

Effect of wheat gluten and extracted, protected soybean meal addition to the diet of cows with different beta-lactoglobulin genotypes on the composition and physical properties of milk

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ABSTRACT: The effect of wheat gluten or extracted soybean meal (300 g of crude protein/head/day) on milk yield, composition and its physical properties was analysed in 53 cows of Polish Holstein-Friesian breed with different milk beta-lactoglobulin (blg) genotypes (AA, AB and BB). The addition of wheat gluten to the diet of cows with the AA and AB blg genotypes caused a significant increase in crude protein (by 0.21; $P \leq 0.05$, and 0.19%; $P \leq 0.01$), casein (by 0.15; $P \leq 0.05$, and 0.15%; $P \leq 0.01$) and dry matter content (0.3 and 0.5%; $P \leq 0.05$) in milk, and a significant increase in beta-casein production (0.6 g/l; $P \leq 0.05$, and 0.6 g/l; $P \leq 0.01$) and kappa-casein yield (0.3 g/l; $P \leq 0.05$) in the case of cows with the AA genotype. Milk acidity and electrical resistance in milk were lowered. The cows with the BB blg genotype did not show any significant changes in milk composition. The supplementation of extracted soybean meal to cows with the AB and BB blg genotype led to a significant increase in milk protein (0.26% and 0.21%; $P \leq 0.01$) and casein content (0.2 and 0.15%; $P \leq 0.01$), beta-casein production (0.60 and 0.50 g/l; $P \leq 0.01$) and to a decrease in kappa-casein content (by 0.7%; $P \leq 0.05$) in cows with the BB genotype. The production of kappa-casein increased in cows with the AB genotype (by 0.2 g/l; $P \leq 0.01$). In the case of all genotypes, an increase in milk active acidity and thermostability and a decrease in electrical resistance in milk were noted. It was observed that cows with different blg genotypes differently utilised the protein from given supplements for the production of individual milk components, which may be used in rationalisation of their feeding and improvement of milk content.

Keywords: nutri-genomics; wheat gluten; extracted soybean meal; milk properties

The analysis of relationships and cooperation between quantitative features of genes, determining their products and looking for those of a high influence on the activity of quantitative trait locus (QTL) are possible thanks to the identification of animal genotypes by DNA analysis and recognition of its polymorphism. The determination of the role of genes and their effect on the processes of transformation, and also the production ones in animals, is one of the aspects of functional genomics that has been developing in the last years (Freyer and Vukasinovic, 2005; Kamiński et al.,

2005; Matějček et al., 2007, 2008; Walawski et al., 2004).

Phenotypic specifications of the composition and physical properties of milk result from an expression of genes coding its particular components. The activity of particular mRNA replications may differ depending on the genotype, kind and composition of feeds used in the diet, stage of lactation and other environmental factors like feeding, management and utilisation. The recognition of this variability may enable the regulation of the composition and physical properties of milk and affect its biologi-

cal value, processing efficiency and the quality of consumer products. It may also be of assistance in the selection of feeds for animals and be used in the selection. The results of research by Ng-Kwai-Hang et al. (1990), Walawski et al. (1994), Kučerová et al. (2006) and others document that cows with the AA beta-lactoglobulin genotype are characterised by higher milk yield, while cows with the BB beta-lactoglobulin genotype by higher protein and casein content in milk. Milk from cows with the BB beta-lactoglobulin genotype is characterised by shorter coagulation time, better clot conciseness and higher cheese yield.

No prior studies concerning gene expression in cows with different milk beta-lactoglobulin genotypes, receiving feed supplemented with wheat gluten or extracted soybean meal, were found in the published literature. No studies in this range concerning other fodders were conducted either.

The present study aimed to determine changes in the composition and physical properties of milk produced by cows with different milk beta-lactoglobulin genotypes (AA, AB, BB) receiving feed supplemented with wheat gluten or extracted soybean meal. It was assumed that such cows might differently utilise the addition of protein from various feed supplements for the production of milk components, including caseins, and influence its physical value as well.

MATERIAL AND METHODS

Selection of animals

The study was conducted on Polish Holstein-Friesian cows in a herd of 300 head with the mean annual yield amounting to 8 500 kg of milk per cow, with a fat content of 3.99% and crude protein content of 3.40%. All the cows in the herd were examined for beta-lactoglobulin (blg) genotype using PCR method (Ziemiński et al., 2002). On the basis of the results obtained, 53 cows with specific genotypes for milk proteins were selected (18 cows with the AA genotype, 17 with the AB genotype and 18 with the BB genotype). Within the genotypes the cows were qualified for the experiment on the basis of analogues, taking into consideration their milk yield in previous milking, subsequent lactation and month of lactation. Cows in lactation 2–5 were qualified for the experiment on the basis of their similar milk yield in the last sample milking. Cows

in a period 30–100 days of lactation were included in the first experiment, while in the second one cows in a period 60–120 days of lactation.

Feeding

Throughout the year cows in the herd were fed using the total mixed ration (TMR) system. The mean daily content of particular forages in TMR per cow was as follows: maize silage – 21 kg, lucerne silage – 9 kg, beet pulp silage – 12 kg, hay – 1 kg, and extracted rapeseed meal – 1 kg. The feed offered contained 45.52% of dry matter (DM), including 12.43% ash, 15.75% crude protein, and 18.75% crude fibre in the DM. The quality of silage was assessed by 77 points according to Flieg's score. The basic ration was calculated for the production of 18 kg of milk. Cows with daily milk yield exceeding 18 kg received 1 kg of concentrate, containing 7.5 MJ of net energy for lactation (NEL) and 185 g of crude protein for each 2 kg of additional milk. During experiment I and II, over a period of 12 days, the cows additionally received on top of the TMR mixture:

- experiment I – 450 g of wheat gluten concentrate containing 300 g of crude protein per head per day;
- experiment II – 670 g of extracted soybean meal containing 300 g of crude protein per head per day.

Milk analysis

The milk yield control was performed before the beginning and after the 12th day of supplementation on the basis of double milking. Experiment II was conducted on the same cows, with the same basic TMR mixture, 2 weeks after the end of Experiment I. The milk samples were analysed for: crude protein, fat, lactose and dry matter content using the Milko-Scan 133B apparatus (ASN FOSS-Electric, Denmark); casein content by Walker's method (PN-68/A-86122, 1968); active acidity using a pH meter; potential acidity according to the method of Soxhlet-Henkel (PN-68/A-86122, 1968); thermostability by the alcohol test (PN-68/A-86122, 1968); somatic cell count (SCC) by the flow cytometry method using the Somacount 150 apparatus (Bentley, USA); total number of microorganisms by flow cytometry using the Bactocount 70

Table 1. Chemical content and casein protein proportion in milk from cows with different blg genotypes before and after wheat gluten supplementation

Specification	Analysis	Beta-lactoglobulin genotype						Total	
		AA		AB		BB			
		18		17		18		53	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
Daily yield	*	30.7	4.1	35.5	6.0	34.5	6.3	33.4	5.8
	**	30.6	4.1	35.1	5.7	34.0	5.9	33.2	5.5
Fat (%)	*	4.23	1.20	3.66	0.71	4.63	1.59	4.18	1.27
	**	4.28	0.79	4.05	0.94	4.39	0.98	4.25	0.90
Protein (%)	*	3.56 ^a	0.32	3.17 ^A	0.36	3.46	0.42	3.41 ^a	0.40
	**	3.77 ^b	0.46	3.36 ^B	0.39	3.62	0.57	3.59 ^b	0.50
Casein (%)	*	2.73 ^a	0.23	2.42 ^A	0.27	2.65	0.32	2.60 ^a	0.30
	**	2.88 ^b	0.35	2.57 ^B	0.30	2.74	0.45	2.74 ^b	0.39
α -CN (% of CN)	*	56.2	3.5	55.9	2.4	55.2	3.5	55.8	3.1
	**	55.1	3.1	55.5	2.6	54.4	2.8	55.0	2.9
α -CN (g/l)	*	15.3	1.4	13.5 ^a	1.6	14.6	2.1	14.5	1.8
	**	15.9	2.2	14.3 ^b	1.8	14.9	2.6	15.1	2.3
β -CN (% of CN)	*	29.0	2.32	29.0	2.03	29.3	2.80	29.1	2.4
	**	29.4	1.78	29.5	1.94	29.7	2.22	29.5	2.0
β -CN (g/l)	*	7.90 ^a	0.9	7.00 ^A	0.9	7.8	1.3	7.60 ^a	1.1
	**	8.50 ^b	1.0	7.60 ^B	1.1	8.1	1.5	8.10 ^b	1.3
κ -CN (% of CN)	*	14.8	2.9	15.1	1.6	15.5	2.1	15.1	2.3
	**	15.4	2.6	14.9	2.2	15.9	2.1	15.4	2.3
κ -CN (g/l)	*	4.10 ^a	1.0	3.7	0.6	4.10 ^a	0.6	3.90 ^a	0.8
	**	4.40 ^b	1.0	3.8	0.6	4.40 ^b	1.0	4.20 ^b	0.9
Lactose (%)	*	4.65	0.35	4.73	0.20	4.67	0.23	4.65	0.28
	**	4.65	0.21	4.74	0.16	4.54	0.41	4.64	0.29
Dry matter (%)	*	13.0	1.27	12.2 ^a	0.92	13.3	1.77	12.9	1.43
	**	13.3	1.03	12.7 ^b	1.17	13.2	1.28	13.1	1.16
Solids-non-fat (%)	*	8.76 ^A	0.36	8.51 ^A	0.45	8.72	0.29	8.67	0.38
	**	8.98 ^B	0.45	8.68 ^B	0.36	8.76	0.56	8.81	0.47
Urea (mg/l)	*	261	75	276	103	217 ^A	90	251	91.0
	**	255	44	268	49	282 ^B	86	268	62.5

*before wheat gluten supplementation; **after wheat gluten supplementation;

statistical comparison before and after supplementation (in columns): ^{A,B} $P \leq 0.01$; ^{a,b} $P \leq 0.05$

apparatus (Bentley, USA); urea content by the near infrared spectroscopy method PIRS on the AA II analyser (Bran + Luebbe, Germany); coagulability

according to the method of Scharb using 1% rennin (Pijanowski, 1980); electrical resistance using the apparatus of Dрамиński Company (Poland);

and the proportion of casein protein fractions (alpha-casein, beta-casein and kappa-casein) according to the electrophoretic method described by Laemmli (1970) on polyacrylamide gel in the presence of SDS composed of 12% of separating gel and 4% of condensing gel. Before separation milk samples were defatted by centrifugation, and an excess of salt was removed via dialysis in special viscose tubes (Visking Tubes). Before the separation, protein included in samples was denatured by an addition of 2% of SDS and incubated at a temperature of 100°C for 5 min. In order to break disulphide bonds, a reducing agent, i.e. 5% mercaptoethanol and 0.0625 M buffer of pH 6.75, was added to samples. Glycerol (19%) was added to increase the density of samples and bromphenol blue (0.25%) was introduced to obtain a colour. To remove all insoluble impurities, samples were centrifuged directly before putting on a gel. The qualitative analysis of protein separation was done

according to Kim and Jimnez-Flores (1994). The quantitative participation of analysed fractions in a scanned electrophoretic picture, based on particle detection, was determined using Bio1D software (Viber Lourmat, France). The production of casein fractions (in g/l) in milk was calculated as the quotient of their content determined in an electrophoregram and the content of casein in milk.

The obtained results were processed statistically using analysis of variance with Duncan's test in order to determine the significance of the differences between groups (Statistica 6.1 software).

RESULTS AND DISCUSSION

The supplementation of cows' TMR dose with 450 g of wheat gluten including 300 g of protein caused some changes in milk composition and its physical properties. In all genotypes an increase in crude

Table 2. Technological properties of milk from cows with different blg genotypes before and after wheat gluten supplementation

Specification	Analysis	Beta-lactoglobulin genotype						Total	
		AA		AB		BB			
		18		17		18		53	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
TBC × 1 000	*	39 ^A	41	45 ^A	33	152	302	79	181
	**	107 ^B	98	111 ^B	68	149	275	122	171
SCC × 1 000	*	386	528	397	543	304	496	362	514
	**	257	315	264	303	462	683	328	469
Active acidity (pH)	*	6.69 ^A	0.05	6.70 ^a	0.06	6.68	0.08	6.69	0.06
	**	6.64 ^B	0.07	6.68 ^b	0.05	6.70	0.07	6.67	0.07
Potential acidity (°SH)	*	6.40	0.60	6.12	0.69	6.49 ^a	0.61	6.34	0.64
	**	6.57	0.68	6.02	0.75	6.04 ^b	0.74	6.22	0.76
Coagulability (min)	*	6.84	4.15	6.47 ^a	3.43	6.67	3.83	6.67	3.76
	**	5.53	1.58	5.00 ^b	0.00	6.39	3.35	5.65	2.18
Thermostability (cm ³)	*	2.63 ^a	1.13	2.30	0.69	2.19	1.17	2.38	1.03
	**	2.06 ^b	0.54	2.21	0.54	1.99	0.51	2.09	0.53
Resistance (Ω)	*	491	98.7	529 ^a	96.9	546 ^A	56.4	521 ^A	87.8
	**	484	73.3	488 ^b	46.2	466 ^B	66.2	479 ^B	63.0

*before wheat gluten supplementation; **after wheat gluten supplementation;

statistical comparison before and after supplementation (in columns): ^{A,B}P ≤ 0.01; ^{a,b}P ≤ 0.05

Table 3. Chemical content and casein protein proportion in milk from cows with different blg genotypes before and after extracted, protected soybean meal supplementation

Specification	Analysis	Beta-lactoglobulin genotype						Total	
		AA		AB		BB		53	
		16		18		19		53	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
Daily yield	*	30.5	4.2	34.7	4.9	33.2	5.1	32.9	5.0
	**	30.1	4.1	34.4	4.7	33.0	4.8	32.6	4.8
Fat (%)	*	4.27	1.21	3.71	0.92	4.17	1.24	4.04 ^a	1.14
	**	4.69	1.02	4.17	1.03	4.60	0.97	4.48 ^b	1.01
Protein (%)	*	3.87	0.37	3.45 ^A	0.37	3.73 ^A	0.41	3.68 ^a	0.41
	**	4.00	0.41	3.71 ^B	0.46	3.94 ^B	0.55	3.88 ^b	0.49
Casein (%)	*	2.96	0.28	2.64 ^A	0.28	2.86 ^A	0.32	2.82 ^a	0.32
	**	3.07	0.31	2.84 ^B	0.35	3.01 ^B	0.42	2.97 ^b	0.37
α -CN (% of CN)	*	55.6	3.82	56.2	2.26	55.3	3.14	55.7	3.1
	**	55.8	3.69	56.3	1.98	55.9	2.92	56.0	2.9
α -CN (g/l)	*	16.5	1.90	14.80	1.50	15.8	2.10	15.7 ^a	2.0
	**	17.1	0.23	16.0	2.00	16.9	2.80	16.7 ^b	2.4
β -CN (% of CN)	*	29.1	2.02	29.4	2.08	28.7	2.33	29.0	2.1
	**	29.3	1.97	29.4	1.76	28.9	2.02	29.2	1.9
β -CN (g/l)	*	8.60	1.00	7.80 ^A	1.10	8.20 ^A	0.90	8.20 ^a	1.0
	**	9.00	1.00	8.40 ^B	1.20	8.70 ^B	1.30	8.70 ^b	1.2
κ -CN (% of CN)	*	15.3	3.26	14.5	1.81	16.0 ^a	2.34	15.3	2.5
	**	14.9	3.08	14.3	1.57	15.3 ^b	2.22	14.8	2.3
κ -CN (g/l)	*	4.50	1.10	3.80 ^A	0.70	4.60	0.90	4.30	0.90
	**	4.50	0.90	4.00 ^B	0.70	4.60	0.70	4.40	0.80
Lactose (%)	*	4.60 ^A	0.29	4.65	0.18	4.55	0.42	4.60	0.31
	**	4.47 ^B	0.27	4.56	0.26	4.52	0.29	4.52	0.27
Dry matter (%)	*	13.4	1.34	12.4	1.13	13.0 ^a	1.20	12.9 ^a	1.26
	**	13.8	1.37	13.0	1.37	13.6 ^b	1.24	13.5 ^b	1.34
Solids-non-fat (%)	*	9.08	0.38	8.69	0.35	8.87 ^a	0.32	8.87	0.38
	**	9.06	0.34	8.73	0.62	9.04 ^b	0.46	8.94	0.51
Urea (mg/l)	*	220	69	225	64	211	60	219	63
	**	231	57	234	58	225	53	230	55

*before extracted protected soybean meal supplementation; **after extracted protected soybean meal supplementation; statistical comparison before and after supplementation (in columns): ^{A,B} $P \leq 0.01$; ^{a,b} $P \leq 0.05$

protein and casein content in milk was observed, however a significant increase was proved only in the case of cows with the AB ($P \leq 0.01$) and the AA

blg ($P \leq 0.05$) genotype. In cows with the AB blg genotype there was also an increase in alpha-casein ($P \leq 0.05$) and beta-casein ($P \leq 0.01$) yield,

dry matter ($P \leq 0.05$) and solids-non-fat ($P \leq 0.01$) content. A significant decrease in active acidity, milk coagulability and electrical resistance was also noted, however all values are within the standards. In milk from cows with the AA blg genotype, after 12 days of wheat gluten supplementation, there was a significant ($P \leq 0.05$) increase in crude protein and casein content from 3.56% and 2.73% to 3.77% and 2.88%, respectively. Milk produced by these cows contained statistically significantly ($P \leq 0.05$) more solids-non-fat (by 0.22%) compared to milk produced before the beginning of supplementation. A significant ($P \leq 0.05$) increase in beta-casein and kappa-casein production, and a significant decrease in milk active acidity ($P \leq 0.01$) and thermostability ($P \leq 0.05$) were also observed. Except scarce cases, no significant influence of wheat gluten on physical properties of milk produced by cows with the BB blg genotype was noted.

The addition of wheat gluten to the diet of cows with the AA and AB blg genotype increased the

expression of genes responsible for milk protein and casein production, which indicates the better utilisation of amino acids included in the fodders for milk protein production, especially alpha-casein and beta-casein. The kappa-casein proportion was significantly increased only in the case of cows with the AA ($P \leq 0.05$) and AB ($P \leq 0.01$) blg genotype. Wohlt et al. (1991) observed an increase in daily milk yield and fat content, and a decrease in protein content in a period of 4–18 weeks of lactation after the addition of maize gluten. Similarly, an increase in yield, fat and protein content of milk was noted by Schroeder (2003) while Boddugari et al. (1999) observed a decrease in protein proportion and did not note an increase in milk yield. Gunderson et al. (1988) did not observe any changes in milk yield and composition. The observed differences in cows fed the same TMR mixture may be a result of better utilisation of wheat gluten by cows with the AA and AB blg genotype. The changes in physical properties of milk, in spite of some significant cases,

Table 4. Technological properties of milk from cows with different blg genotypes before and after extracted, protected soybean meal supplementation

Specification	Analysis	Beta-lactoglobulin genotype						Total	
		AA		AB		BB			
		16		18		19		53	
		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
TBC \times 1 000	*	117	191	175	389	318	464	209	377
	**	163	76	158	118	160	140	160	114
SCC \times 1 000	*	248 ^a	243	417	723	933	1 192	551	876
	**	398 ^b	278	685	916	479	455	524	619
Active acidity (pH)	*	6.60 ^A	0.09	6.64 ^A	0.06	6.66 ^A	0.10	6.63 ^A	0.09
	**	6.71 ^B	0.08	6.75 ^B	0.11	6.73 ^B	0.07	6.73 ^B	0.09
Potential acidity (°SH)	*	6.50	0.77	6.13	0.61	6.11	0.72	6.23	0.71
	**	6.25	0.67	6.09	0.63	6.06	0.61	6.13	0.63
Coagulability (min)	*	5.31	1.25	5.83	1.92	7.37	3.86	6.23	2.76
	**	5.31	1.25	6.67	2.97	6.05	2.09	6.04	2.27
Thermostability (cm ³)	*	2.62 ^A	0.71	2.63 ^A	0.56	2.05 ^A	0.54	2.42 ^A	0.65
	**	4.23 ^B	1.84	3.44 ^B	1.18	3.33 ^B	0.97	3.64 ^B	1.38
Resistance (Ω)	*	477 ^A	42.1	460 ^A	58.1	443	94.4	459 ^A	69.9
	**	423 ^B	47.1	392 ^B	76.0	418	56.6	411 ^B	61.8

*before extracted protected soybean meal supplementation; **after extracted protected soybean meal supplementation; statistical comparison before and after supplementation (in columns): ^{A,B} $P \leq 0.01$; ^{a,b} $P \leq 0.05$

were small, and their values were within the range of standards. A significant decrease in electrical resistance and somatic cell count points to the improvement of the health status of cows' udders.

The supplementation of extracted soybean meal to cows with the AB and BB blg genotype caused a significant ($P \leq 0.01$) increase in protein (by 0.26% and 0.21%) and casein content (by 0.20% and 0.15%). There was also a significant ($P \leq 0.01$) increase in beta-casein amount produced in these groups, and in the case of cows with the AB blg genotype, there was also a significant increase in kappa-casein content. It should be emphasised that the proportion of kappa-casein was lowered in all groups. In analysed groups there was a significant ($P \leq 0.01$) increase in active acidity, thermostability and a decrease in electrical resistance in milk. No statistically significant changes in the composition of milk from cows with the AA blg genotype were observed.

Milk from cows with the AA blg genotype contained 4.27% of fat, 3.87% of crude protein and 2.96% of casein before the addition of extracted soybean meal. After 12 days of the experiment the content of the above-mentioned components was 4.69%, 4.00% and 3.07% respectively, which documents the stimulating activity of the supplement, and probably higher lysine and threonine supply. Any significant changes in protein casein fractions in milk from cows of particular genotypes were observed, and statistically significant ($P \leq 0.01$) differences in yield in the AB and BB blg genotypes were due to increased total casein production in these genotypes. The statistical analysis showed a significant decrease in lactose content in milk, which may be due to the better energy utilisation for protein and fat production since the cows did not show any mammary gland disorders, and a decrease in electrical resistance shows the improvement of their udders' health status. Olmos Colmenero and Broderick (2006) observed an increase in milk yield and fat content, and obtained a constant level of protein content while extracted soybean meal was supplemented. Research conducted by Broderick (2003) also demonstrated an increase in milk yield, fat and protein content in milk. However, Nakamura et al. (1992) and Atwal et al. (1995) observed a decrease in yield with an increase in milk fat and protein content when extracted soybean meal was supplemented.

The results of our research indicate a differentiated expression of milk protein genes in cows with

different beta-lactoglobulin genotypes receiving a diet supplemented with glucose from wheat or extracted soybean meal. A different influence of wheat gluten and extracted soybean meal addition was demonstrated in cows with different blg genotypes, which may be of practical value in feeding rationalisation and improvement of milk composition.

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