

THE RELATIONSHIP BETWEEN GENETIC VARIANTS OF *B*-LACTOGLOBULIN AND *K*-CASEIN AND SELECTED PARAMETERS OF THE SUITABILITY OF MILK FOR CHEESE PRODUCTION

Anna Wolanciuk¹✉, Joanna Barłowska¹,
Zygmunt Litwińczuk², Wioletta Sawicka-Zugaj²

¹Department of Commodity Sciences and Processing of Animal Raw Materials, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

²Department of Breeding and Protection of Genetic Resources of Cattle, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

Abstract. A key stage in cheese production is the coagulation of milk proteins, leading to the formation of a curd. The aim of the study was to determine how genetic variants of β -lactoglobulin (*BLG*) and κ -casein (*CSN3*) are linked to milk coagulability, fat dispersion and the texture of rennet curds. The material for the study consisted of blood and milk samples collected from 213 cows of four breeds: 63 PHF HO, 50 JE, 50 RP and 50 BG. *BLG* and *CSN3* genotypes were determined by PCR-RFLP. A total of 741 milk samples were evaluated, in which chemical composition, the degree of fat dispersion, and coagulation properties were determined. The texture of 228 rennet curds was evaluated. The *BLG A* allele was associated with significantly ($P \leq 0.01$) higher content of non-fat dry matter in the milk and a lower degree of fat dispersion. The presence of the *CSN3 B* allele was associated with significantly ($P \leq 0.01$) higher content of non-fat dry matter and significantly ($P \leq 0.01$) shorter coagulation time. The curds from such milk were characterized by greater springiness, gumminess and chewiness ($P \leq 0.05$). The most favourable coagulation properties were noted for the milk of cows with the *CSN3 B* allele. The association between the *BLG* alleles and the parameters of the suitability of the raw material for processing is less clear.

Key words: milk protein polymorphism, rennet curd, fat dispersion

✉anna.wolaciuk@up.lublin.pl

INTRODUCTION

A key stage in cheese production is the coagulation of milk proteins, leading to the formation of a curd. The rheological properties of the curd determine the further course of technological processes, which in turn is reflected in the cheese yield and the quality of the final product.

The results of numerous studies [Molina et al. 2006a, Ahmadi et al. 2008, Heck et al. 2009, Mao et al. 2015] indicate a relationship between genetic variants of certain milk proteins and the productivity of cows, as well as the chemical composition and physicochemical properties of the milk. Significant differences in the coagulation process are influenced by variation in the nucleotide sequence and phosphorylation and glycosylation sites depending on the genetic variant of the protein [Jensen et al. 2012]. This mainly involves genetic variants of two genes: the β -lactoglobulin gene (*BLG*) and the κ -casein gene (*CSN3*). Hallen et al. [2007] emphasize that the genetic variation in κ -casein mainly affects the structure and function of the proteins, whose influence on the coagulation properties of the milk is much greater than that of the content of the protein. Phosphorylation sites of κ -casein are associated with varying ability to bind to calcium phosphate, and in later stages of coagulation, with the process of cross-linking and hydration of proteins [Mamone et al. 2003]. Many authors suggest that polymorphism within the *CSN3* gene may also be a significant factor differentiating the texture and functional properties of cheese [Hallen et al. 2007, Jensen et al. 2012]. Amenu and Deeth [2007] report that casein is responsible for forming the original structure of the curd and its ability to retain water and fat. The structure of curds is also determined by the degree of milk fat dispersion. Fat globules do not have an active role in curd formation, but are 'trapped' in a network of aggregated casein micelles, filling the pores in the forming gel (to varying degrees, depending on their size) [Logan et al. 2014]. Although β -lactoglobulin is not directly involved in the process of enzymatic coagulation of milk, its genetic variants may also be associated with the parameters of this process.

The main objective of the study was to determine how genetic variants of β -lactoglobulin (*BLG*) and κ -casein (*CSN3*) are linked to selected parameters influencing the suitability of milk for cheese production, including milk coagulability, fat dispersion, and the texture of rennet curds.

MATERIAL AND METHODS

The material for the study consisted of samples of blood and milk collected from 213 cows of 4 breeds, including two dairy breeds, Polish Black-and-White Holstein-Friesian (PHF HO) – 63 and Jersey (JE) – 50, and two native

breeds, Polish Red (RP) – 50 and White-Backed (BG) – 50. The Jersey and Polish Holstein-Friesian cows were housed in free-stall barns and fed in a TMR (Total Mixed Ration) system; the main components of the feed ration were maize silage, haylage and straw, as well as post-extraction meal (soya and rapeseed) and grain meal. The White-Backed and Polish Red cows were housed in tie-stall barns and their diet was based on on-farm fodder; during the autumn and winter the main components were haylage and hay, and the feed ration was supplemented with on-farm grain meal.

Blood samples were taken once during routine epidemiological testing, carried out by veterinarians caring for the animals in the herds studied. DNA was isolated from the samples using a QIAamp DNA Blood Mini Kit by QIAGEN. β -Lactoglobulin and κ -casein genotypes were determined by PCR-RFLP according to Medrano and Aquilar-Cordova [1990a,b].

Samples of milk obtained from a complete milking procedure were collected in the autumn/winter period (November to March), individually from each cow during test-day milking (AT4 method), and then refrigerated and transported to the laboratory. They were collected no earlier than 90 days and no later than 210 days after calving. Data on the daily milk yield of the cows in the months when the milk was collected were obtained from documentation (RW-2 reports) conducted by the Polish Federation of Cattle Breeders and Dairy Farmers [2014]. To eliminate milk samples from cows with diseased udders (SCC over 400.000 per ml), the somatic cell count (SCC) was determined using a Somacount 150 apparatus by Bentley.

In total 741 milk samples were evaluated, including 210 from cows of the PHF HO breed, 188 from JE, 181 from RP and 162 from BG, in which the basic chemical composition of the milk was determined with an Infrared Milk Analyzer (Bentley) and used to calculate the content of non-fat dry matter and the protein-to-fat ratio. The coagulation time was determined by Schern's method, in which a 250 μ l volume of rennet solution (Fromase[®] 2,200 IMCU/g Granulate, DSM Food Specialities, France) at a concentration of 0.66 g/100 ml (w/v) was added to 25 ml of milk heated to 35°C in a water bath and maintained at that temperature (35°C \pm 0.5°C). The sample was observed until the moment when the first casein flocules formed. Milk fat dispersion (expressed as the mean surface area, circumference and mean diameter of the fat globules) was determined in 518 milk samples, including 146 from cows of the PHF HO breed, 119 from JE, 141 from RP and 112 from BG. The analysis was conducted under a microscope at 1,000 \times magnification in slides stained with Sudan III (in three fields of view) using Motic Images Plus 2.0 software. Texture parameters of 228 rennet curds were determined, including 100 obtained from the milk of cows of the PHF HO breed, 62 from PC and 66 from BG. Rennet curds were prepared according to the method described by Ziarno [2006] with our own modifications. The milk was heated in a

water bath at 37°C and then rennet was added at a strength of 2,200 IMCU · g⁻¹ (0.66 g · 100 ml⁻¹ (w/v); Fromase 220TL Granulate, DSM Food Specialities, France). Parameters defining the texture of the curds obtained, i.e. their hardness, springiness, gumminess, chewiness and cohesiveness, were determined using a Zwick/Roell Proline BDO-FB0.5TS universal testing machine (Zwick GmbH and Co, Ulm, Germany). The measurements were performed in two replications and the results were registered in TestXpert® II software.

StatSoft Inc. STATISTICA ver. 6 software was used for the statistical analysis. The analysis was based on a General Linear Model (GLM) – an ANOVA procedure for factorial designs with interaction:

$$Y_{ikl} = \mu + G_i + P_k + (G_i \times P_k) + e_{ikl}$$

where:

μ – overall mean, G_i – cow's breed ($i = \text{PHF HO, JE, RP, BG}$), P_k – *BLG* genotype ($k = \text{AA, AB, BB}$), $(G_i \times P_k)$ – cow's breed × *BLG* genotype interaction, e_{ikl} – effect of random error, or:

$$Y_{imn} = \mu + G_i + F_n + (G_i \times F_n) + e_{imn}$$

where:

μ – overall mean, G_i – cow's breed ($i = \text{PHF HO, JE, RP, BG}$), F_n – *CSN3* genotype ($n = \text{AA, AB, BB}$), $(G_i \times F_n)$ – cow's breed × *CSN3* genotype interaction, e_{imn} – effect of random error.

Significance of differences between means was determined by Tukey's test, at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

The data in Table 1 indicate that the *BLG A* allele was associated with significantly ($P \leq 0.01$) higher content of non-fat dry matter in the milk, while milk obtained from *AB* heterozygotes had a significantly ($P \leq 0.05$) shorter rennet coagulation time. No relationship was found between the occurrence of polymorphic forms of the *BLG* gene and the protein-to-fat ratio. Analysis of selected parameters of the suitability of the milk for cheese production in relation to genetic variants of *BLG* for each breed separately generally found confirmation in the results obtained for the entire evaluated population of cows. Significant breed × *BLG* genotype interactions were demonstrated for content of non-fat dry matter and rennet coagulation time ($P \leq 0.05$). The results pertaining to the content of non-fat dry matter are in agreement with those obtained by Pytlewski et al. [2004], who also linked higher content of non-fat dry matter to the presence of the

BLG A allele. Literature reports on the relationship between genetic variants of β -lactoglobulin and the coagulation properties of milk are unclear. Molina et al. [2006a] found (as in the present study) the most beneficial coagulation properties in milk obtained from *BLG AB* heterozygotes. On the other hand, Ikonen et al. [1999] and Celik [2003] linked the most beneficial milk coagulation properties with the *BLG AA* genotype, while Lodes et al. [1997] and Barłowska et al. [2007] found no relationship between *BLG* variants and milk coagulation properties.

In the case of κ -casein (Table 2), the presence of the *B* allele (in both the homo- and heterozygous state) was associated with significantly ($P \leq 0.01$) higher content of non-fat dry matter, with the fastest rennet coagulation time noted for the milk of cows with the *CSN3 B* genotype ($P \leq 0.01$). The first casein flocules formed considerably later (by 1:31 min) in the milk from cows with the *CSN3 AA* genotype. No relationship was found between polymorphism of the gene *CSN3* and the protein-to-fat ratio. As in the case of *BLG*, significant breed \times *CSN3* genotype interactions were observed for content of non-fat dry matter and coagulation time ($P \leq 0.01$). The results obtained in this regard are generally consistent with data reported by other authors [Barłowska et al. 2007, Sulimova et al. 2007, Azevedo et al. 2008]. According to Botaro et al. [2009], the *B* κ -casein genotype contributes to better stability of casein micelles, shorter rennet coagulation time, formation of firmer curds, and higher cheese yield.

Evaluation of the relationship between genetic variants of β -lactoglobulin and milk fat dispersion (Table 1) showed that the milk of cows with the *BLG AA* genotype had the largest fat globules (greatest mean diameter, membrane circumference and surface area). However, the differences were not confirmed statistically for the entire population, but only for the Jersey breed. The milk from cows of this breed with the *BLG AA* genotype had significantly ($P \leq 0.01$) larger fat globules than in the case of *AB* or *B* (3.19, 2.84 and 2.77 μm , respectively). Similar analysis of the relationship between κ -casein variants (Table 2) shows that the presence of the *CSN3 B* allele (in both the homo- and heterozygous state) was associated with larger fat globules. The milk of cows with *CSN3 B* and *AB* genotypes had fat globules with a significantly ($P \leq 0.01$) longer mean diameter and thus a greater circumference and surface area. These relationships were also confirmed for each breed, and were statistically significant in the case of the Jersey and White-Backed breeds. Significant ($P \leq 0.01$) breed \times *CSN3* variant interactions were found for all analysed parameters defining milk fat dispersion. A more in-depth analysis of these relationships is difficult because no studies on this subject were found in the available literature.

The data presented in Tables 3 and 4 show statistically significant relationships between parameters characterizing curd texture and genetic variants of *BLG* and *CSN3*. The curds obtained from the milk of cows with the *BLG A* allele were

Table 1. Relationship between genetic variants of β -lactoglobulin and selected indices of the suitability of milk for cheese production

Tabela 1. Związek wariantów genetycznych β -laktoglobuliny z wybranymi wskaźnikami przydatności technologicznej mleka

Breed Rasa	Genotype Genotyp	n (milk samples) n (próbek mleka)	Non-fat dry matter, Sucha masa beztłuszczowa, %			Protein-to-fat ratio Proporcja białko/tłuszcz			Clotting time, min Czas krzepnięcia, min			Surface area of fat globules in field of view, μm Powierzchnia kuleczek w polu widzenia, μm^2			Circumference of fat globules in field of view, μm Obwód otoczek kuleczek tłuszczowych w polu widzenia, μm			Mean diameter of fat globules, μm Przeciętna średnica kuleczek, μm		
			\bar{x}	SD	n	\bar{x}	SD	n	\bar{x}	SD	n	\bar{x}	SD	n	\bar{x}	SD	n	\bar{x}	SD	n
PHF HO	AA	62	9.01	0.43	0.82	0.09	6.06 ^b	2.14	46	5.89	2.00	7.80	1.40	2.48	0.45					
	AB	78	9.00	0.48	0.84	0.09	5.11 ^a	2.05	46	5.50	1.78	7.59	1.15	2.41	0.37					
	BB	70	8.92	0.33	0.83	0.12	6.17 ^b	2.40	54	5.05	1.37	7.24	1.01	2.30	0.32					
JE	AA	52	9.72 ^b	0.37	0.82	0.10	4.55 ^b	1.40	41	10.08 ^B	4.76	10.02 ^B	2.49	3.19 ^B	0.79					
	AB	68	9.58 ^A	0.36	0.80	0.42	4.25 ^a	1.25	57	7.89 ^A	3.49	8.91 ^A	1.99	2.84 ^A	0.63					
	BB	24	9.54 ^A	0.37	0.73	0.12	4.26 ^a	1.37	21	7.49 ^A	2.86	8.71 ^A	1.82	2.77 ^A	0.58					
PR	AA	8	9.05	0.24	0.80	0.10	2.47 ^a	1.17	6	8.37	2.72	9.14	1.84	2.93	0.56					
	AB	72	8.97	0.43	0.79	0.12	4.31 ^{ab}	2.24	56	8.21	3.85	9.17	1.98	2.92	0.63					
	BB	101	8.92	0.39	0.80	0.12	4.12 ^b	2.00	79	7.75	2.58	8.92	1.57	2.81	0.50					
BG	AA	42	9.00 ^b	0.52	0.84	0.16	4.45	2.18	32	5.45	2.44	8.12	1.83	2.57	0.58					
	AB	64	8.82 ^{ab}	0.51	0.83	0.20	3.58	1.45	49	5.34	2.11	7.73	2.97	2.44	0.95					
	BB	34	8.76 ^a	0.59	0.81	0.18	4.51	2.01	31	4.56	1.46	7.34	1.71	2.32	0.54					
Total	AA	164	9.20 ^b	0.52	0.82	0.11	4.54 ^b	2.21	125	7.39	4.18	8.61	2.26	2.74	0.77					
Łącznie	AB	282	9.12 ^B	0.51	0.82	0.19	4.35 ^a	2.08	208	6.91	3.54	8.38	2.00	2.66	0.65					
	BB	229	8.98 ^A	0.46	0.80	0.13	4.46 ^{ab}	2.16	185	6.61	2.74	8.20	1.83	2.60	0.57					
	Effect of factor	Breed – Rasa	***	***	***	***	***	***	***	***	***	***	***	***	***					
Wpływ czynnika	Genotype – Genotyp	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns						
	Breed x genotype	*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns						
	Rasa x genotyp																			

A, B – różnice istotne przy $P \leq 0.01$; a, b – różnice istotne przy $P \leq 0.05$; Effect of factor: * – at $P \leq 0.05$; ** – at $P \leq 0.01$; *** – at $P \leq 0.001$; ns – not found.
A, B – różnice istotne przy $P \leq 0.01$; a, b – różnice istotne przy $P \leq 0.05$; Wpływ czynnika: * – przy $P \leq 0.05$; ** – przy $P \leq 0.01$; *** – przy $P \leq 0.001$; ns – nie stwierdzono.

Table 2. Relationship between genetic variants of κ -casein and selected indicators of the suitability of milk for cheese production
 Tabela 2. Związek wariantów genetycznych κ -kazeiny z wybranymi wskaźnikami przydatności technologicznej mleka

Breed Rasa	Genotype Genotyp	n (milk samples) n (próbek mleka)	Non-fat dry matter, Sucha masa beztłuszczowa, %		Protein-to-fat ratio Proporcja białko/tłuszcz		Clotting time, min Czas krzepnięcia, min		n (milk samples) n (próbek mleka)		Milk fat dispersion – Stan dyspersji tłuszczu mlekowego		Circumference of fat globule membranes Mean diameter of fat globules, μ m		
			\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}
PHF HO	AA	104	8.82 ^A	0.37	0.80	0.10	6:26 ^C	2:28	74	5.30	2.32	7.23	1.49	2.30	0.47
	AB	90	9.05 ^{AB}	0.45	0.81	0.09	5:22 ^B	2:09	60	5.16	1.48	7.34	1.09	2.34	0.35
	BB	12	9.17 ^B	0.43	0.84	0.09	5:08 ^A	2:31	12	5.85	1.87	7.82	1.24	2.49	0.39
JE	AA	12	9.54	0.38	0.79	0.10	5:28 ^C	1:31	15	6.87 ^A	2.60	8.37 ^A	1.60	2.66 ^A	0.51
	AB	80	9.65	0.38	0.80	0.22	4:52 ^B	1:33	69	7.36 ^A	2.39	8.51 ^A	2.31	2.71 ^A	0.73
	BB	96	9.69	0.32	0.82	0.13	4:12 ^A	1:00	35	9.56 ^B	2.86	9.83 ^B	2.10	3.13 ^B	0.67
PR	AA	42	8.89	0.45	0.76	0.11	4:59 ^C	1:39	30	7.66	2.57	8.85	1.53	2.82	0.48
	AB	91	8.98	0.34	0.80	0.12	4:08 ^B	1:53	68	8.04	3.43	9.05	1.87	2.88	0.59
	BB	48	9.02	0.34	0.83	0.11	3:51 ^A	1:51	43	8.22	3.26	9.23	1.80	2.94	0.57
BG	AA	52	8.61 ^A	0.48	0.82	0.19	4:44	2:11	40	3.97 ^A	0.34	5.93 ^A	1.39	2.23 ^A	0.12
	AB	75	8.82 ^A	0.54	0.83	0.18	4:24	2:08	48	5.47 ^B	3.22	7.06 ^B	2.18	2.58 ^B	0.69
	BB	35	9.34 ^B	0.34	0.85	0.11	3:25	1:41	20	6.29 ^C	3.22	7.71 ^C	2.29	2.79 ^C	0.73
Total	AA	210	8.86 ^A	0.45	0.80	0.20	5:36 ^C	2:01	159	5.94 ^A	2.64	7.84 ^A	1.69	2.47 ^A	0.54
Łącznie	AB	336	9.11 ^{AB}	0.52	0.81	0.13	4:43 ^B	2:16	245	7.49 ^B	3.69	8.70 ^B	2.18	2.71 ^{AB}	0.69
	BB	191	9.36 ^B	0.47	0.83	0.11	4:05 ^A	2:08	110	6.97 ^{AB}	3.51	8.34 ^{AB}	2.03	2.91 ^B	0.64
Effect of	Breed – Rasa		***		***		***			***		***		***	
factor	Genotype – Genotyp		*		ns		***			***		***		***	
Wpływ	Breed x genotype		**		ns		**			**		**		**	
czynnika	Rasa x genotyp														

A, B – significant differences at $P \leq 0.01$; a, b – significant differences at $P \leq 0.05$; Effect of factor: * – at $P \leq 0.05$; ** – at $P \leq 0.01$; *** – at $P \leq 0.001$; ns – not found.
 A, B – różnice istotne przy $P \leq 0.01$; a, b – różnice istotne przy $P \leq 0.05$; Wpływ czynnika: * – przy $P \leq 0.05$; ** – przy $P \leq 0.01$; *** – przy $P \leq 0.001$; ns – nie stwierdzono.

significantly ($P \leq 0.05$) harder and gummier, while the *BLG* allele determined greater springiness ($P \leq 0.05$). No significant relationships were found between the polymorphic forms of the *BLG* gene and the chewiness or cohesiveness of the curds. Similar relationships as for the entire study population were also found for each breed of cow. However, in the case of curds from the milk of the White-Backed cows, the differences in hardness and gumminess were not confirmed statistically. The springiest curds were obtained from the milk of the cows with the *BLG B* genotype (Table 3). In each of the breeds analysed the evaluation of the simultaneous effect of breed and *BLG* variant (interaction) on the texture parameters of rennet curds showed significant interactions for hardness ($P \leq 0.01$) and springiness ($P \leq 0.05$) – Table 3. The presence of the *CSN3 A* allele was found to be linked to harder ($P \leq 0.05$) rennet curds (Table 4), while the *B* allele was associated with greater springiness, gumminess and chewiness ($P \leq 0.05$). Two-way analysis of variance taking into account the effect of *CSN3* polymorphism and the breed of cow confirmed the statistically significant differences for the hardness of curds obtained from the milk of native cows. Curds from the milk of Polish Red and White-Backed cows with the *AA* genotype were significant-

Table 3. Relationship between genetic variants of β -lactoglobulin and selected texture parameters of rennet curds

Tabela 3. Związek wariantów genetycznych β -laktoglobuliny z wybranymi parametrami tekstury skrzepów podpuszczkowych

Breed Rasa	Genetic variant Wariant genetyczny	n (curd samples) n (próbek skrzepów)	Hardness, N Twardość, N		Springiness, N Sprężystość, N		Gumminess, N Gumowatość, N		Chewiness, N Żuwalność, N		Cohesiveness, N Spójność, N	
			\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
PHF HO	AA	28	5.74 ^a	2.72	1.78 ^a	0.67	0.62 ^b	0.31	0.95	0.41	0.11	0.04
	AB	30	6.21 ^b	1.86	1.70 ^a	0.66	0.58 ^b	0.26	0.85	0.23	0.09	0.02
	BB	42	5.65 ^a	2.10	2.00 ^b	0.85	0.48 ^a	0.20	0.84	0.31	0.09	0.03
PR	AB	28	4.87 ^b	2.11	2.07 ^a	1.16	0.58 ^b	0.31	1.10	0.32	0.13	0.05
	BB	34	3.73 ^a	1.49	2.44 ^b	1.19	0.47 ^a	0.20	1.04	0.41	0.13	0.08
BG	AA	14	5.12	2.51	1.92 ^a	0.85	0.57	0.21	0.99	0.24	0.11	0.02
	AB	28	4.65	2.10	2.01 ^a	0.92	0.52	0.29	0.92	0.38	0.11	0.04
	BB	14	4.41	1.90	2.21 ^b	1.11	0.58	0.19	0.81	0.26	0.12	0.05
Total Łącznie	AA	42	5.54 ^b	2.67	1.82 ^a	0.72	0.61 ^b	0.31	0.96	0.41	0.11	0.03
	AB	86	5.28 ^{ab}	2.09	1.94 ^{ab}	0.91	0.58 ^{ab}	0.28	0.95	0.49	0.11	0.04
	BB	90	4.74 ^a	2.10	2.21 ^b	1.01	0.49 ^a	0.20	0.91	0.38	0.11	0.06
Effect of factor Wpływ czynnika	Breed – Rasa		***		*		ns		ns		*	
	Genotype – Genotyp		*		*		*		ns		ns	
Breed x genotype Rasa x genotyp		**		*		ns		ns		ns		

a, b – significant differences at $P \leq 0.05$; Effect of factor: * – at $P \leq 0.05$; ** – at $P \leq 0.01$, *** – at $P \leq 0.001$; ns – not found.

a, b – różnice istotne przy $P \leq 0,05$; Wpływ czynnika: * – przy $P \leq 0,05$; ** – przy $P \leq 0,01$, *** – przy $P \leq 0,001$; ns – nie stwierdzono.

Table 4. Relationship between genetic variants of κ -casein and selected texture parameters of rennet curdsTabela 4. Związek wariantów genetycznych κ -kazeiny z wybranymi parametrami tekstury skrzepów podpuszczkowych

Breed Rasa	Genetic variant Wariant genetyczny	n (curd samples) n (próbek skrzepów)	Hardness, N Twardość, N		Springiness, N Sprężystość, N		Gumminess, N Gumowatość, N		Chewiness, N Zuwalność, N		Cohesiveness, N Spójność, N	
			\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD
PHF HO	AA	20	5.98	2.37	1.70	0.86	0.55	0.21	0.78 ^a	0.24	0.10	0.03
	AB	48	5.83	2.34	1.76	0.71	0.52	0.22	0.83 ^{ab}	0.48	0.09	0.03
	BB	32	5.71	1.62	2.03	0.73	0.59	0.17	1.01 ^b	0.32	0.10	0.03
PR	AA	16	5.20 ^b	1.64	2.25	0.77	0.40	0.08	0.88 ^A	0.29	0.12	0.04
	AB	28	4.06 ^{ab}	2.10	2.30	1.36	0.55	0.38	1.04 ^{AB}	0.45	0.13	0.05
	BB	18	3.62 ^a	1.80	2.29	1.24	0.58	0.15	1.27 ^B	0.71	0.14	0.08
BG	AA	20	5.16 ^b	2.24	1.74	1.61	0.46	0.18	0.84	0.32	0.10 ^a	0.03
	AB	30	4.74 ^{ab}	1.42	1.98	0.84	0.58	0.33	0.95	0.32	0.12 ^{ab}	0.04
	BB	16	4.11 ^a	2.11	2.42	0.69	0.60	0.30	0.94	0.45	0.14 ^b	0.02
Total Łącznie	AA	56	5.48 ^b	1.82	1.88 ^a	1.12	0.48 ^a	0.21	0.84 ^a	0.32	0.11	0.04
	AB	106	5.07 ^{ab}	2.43	2.00 ^{ab}	0.93	0.55 ^{ab}	0.30	0.91 ^a	0.38	0.11	0.05
	BB	66	4.76 ^a	2.18	2.23 ^b	0.96	0.59 ^b	0.37	1.05 ^b	0.60	0.11	0.04
Effect of factor Wpływ czynnika	Breed – Rasa		***		ns		ns		ns		***	
	Genotype – Genotyp		*		ns		ns		*		ns	
	Breed x genotype		ns		ns		ns		*		ns	
	Rasa x genotyp		ns		ns		ns		*		ns	

A, B – significant differences at $P \leq 0.01$; a, b – significant differences at $P \leq 0.05$; Effect of factor: * – at $P \leq 0.05$; ** – at $P \leq 0.01$, *** – at $P \leq 0.001$; ns – not found.

A, B – różnice istotne przy $P \leq 0,01$; a, b – różnice istotne przy $P \leq 0,05$; Wpływ czynnika: * – przy $P \leq 0,05$; ** – przy $P \leq 0,01$, *** – przy $P \leq 0,001$; ns – nie stwierdzono.

tly ($P \leq 0.05$) harder, and in the case of the White-Backed breed, they were also significantly ($P \leq 0.05$) more cohesive than curds from the milk of *CSN3 B* homozygotes. Analysis of the simultaneous effect of breed and genetic variant of *CSN3* on parameters characterizing rennet curd texture revealed a significant interaction ($P \leq 0.05$) only for chewiness (Table 4).

The available literature lacks studies analysing texture parameters of rennet curds. Authors investigating texture focus mainly on analysing these parameters with respect to the final product, i.e. various types of cheeses. The quality of the curd is usually characterized according to two parameters: A_{30} defining curd firmness 30 min after the coagulating enzyme is added, and K_{20} indicating the rate at which the gel attains a given degree of firmness.

The results of the present study are in some sense confirmed by results obtained by Jõudu et al. [2009]. The authors analysed the coagulation properties of the milk of Estonian Holstein-Friesian and Estonian Red cows with different variants of the *CSN3* gene and showed that curds obtained from the milk of cows with the *CSN3 B* genotype attained greater firmness 30 min after the coagulating enzyme

was added in comparison with AA homozygotes (40 mm and 27 mm, respectively). Saccà et al. [2003] also report significantly ($P \leq 0.01$) greater firmness of curds obtained from the milk of B homozygotes in comparison with CSN3 AA (32.9 mm and 25.2 mm, respectively). Molina et al. [2006b], on the other hand, showed no differences in the firmness of curds obtained from the milk of cows with extreme CSN3 genotypes. However, when the authors analysed this parameter in the context of polymorphism of the gene *BLG* they found that curds from the milk of cows with the AB genotype were significantly ($P \leq 0.05$) firmer than in the case of AA.

CONCLUSIONS

In conclusion, polymorphic variants of β -lactoglobulin and κ -casein substantially differentiate the suitability of milk for cheese production. In the case of CSN3, the best coagulation parameters were noted for the raw material obtained from cows with the B allele. Its presence is linked to significantly higher content of non-fat dry matter in the milk and shorter coagulation time, and the curd formed is springier, gummier and chewier. The presence of this allele is also associated with the lowest milk fat dispersion. The relationship between genetic variants of β -lactoglobulin and parameters defining the suitability of milk for processing is less clear.

REFERENCES

- Ahmadi, M., Mohammadi, Y., Darmani Kahi, H., Osfoori, R., Qanbari, S. (2008). Association of milk protein genotypes with production traits and somatic cell count of Holstein Cows. *Int. J. Biol. Sci.*, 8(7), 1231–1235.
- Amenu, B., Deeth, H.C. (2007). The impact of milk composition on cheddar cheese manufacture. *Aust. J. Dairy Technol.*, 62, 171–184.
- Azevedo, A.L.S., Nascimento, C.S., Steinberg, R.S., Carvalho, M.R.S., Peixoto, M.G.C.D., Teodoro, R.L., Verneque, R.S., Guimarães, S.E.F., Machado, M.A. (2008). Genetic polymorphism of the kappa-casein gene in Brazilian cattle. *Genet. Mol. Res.*, 7(3), 623–630.
- Barłowska, J., Litwińczuk, Z., Król, J., Kędzierska-Matysek, M. (2007). Relationship between β -lactoglobulin and κ -casein genetic variants and the selected indicators of technological usefulness of milk from polish red and whitebacked cows. *Ann. Anim. Sci.*, 1, 43–47.
- Botaro, B.G., de Lima, Y.V.R., Cortinhas, C.S., Silva, L.F.P., Renno, F.P., dos Santos, M.V. (2009). Effect of the kappa-casein gene polymorphism, breed and seasonality on physicochemical characteristics, composition and stability of bovine milk. *Rev. Bras. Zootech.*, 38(12), 2447–2454.

- Celik, S. (2003). β -lactoglobulin genetic variants in Brown Swiss breed and its association with compositional properties and rennet clotting time of milk. *Int. Dairy J.*, 13, 727–731.
- Hallen, E., Allmere, T., Naslund, J., Andren, A., Lunden, A. (2007). Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. *Int. Dairy J.*, 17, 791–799.
- Heck, J.M.L., Schennink, A., van Valenberg, H.J.F., Bovenhuis, H., Visker, M.H.P.W., van Arendonk, J.A.M., van Hooijdonk, A.C.M. (2009). Effects of milk protein variants on the protein composition of bovine milk. *J. Dairy Sci.*, 92, 1192–1202.
- Ikonen, T., Ahlfors, K., Kempe, R., Ojala, M., Ruottinen, O. (1999). Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish dairy cows. *J. Dairy Sci.*, 82, 205–2014.
- Jensen, H.B., Holland, J.W., Poulsen, N.A., Larsen, L.B. (2012). Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. *J. Dairy Sci.*, 95, 2891–2903.
- Jõudu, I., Henno, M., Väriv, S., Viinalass, H., Püssa, T., Kaart, T., Arney, D., Kärt, O. (2009). The effect of milk proteins on milk coagulation properties in estonian dairy breeds. *Veter. Med. Zootech.*, 46 (68), 14–19.
- Lodes, A., Buchberger, J., Krause, I., Klostermeyer, H. (1997). The influence of rare genetic variants of milk proteins on compositional properties of milk, rennetability, casein micelle size and content of non-glycosylated kappa-casein. Milk protein polymorphism. *Bulletin of the IDF. IDF Brussels, Belgium*, 158–161.
- Logan, A., Day, L., Pin, A., Auldish, M., Leis, A., Puvanenthiran, A., Augustin, M.A. (2014). Interactive Effects of Milk Fat Globule and Casein Micelle Size on the Renneting Properties of Milk. *Food Bioproc. Technol.*, 7, 3175–3185.
- Mamone, G., Cairra, S., Garro, G., Nicolai, A., Ferranti, P., Picariello, G. (2003). Casein phosphoproteome: identification of phosphoproteins by combined mass spectrometry and two-dimensional gel electrophoresis. *Electrophoresis*, 24, 2824–2837.
- Mao, Y., Zhu, X., Xing, S., Zhang, M., Zhang, H., Wang, X., Karrow, N., Yang, L., Yang, Z. (2015). Polymorphisms in the promoter region of the bovine lactoferrin gene influence milk somatic cell score and milk production traits in Chinese Holstein cows. *Res. Vet. Sci.*, 103, 107–112.
- Medrano, J.F., Aguilar-Cordova, E. (1990a). Genotyping of bovine kappa-casein loci following DNA sequence amplification. *Nat. Biotechnol.*, 8, 144–146.
- Medrano, J.F., Aguilar-Cordova, E. (1990b): Polymerase chain reaction amplification of bovine β -lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. *Anim. Biotechnol.*, 59, 180–182.
- Molina, L.H., Benavides, T., Brito, C., Carrillo, B., Molina, I. (2006b). Relationship between A and B variants of κ -casein and β -lactoglobulin and coagulation properties of milk (Part II). *Int. J. Dairy Technol.*, 59 (3), 188–191.
- Molina, L.H., Kramm, J., Brito, C., Carrillo, B., Pinto, M., Ferrando, A. (2006a). Protein composition of milk from Holstein-Friesian dairy cows and its relationship with the genetic variants A and B of κ -casein and β -lactoglobulin (Part I). *Int. J. Dairy Technol.*, 56(3), 183–187.
- Polska Federacja Hodowców Bydła i Producentów Mleka (PFHBiPM). (2014). Wyniki oceny wartości użytkowej bydła ras mlecznych. Raport: Sytuacja na rynku mleka

- [Polish Federation of Cattle Breeders and Dairy Farmers (PFCBDF). The results of milk recording for dairy cattle. Report: The situation on the milk market] [in Polish].
- Pytlewski, J., Antkowiak, I., Dorynek, Z., Stanisławski, D. (2004). Polimorfizm LGB i CSN3 a dzienna wydajność, skład i wybrane wskaźniki fizyczne mleka krów czarno-białych [Polymorphism of LGB and CSN3 and the daily milk yield, chemical composition and selected physical indicators of milk of black and white cows]. Zesz. Nauk. Prz. Hod., 72(1), 195–204 [in Polish].
- Saccà, E., Bovolenta, S., Ventura, W., Gaiarin, G. (2003). Coagulation properties and Nostrano di Primiero cheese yield of milk from Brown grazing cows of different κ -casein genotype. Ital. J. Anim. Sci., 2(1), 284–286.
- StatSoft Inc. (2003). STATISTICA (data analysis software system), v. 6. www.statsoft.com
- Sulimova, G.E., Ahani Azari, M., Rostamzadeh, J., Mohammad Abadi, M.R., Lazeby, O.E. (2007). κ -casein gene (CSN3) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker. Russ. J. Genet., 43(1), 73–79.
- Ziarno, M. (2006). Otrzymywanie i charakterystyka skrzepów kwasowego i podpuszczkowego. Wybrane zagadnienia z technologii żywności. Red. M. Mitek, M. Słowińska [Obtaining rennet and acid curd and their characterization. Selected issues in food technology. Eds. M. Mitek, M. Słowińska]. Wyd. SGGW, Warszawa [in Polish].

ZWIĄZEK WARIANTÓW GENETYCZNYCH B -LAKTOGLOBULINY I K -KAZEINY Z WYBRANYMI PARAMETRAMI PRZYDATNOŚCI SEROWARSKIEJ MLEKA

Streszczenie. W technologii wytwarzania serów kluczowym etapem procesu produkcyjnego jest koagulacja białek mleka, prowadząca do powstania skrzepu. Celem pracy było określenie związku wariantów genetycznych β -laktoglobuliny (BLG) i κ -kazeiny ($CSN3$) z krzepliwością mleka, stanem dyspersji tłuszczu i teksturą skrzepów podpuszczkowych. Materiał do badań stanowiły próby krwi i mleka pobrane od 213 krów 4 ras: 63 PHF HO, 50 JE, 50 RP i 50 BG. Genotypy BLG i $CSN3$ określono metodą PCR-RFLP. Oceniono 741 próbek mleka, w których oznaczono skład chemiczny, stopień dyspersji tłuszczu i właściwości koagulujące. Oceniono teksturę 228 skrzepów podpuszczkowych. Allel A BLG był związany istotnie ($P \leq 0,01$) z wyższą zawartością suchej masy beztłuszczowej w mleku i niższym stopniem dyspersji tłuszczu. Obecność allelu B $CSN3$ była związana istotnie ($P \leq 0,01$) z wyższą zawartością suchej masy beztłuszczowej istotnie ($P \leq 0,01$) i krótszym czasem krzepnięcia mleka. Skrzepy z takiego mleka wykazywały większą sprężystość, gumowatość i żujność ($P \leq 0,05$). Najlepszymi właściwościami koagulującymi charakteryzuje się mleko krów posiadających allel B $CSN3$. Związek alleli BLG z analizowanymi parametrami przydatności surowca do przetwórstwa jest mniej jednoznaczny.

Słowa kluczowe: polimorfizm białek mleka, skrzep podpuszczkowy, stan dyspersji tłuszczu

Accepted for print: 15.10.2016

For citation: Wolanciuk, A., Barłowska, J., Litwińczuk, Z., Sawicka-Zugaj, W. (2016). The relationship between genetic variants of β -lactoglobulin and κ -casein and selected parameters of the suitability of milk for cheese production. *Acta Sci. Pol. Zootechnica*, 15(3), 53–66. DOI: 10.21005/asp.2016.15.3.05

