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## **A comparison of protein polymorphisms in milk produced by two dairy farms in West Pomerania**

### **Abstract**

The aim of the study was to compare the frequencies of protein polymorphisms in milk produced by cows with various proportion of HF genes within their genotypes, managed in two farms.

Frequency analyses of beta-lactoglobulin (BLG) and three casein fractions (CSN1S1, CSN2, and CSN3) in individual genetic groups of animals were carried out in milk of 342 cows. Also, milk content of casein and whey proteins was assayed.

The highest level of casein was found in farm B, in all the genotypic groups of cows, while the proportion of whey proteins in milk was highest in farm A, in the genotypic group of cows with 25% to 50% of HF genes. As far as the remaining groups of cows are concerned, the level of whey proteins was equal in both studied farms.

A genetic differentiation of polymorphisms in milk protein was found in the analysed farms. Frequency of kappa-casein B (CSN3 B) genetic variants, which are desired in processing, was higher in farm A.

Key Words: polymorphism, gene frequency, milk protein, casein

### **Zusammenfassung**

**Titel der Arbeit: Vergleich von Polymorphismen der Milchproteine aus zwei Betrieben in Westpommern**

Das Ziel dieser Arbeit war ein Vergleich der Polymorphismen von Milcheiweiß in Kuhpopulationen mit verschiedenem Holstein-Friesian-Genanteil in zwei Betrieben.

Die Untersuchungen über das Auftreten von Genotypen Beta-Laktoglobulin (BLG) und drei Kaseinfraktionen (CSN1S1, CSN2 und CSN3) in einzelnen genetischen Gruppen wurden an Milchproben von 342 Kühen durchgeführt, wobei auch der Gehalt an Kasein und Molkeprotein in der Milch bestimmt wurde.

Der größte Kaseingehalt in der Milch wurde im Betrieb B in allen Genotypgruppen festgestellt; der prozentuelle Anteil an Molkeeiweiß in der Milch war dagegen im Betrieb A in den Genotypgruppen mit einem HF-Anteil von 25% bis 50% am höchsten; in den übrigen Genotypgruppen war er in beiden untersuchten Betrieben gleich.

In den ausgewerteten Betrieben wurde eine genetische Differenzierung der Polymorphismen von Milcheiweiß festgestellt. Die Frequenz des Auftretens von genetischen Varianten des Kappa-Kaseins B (CSN3 B), die günstig hinsichtlich einer technologischen Eignung ist, war im Betrieb A größer.

Schlüsselwörter: Polymorphismus, Genfrequenz, Milcheiweiß, Kasein

### **Introduction**

The studies on protein polymorphism application in cattle genetics, which have been extensive over the recent years, indicate that milk proteins can be utilised in both breeding practice and milk processing (LITWIŃCZUK et al., 1998b; GRAML and PIRCHNER, 2003). Presence and frequencies of milk protein encoding alleles are different in individual breeds of cows (JANN et al., 2002), the differences being detectable between herds of cattle within a single breed (JUSZCZAK et al., 2001; FREYER et al., 1998).

Nutritive value of milk is determined primarily by total protein content, whereas its technological value depends on the proportion of its desired (genetically determined)

fractions, particularly casein, which represents about 75-85% of total milk proteins. The aim of the study was to compare the frequencies of polymorphisms in proteins of milk obtained from two populations of cows, with various HF genes proportion in the genotype and managed in two large-herd farms.

### Material and methods

The frequency analyses of beta-lactoglobulin (BLG), alpha-casein S1 (CSN1S1), beta-casein (CSN2), and kappa-casein (CSN3) were carried out on 342 Black-and-White (BW) cows with varying proportion of Holstein-Friesian (HF) genes in their genotype. The cows in the farm A were managed in pasture-housing system, while the cows in the farm B were kept only indoors, in confinement.

Milk samples were collected during test days from cows being in milk between 60 and 120 days. The samples were placed in 100-ml plastic containers. The analyses were done immediately on having transported the samples to the laboratory. The analyses did not include samples taken from cows showing symptoms of mastitis, or samples of milk that displayed abnormal properties observed during the precipitation of the proteins.

Both beta lactoglobulin and the three mentioned casein phenotypes were tested twice on starch gel in alkaline environment, according to SCHMIDT (1964), modified by MICHALAK (1969), and in acid environment, according to PETERSON and KOPFLER (1966).

Casein content was arrived at from the difference between total protein and the content of whey proteins.

Total protein was assayed using a Pro-Milk instrument (Foss Electric, Denmark), while whey proteins were assayed using the calorimetric technique with 10 B amide black, according to ASCHAFFENBURG and DREWRY (1959). The standardisation curve for total whey protein was created with the Kjeldahl method.

The obtained results enabled an estimation of the number of all phenotypes of casein and beta lactoglobulin, which in turn allowed estimating the frequencies of genes and genotypes. Genetic equilibrium was analysed according to the Hardy-Weinberg law, while the chi-square test was used for testing the fitness of the empirical data to their theoretical distribution (RUSZCZYC, 1978).

### Results

Table 1 presents protein content and frequencies of milk protein polymorphisms in relation to genotypes of cows in farms A and B.

The highest level of total protein (3.09%) was obtained in farm B from cows of 75-100% HF, whereas the lowest (2.93%) was found in barn A from cows of 50-75% HF. The highest casein content (2.47%) was recorded in farm B in cows with more than 75.1% HF genes, while the lowest was in farm A (2.31%) in the group of cows with 50-75% HF. The highest content of whey proteins was observed in farm A (0.66%) in cows of the genotype 25-50% HF, while the lowest was obtained in farm B (0.57%) in the same group of cows.

The highest frequency of CSN1S1 phenotypes was found in milk of cows with 75% to 100% HF genes in farm B, where two variants were observed, B and BC, depending on the presence of two alleles, i.e. CSN1S1 B and CSN1S1 C. In the genotypic group

of cows with 25-50% HF, only one phenotype of this protein, B, was found with no BC phenotype in either farm. The total number of CSN1S1 B phenotypes was higher in farm A within the genotypic group of 50-75% HF.

Table 1

Number of milk protein polymorphism phenotypes by cow genotype in the studied farms (Anzahl Phänotypen der Milchproteinstrukturen unter Berücksichtigung der Kuhgenotypen in untersuchten Betrieben)

Genotype groups % HF	n	Protein %			Casein CSN									BLG				
					B	BC	A1			A2			A	AB	B	A	AB	B
							A1	A2	A1A2	A1B	A2B	A2A3						
<b>Farm A</b>																		
25-50	12	3.05	2.40	0.66	12	0	4	6	0	1	1	0	7	4	1	4	5	3
50-75	57	2.93	2.31	0.60	54	3	11	17	22	4	3	0	31	24	2	0	27	30
75-100	102	3.03	2.40	0.62	101	1	21	29	37	8	6	1	56	39	7	8	54	40
<b>Total</b>	171	-	-	-	<b>167</b>	<b>4</b>	<b>36</b>	<b>52</b>	<b>59</b>	<b>13</b>	<b>10</b>	<b>1</b>	<b>94</b>	<b>67</b>	<b>10</b>	<b>12</b>	<b>86</b>	<b>73</b>
<b>Farm B</b>																		
25-50	19	3.00	2.43	0.57	19	0	5	4	9	0	1	0	17	2	0	1	8	10
50-75	41	3.05	2.45	0.60	39	2	8	12	12	2	6	1	26	15	0	2	23	16
75-100	111	3.09	2.47	0.62	106	5	18	27	55	6	4	1	80	27	4	19	46	46
<b>Total</b>	171	-	-	-	<b>164</b>	<b>7</b>	<b>31</b>	<b>43</b>	<b>76</b>	<b>8</b>	<b>11</b>	<b>2</b>	<b>123</b>	<b>44</b>	<b>4</b>	<b>22</b>	<b>77</b>	<b>72</b>
Grand total	342				331	11	67	95	135	21	21	3	217	111	14	34	163	145

Within the CSN2 of milk, the following six variants were found: A1, A2, A1A2, A1B, A2B, and A2A3, encoded by four alleles, i.e. CSN2 A1, CSN2 A2, CSN2 A3, CSN2 B. The A2A3 phenotype was not found in the groups 25-50% or 50-75% HF in farm A, while in farm B in the group 25-50% HF.

Within the CSN3 of milk, three variants were found, A, AB, and B, whose occurrence was controlled by two alleles, CSN3 A and CSN3 B. In farm B the phenotype B was not found in the groups 25-50% HF and 50-75% HF. The total number of CSN3 A phenotypes was higher in farm B, while CSN3 AB were more frequent in farm A.

Three phenotypes were found in the BLG polymorphism, A, AB, and B, determined by two BLG alleles, BLG A and BLG B. The phenotype A was not found in the group 50-75% HF in farm A. The total frequency of BLG phenotypes, AB and B, was higher at farm A, while BLG A phenotypes were more frequent at farm B.

Table 2 presents frequency of the genes encoding for milk protein polymorphisms as well as empirical and theoretical distribution of genotypes in the studied population of cows with varying content of HF genes at farm A.

As far as CSN3 is concerned, 58.33% of the 25-50% HF individuals were homozygotes AA, which was a consequence of a high frequency of the CSN3 A allele (0.75). A2A2 homozygous individuals also prevailed in CSN2, constituting 50% of the population. For polymorphisms in CSN3 and CSN2, a lack of genetic equilibrium was found, which resulted from a significant difference between empirical and theoretical genotype distributions; in the remaining cases the chi-square values were lower than theoretical one. In the polymorphism of BLG, heterozygous AB animals constituted

for 41.67%.

Table 2

Frequencies of genes controlling milk protein polymorphism as well as observed and theoretical distribution of genotypes with different proportion of HF cattle genes in farm A (Genfrequenz des Milchproteinpolymorphismus sowie beobachtete und theoretische Genotypenverteilung in der untersuchten Kuhpopulation mit verschiedenem HF- Genanteil im Betrieb A)

%HF	Milk protein	Genotypes	Observed number of individuals	%	Gene frequency	Genotype frequency	Theoretical number of individuals	Chi2
20-50	CSN3	AA	7	58.33	A= 0.75	0.5625	6.75	16.815*
		AB	1	8.33	B= 0.25	0.3750	4.50	
		BB	4	33.33		0.0625	0.75	
	CSN2	A1	4	33.33	A1= 0.375	0.1406	1.69	13.591*
		A2	6	50.00	A2= 0.542	0.2938	3.53	
		A1A2	0	0.00	A3= 0	0.4065	4.47	
		A1B	1	8.33	B= 0.083	0.0623	0.87	
		A2B	1	8.33		0.0900	6.03	
		A2A3	0	0.00		0.0000	0.00	
	BLG	AA	4	33.33	A= 0.542	0.2938	3.53	0.311
		AB	5	41.67	B= 0.458	0.4965	5.96	
		BB	3	25.00		0.2098	2.52	
50-75	CSN1S1	BB	54	94.74	B= 0.974	0.9487	54.07	0.043
		BC	3	5.26	C= 0.026	0.0506	2.89	
		CC	0	0.00		0.0007	0.04	
	CSN3	AA	31	54.39	A= 0.754	0.5685	32.41	1.055
		AB	24	42.11	B= 0.246	0.3710	21.15	
		BB	2	3.51		0.0605	3.45	
	CSN2	A1	11	19.30	A1= 0.421	0.1772	10.10	2.397
		A2	17	29.82	A2= 0.518	0.2683	12.34	
		A1A2	22	38.60	A3= 0	0.4362	24.42	
		A1B	4	7.02	B= 0.061	0.0514	3.29	
		A2B	3	5.26		0.0632	3.79	
		A2A3	0	0.00		0.0000	0.00	
	BLG	AA	0	0.00	A= 0.237	0.0562	3.20	5.485
		AB	27	47.37	B= 0.763	0.3617	20.61	
		BB	30	52.63		0.5822	33.18	
75-100	CSN1S1	BB	101	99.02	B= 0.995	0.9900	100.98	0.003
		BC	1	0.98	C= 0.005	0.0100	1.01	
		CC	0	0.00		0.0000	0.00	
	CSN3	AA	56	54.90	A= 0.74	0.5476	55.86	0.205
		AB	39	38.24	B= 0.246	0.3641	37.14	
		BB	7	6.86		0.0605	6.17	
	CSN2	A1	21	20.59	A1= 0.426	0.1815	18.51	5.627
		A2	29	28.43	A2= 0.5	0.2500	22.25	
		A1A2	37	36.27	A3= 0.005	0.4260	48.56	
		A1B	8	7.84	B= 0.069	0.0588	6.88	
		A2B	6	5.88		0.0690	7.52	
		A2A3	1	0.98		0.0050	0.52	
BLG	AA	8	7.84	A= 0.343	0.1176	12.00	3.104	
	AB	54	52.94	B= 0.657	0.4507	45.97		
	BB	40	39.22		0.4316	44.03		

\* P ≤ 0,05

For the cattle with 50-75% HF, homozygous BB individuals clearly prevailed within

the CSN1S1 polymorphism, reaching 94.74%. Similarly, homozygous animals (AA) were prevalent in the polymorphism of CSN3, whereas heterozygotes prevailed in the polymorphisms of the milk proteins CSN2 and homozygotes prevailed for BLG.

Table 3

Frequencies of genes controlling milk protein polymorphism as well as observed and theoretical distribution of genotypes with different proportion of HF cattle genes in farm B (Genfrequenz des Milchproteinpolymorphismus sowie beobachtete und theoretische Genotypenverteilung in der untersuchten Kuhpopulation mit verschiedenem HF-Genanteil im Betrieb B)

%HF	Milk protein	Genotypes	Observed number of individuals	%	Gene frequency	Genotype frequency	Theoretical number of individuals	Chi2
25-50	CSN3	AA	17	89.47%	A= 0.947	0.8968	17.04	0.058
		AB	2	10.53%	B= 0.053	0.1004	1.91	
		BB	0	0.00%		0.0028	0.05	
	CSN2	A1	5	26.32%	A1= 0.5	0.2500	4.75	0.793
		A2	4	21.05%	A2= 0.474	0.2247	3.37	
		A1A2	9	47.37%	A3= 0	0.4740	9.01	
		A1B	0	0.00%	B= 0.026	0.0260	0.52	
		A2B	1	5.26%		0.0246	1.45	
		A2A3	0	0.00%		0.0000	0.00	
	BLG	AA	1	5.26%	A= 0.263	0.0692	1.31	0.140
		AB	8	42.11%	B= 0.737	0.3877	7.37	
BB		10	52.63%		0.5432	10.32		
50-75	CSN1S1	BB	39	95.12%	B= 0.976	0.9526	39.06	0.027
		BC	2	4.88%	C= 0.024	0.0468	1.92	
		CC	0	0.00%		0.0006	0.02	
	CSN3	AA	26	63.41%	A= 0.817	0.6675	27.37	2.054
		AB	15	36.59%	B= 0.183	0.2990	12.26	
		BB	0	0.00%		0.0335	1.37	
	CSN2	A1	8	19.51%	A1= 0.366	0.1340	5.49	4.479
		A2	12	29.27%	A2= 0.524	0.2746	9.61	
		A1A2	12	29.27%	A3= 0.012	0.3836	17.64	
		A1B	2	4.88%	B= 0.098	0.0717	3.59	
		A2B	6	14.63%		0.1027	4.93	
		A2A3	1	2.44%		0.0126	1.86	
	BLG	AA	2	4.88%	A= 0.329	0.1082	4.44	2.992
		AB	23	56.10%	B= 0.671	0.4415	18.10	
		BB	16	39.02%		0.4502	18.46	
75-100	CSN1S1	BB	106	95.50%	B= 0.977	0.9545	105.95	0.059
		BC	5	4.50%	C= 0.023	0.0449	4.99	
		CC	0	0.00%		0.0005	0.06	
	CSN3	AA	80	72.07%	A= 0.842	0.7090	78.70	0.784
		AB	27	24.32%	B= 0.158	0.2661	29.53	
		BB	4	3.60%		0.0250	2.77	
	CSN2	A1	18	16.22%	A1= 0.437	0.1910	21.20	1.610
		A2	27	24.32%	A2= 0.514	0.2642	29.59	
		A1A2	55	49.55%	A3= 0.005	0.4492	58.85	
		A1B	6	5.41%	B= 0.045	0.0393	4.80	
		A2B	4	3.60%		0.0463	5.37	
		A2A3	1	0.90%		0.0051	0.58	
BLG	AA	19	17.12%	A= 0.378	0.1429	15.86	1.575	
	AB	46	41.44%	B= 0.622	0.4702	52.20		
	BB	46	41.44%		0.3869	42.94		

In the group of cows with 75-100% HF, within the CSN1S1, 99.02% of individuals were homozygotes BB, which resulted from a high frequency of the allele CSN1S1B (0.995). Similarly, homozygotes prevailed in the polymorphism of CSN3. A higher frequency of heterozygous individuals was found within the milk proteins CSN2 and BLG, respectively CSN2 A1A2 (36.27%) and BLG AB (52.94%).

Table 3 presents the frequency of genotypes that control milk protein polymorphism, as well as the observed and theoretical distribution of genotypes in the studied population of cows with different share of HF breed in the genotype in farm B.

Three genotypes of CSN3 protein, AA, AB, and BB, were found in the group of cows with 25-50% of HF. Homozygous CSN3 AA animals prevailed, representing 89.47%. In the polymorphism of CSN2, heterozygotes prevailed. Within the BLG, three genotypes were found, i.e. AA, AB, and BB. The highest frequency was represented by BLG BB individuals (52.63%).

In the population of cows with 50-75% HF, within the polymorphism of CSN1S1, homozygous BB individuals represented 95.12%, which was a consequence of a high frequency of the allele CSN1S1 B (0.976). Within CSN3, also homozygous AA individuals represented a majority (63.41%). In the BLG polymorphism, heterozygous AB individuals prevailed (56.10%).

Within the genotypic group of 75-100% HF, the domination of homozygous individuals was recorded in the milk proteins CSN1S1 and CSN3, i.e. 0.977 and 0.842 for BB and AA respectively. Within the CSN2, heterozygotes dominated. Three genotypes were found for BLG (AA, AB, and BB). The percentages of genotypes AB and BB were equal, namely 41.44%.

In all studied milk protein polymorphisms, the empirical distribution of genotypes corresponded to the theoretical distribution, so the entire population of cattle with different HF proportion remained in the genetic equilibrium.

### Discussion

Casein, with its content in milk being about 2.5%, is the most important milk protein for the cheese industry (PANICKE et al., 1998; FELEŃCZAK and SZAREK, 1987).

Milk with higher content of casein is desired because of its better technological value, i.e. shorter time of flocculation (formation of casein clot in the process of cheese production), tougher clots, and an increased cheese production yield (GRAML and PIRCHNER, 2003; HARTUNG and GERNAND, 1997; KRZYŻEWSKI et al., 1997). The performance of technological processes of cheese production depends on the structure of the kappa-casein gene (ZATOŃ, 1999; GERNAND and HARTUNG 1997). In the studied herd, a decreasing frequency of the phenotype CSN3B was observed to accompany an increase in the proportion of HF genes (Table 1). According to KAMIŃSKI (1994), reduced frequency of the CSN3B allele results from a negative effect of still progressive expansion of Holstein-Friesian cattle, for which a low frequency of the desired CSN3B allele is a characteristic trait.

Concentration of individual milk protein fractions and their interrelations depend on the polymorphic form of the given protein determined by a mutation that is expressed through a substitution of one or more amino acids with others at the given site. These relatively small changes in the polypeptide chain have a significant influence on chemical composition and physicochemical properties of total milk protein (GRUPE and SCHWERIN, 1998; KRZYŻEWSKI et al., 1998).

Comparing our studies with those by LITWIŃCZUK et al. (1998b), who studied 351 crossbreds with the Holstein-Friesian breed of cattle, similar phenotypes of milk proteins were found. The frequency of milk protein polymorphism phenotypes in a majority of cases corresponded to the expected frequency. Only in the CSN3 polymorphism in the group of crossbreds with less than 50% of HF genes, significant differences were found between the observed and expected genotype frequencies. Significant differences were also found in the group of 25.1-50.0% HF in the polymorphism of CSN2, which was confirmed with own studies (Table 2).

Investigating into the frequency of CSN3 of milk revealed prevalence of the gene A over B within all the genotypic groups in both farms (Tables 2 and 3). MICHALAK (1997), who studied cattle with a high proportion of the Holstein-Friesian cattle genes, found the frequencies of kappa-casein alleles A and B respectively 0.8092 and 0.1908. LITWIŃCZUK and BARŁOWSKA (1998), who studied crossbreds with the HF breed in private farms, found that heterozygotes CSN3 AB prevailed (55.7%). JUSZCZAK et al. (2001) found homozygotes CSN3 AA to be prevalent. PANICKE et al. (1998), who studied Black-and-White cattle, found kappa-casein genotypes AE and BE. STRZAŁKOWSKA et al. (1998) stated that about 3% of currently managed Black-and-White (BW) cattle were kappa-casein homozygotes BB.

LITWIŃCZUK et al. (1998a), who studied populations of BW cows, Jersey cows, and their crossbreds with various addition of HF, found that the lowest frequency of BLG AA homozygotes was among the cows with 0.1-25% of HF and BW; these were at the same time the groups where the proportion of BLG AB heterozygotes was highest. STRZAŁKOWSKA et al. (1998) reported the frequencies of BLG alleles A and B in populations of BW cattle with a high proportion of HF genes, respectively 0.41 and 0.59. PANICKE et al. (1998), who studied Black-and-White cattle, recorded frequencies betalactoglobulin genotypes AA, AB, and BB at the level of, respectively, 0.11, 0.51, and 0.38. According to KAMIŃSKI et al. (1996), the frequencies of BLG alleles A and B in Holstein-Friesian cattle ranges between 35.1-41% and 59-65%, respectively.

Results of studies on milk protein polymorphism can be used as an additional criterion for selecting sires in the dairy cattle herds. Selection for respective BLG and CSN3 genotypes would contribute to an increased casein content in milk, improved technological quality of milk, and increased cheese production yield. Selection for the mentioned alleles represents a relatively easy task, as they are located on different chromosomes, so no couplings between them occur. Such a direction of selection would not reduce milk yield, but it would significantly contribute to an improvement of milk processing parameters (FREYER et al. 1998; STRZAŁKOWSKA et al. 1998). This procedure will enable collection of cow herds that produce milk of improved processing quality for the needs of the cheese industry.

### Conclusions

A genetic variability of milk protein polymorphisms was found in the studied farms. The highest milk content of casein was found in farm B in all genotypic groups of cows, while the highest percentage milk content of whey proteins was found in farm A in cows with 25-50% HF; in the remaining groups the level was similar in both farms. The frequency of the allele BLG B, which is desired in cheese production, was highest in the group of cows with 50-75% HF in farm A.

The frequency of desired genetic variants of CSN3B, in relation to the technological value of milk for cheese production, was higher in farm A in all genotypic groups of cows.

Identification of desired genes encoding milk proteins is of high importance in the countries and regions where cheese industry represents an important branch of economy and the quality of raw material and its technological properties considerably influence the value of the end product as well as output capacity and economical results.

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Received: 2003-09-05

Accepted: 2004-02-27

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