <sup>B</sup>    | 4.06 | 4.18 <sup>ab</sup> | 1.07      | 3.54 <sup>B</sup>  | 0.51 | 2.44 <sup>B</sup>  | 0.39 | 4.59 <sup>A</sup>  | 0.41       | 13.05 <sup>B</sup>  | 1.40 |             |    |  |                |    |  |
|                         | BB               | 35  | 10.91 <sup>A</sup>    | 4.24 | 4.31 <sup>b</sup>  | 0.66      | 3.72 <sup>B</sup>  | 0.49 | 2.78 <sup>B</sup>  | 0.36 | 4.92 <sup>B</sup>  | 0.26       | 13.85 <sup>C</sup>  | 0.87 |             |    |  |                |    |  |
| Total                   | AA               | 210 | 19.73 <sup>b</sup>    | 7.40 | 4.21 <sup>A</sup>  | 0.70      | 3.41 <sup>A</sup>  | 0.44 | 2.60 <sup>B</sup>  | 0.42 | 4.78 <sup>ab</sup> | 0.26       | 12.97 <sup>A</sup>  | 1.02 |             |    |  |                |    |  |
|                         | AB               | 336 | 17.41 <sup>b</sup>    | 7.57 | 4.49 <sup>A</sup>  | 0.92      | 3.64 <sup>B</sup>  | 0.52 | 2.71 <sup>ab</sup> | 0.44 | 4.73 <sup>a</sup>  | 0.29       | 13.60 <sup>A</sup>  | 1.30 |             |    |  |                |    |  |
|                         | BB               | 191 | 16.26 <sup>a</sup>    | 5.85 | 4.81 <sup>B</sup>  | 0.85      | 3.86 <sup>C</sup>  | 0.49 | 2.89 <sup>b</sup>  | 0.45 | 4.82 <sup>b</sup>  | 0.23       | 14.21 <sup>B</sup>  | 1.20 |             |    |  |                |    |  |
| Influence of the factor | breed            |     | ***                   |      | ***                |           | ***                |      | ***                |      | **                 |            | ***                 |      |             |    |  |                |    |  |
|                         | genotype         |     | *                     |      | *                  |           | *                  |      | *                  |      | *                  |            | *                   |      |             |    |  |                |    |  |
|                         | breed x genotype |     | **                    |      | ns                 |           | **                 |      | ***                |      | ns                 |            | **                  |      |             |    |  |                |    |  |

A, B, C – differences significant at  $p \leq 0.01$ ; a, b – differences significant at  $p \leq 0.05$ . Influence of the factor: \* – at  $p \leq 0.05$ ; \*\* – at  $p \leq 0.01$ , \*\*\* – at  $p \leq 0.001$ ; ns – not found  
PHF HO – Polish Black-and-White Holstein-Friesian, JE – Jersey, RP – Polish Red, BG – White-backed

A allele (0.60) in Polish Red cows—nearly twice that of the B allele (0.33). Barłowska et al. [5], analysing a population of White-backed cows, found that the frequency of the B allele of  $\kappa$ -CN was 0.35. A high frequency of the B allele in local breeds is also reported by Caroli et al. [11]. Their analysis of polymorphism in the CSN3 gene in the local Italian breed Reggiana revealed a frequency of 0.51 for the B allele.

The data in Table 3 show that the presence of the BLG A allele was associated with significantly higher daily yield ( $p \leq 0.01$ ) as well as content of protein ( $p \leq 0.01$ ) and dry matter ( $p \leq 0.05$ ) in the milk. Cows with the AA BLG genotype produced 4.21 kg more milk with higher content of dry matter (by 0.62 p.p.), including protein (by 0.28 p.p.), as compared to the BB homozygote. The analysis performed within individual breeds revealed similar relationships, although they were rarely confirmed statistically. This was most likely due to the smaller numbers of samples in individual genetic groups. In the case of daily yield, these differences were statistically confirmed only in the Holstein-Friesian breed, and in the case of fat and dry matter in the Jersey breed. Moreover, Jersey and White-backed cows with the BB BLG genotype were found to produce milk with statistically ( $p \leq 0.05$ ) higher casein content as compared with the AA homozygote (by 0.14 and 0.16 p.p., respectively).

With respect to the CSN3 gene, the animals with the A allele also produced significantly ( $p \leq 0.05$ ) more milk, but with a lower concentration of dry matter components ( $p \leq 0.05$ ,  $p \leq 0.01$ )—Table 4. BB homozygotes were found to produce 2.9–4.32 kg less milk, but with higher content of dry matter (by 0.29–1.33 p.p.), including fat (by 0.12–0.52 p.p.) and protein (by 0.13–0.51 p.p.), as compared to the CSN3 AA homozygote. The B allele of CSN3 was also linked to higher casein content in the milk. However, this was confirmed statistically only in the case of two breeds, Holstein-Friesian and White-backed, where the content of this milk component was higher in comparison with the CSN3 AA homozygote, by 0.19 and 0.43 p.p., respectively.

Significant interactions of breed  $\times$  BLG genotype were noted for content of fat ( $p \leq 0.01$ ) and dry matter ( $p \leq 0.05$ ), and interactions of breed  $\times$  CSN3 genotype for daily yield and content of protein and dry matter ( $p \leq 0.01$ )—Tables 3 and 4.

Literature data on the relationship between genetic variants of  $\beta$ -lactoglobulin and milk yield and its chemical composition are not conclusive, and are often even conflicting. Miciński and Kluczyński [24] confirm a positive relationship between the BLG AA genotype and milk yield. Different results were obtained by Molina et al. [27], who found that the BLG AB genotype was associated with higher milk yield. Ahmadi et al. [2], on the other hand, found no correlation between genetic variants of BLG and milk yield in the population analysed. Many authors investigating the relationship between genetic variants of BLG and the content of basic milk components link variant B to higher content of protein and fat [2, 12]. Molina et al. [27] and Heck et al. [16], on the other hand, report a positive association between the A variant of BLG and the total protein concentration in milk. Michalcova et al. [23] observed no relationship between polymorphic forms of BLG and the chemical composition of milk. Reasons for the substantial disparities in the results cited may be found in the non-additive interaction of alleles from various loci, or they may be due to the interaction of genotypes with environmental factors. The vast majority of authors link casein content to the BLG BB genotype. The difference in the content of this component reaches about +0.1 p.p. in comparison with the AA genotype [9, 23].

Results pertaining to the relationships between genetic variants of  $\kappa$ -casein (CSN3) and milk performance traits are more consistent. Most studies [2, 16] have confirmed that the A allele of  $\kappa$ -casein (in both the homo- and heterozygous variants) is associated with higher milk yield. The presence of the B allele, on the other hand, is linked to higher protein content (including casein) in the milk, which makes it a better material for cheese production [4, 5, 8, 15, 34, 36]. Debeljak et al. [13], in an attempt to explain the differences between the A and B alleles, claim that expression of the gene is probably regulated at the post-translational level. AB heterozygotes display different synthesis of the two alleles in the mammary gland—more protein is encoded by the B allele. The authors found a higher level of mRNA for the CSN3 B allele, which may result in higher protein content in the milk of cows with the CSN3 BB genotype. Most studies have shown that  $\kappa$ -casein polymorphism is mainly linked to milk yield and its protein content, and also to the yield and content of fat. Ahmadi et al. [2], investigating the relationships between genetic variants of  $\kappa$ -casein and the yield and quality indicators of the milk of Holstein-Friesian cows, found (as in the present study) that AA homozygotes were characterized by significantly ( $p \leq 0.05$ ) higher milk production, but with lower protein content. However, similar relationships were not found for fat content. Botaro et al. [8], on the other hand, found no correlation between the polymorphic form of the CSN3 gene and the concentration of dry matter components in the milk of Brazilian Holstein-Friesians. Only the content of fat was found to be higher in the milk of the CSN3 AB heterozygote than in that of the other genotypes (3.38% vs 3.25% and 3.14%, respectively). Barłowska et al. [5] also found no association between the presence of polymorphic forms of the gene CSN3 and the chemical composition of the milk of Polish Red cows. In the case of White-backed cows, however, they found significantly ( $p \leq 0.05$ ) higher protein content in the milk of animals with CSN3 BB and AB genotypes as compared to AA (4.09% and 3.67% vs 3.34%, respectively).

To sum up, the analysis of polymorphism of the  $\beta$ -lactoglobulin and  $\kappa$ -casein genes revealed considerable genetic differentiation between the cow breeds analysed. Moreover, the genetic variants of these proteins were found to be associated with daily yield and the concentration of milk components. The presence of the BLG A allele was linked to higher yield and higher content of fat and dry matter in the milk. In the case of the CSN3 gene, the presence of the B allele was generally correlated with lower yield, but with higher content of dry matter and protein, including casein, in the milk.

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