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Effect of wheat gluten and extracted soybean meal added to the diet of cows with different kappa-casein genotypes on the composition and physical properties of milk

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The effect of adding wheat gluten or extracted soybean meal (300 g of crude protein/head/day) on milk yield, composition and physical properties, was analysed on 53 Polish Holstein-Friesian cows with different milk kappa-casein genotypes (AA, AB and BB). The addition of wheat gluten to the diet of cows with the AB kappa-casein genotype caused significant increase in fat, crude protein, and casein content of milk, and alpha-casein, beta-casein and kappa-casein yields. It also resulted in an increase in total solids content and total bacteria count (TBC), and decrease in thermostability, alpha-casein content and electrical resistance of milk. In the case of cows with the AA kappa-casein genotype, only significant increase in crude protein, casein content, kappa-casein yield and total solids was observed as a result of wheat gluten addition. Furthermore, a decrease in TBC, and a reduction in the coagulation time were noted. The addition of extracted soybean meal to the diet of cows with the AA and AB kappa-casein genotypes caused significant changes in milk composition and physical properties. These included significant increase in fat, crude protein, casein, and total solids content, and alpha-casein and beta-casein yields, as well as milk acidity and thermostability. Cows with the BB kappa-casein genotype did not reveal any distinct changes in milk composition and its physical properties as a result of supplementation with either wheat gluten or extracted soybean meal. Results of the study show that cows with particular kappa-casein genotypes utilize feed components of the feeding ration in a different manner that may be significant in rationalization of feeding and improvement of milk composition.

KEY WORDS: milk / kappa-casein / nutrigenomics / protein / soybean meal / wheat gluten

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Identification of genotypes of animals by analysis of DNA and a detection of its polymorphism creates the possibilities of analysis of relationships and cooperation of genes of quantitative traits, determining their products and searching for those of a large effect of activity. Functional genomics that has been developing in last years heads towards the determination of functions of genes and their influence on transformation processes, including production ones in animals [Schwerin *et al*. 2004, Walawski *et al* 2004, Freyer and Vukasinovic 2005, Kamiński *et al.* 2005, Smaragdov *et al*. 2006, Matějíček *et al.* 2007, 2008].

Phenotypic specifications of composition and physical properties of cows' milk result from an expression of genes coding its particular components. The activity of particular mRNA replication may differ depending on the genotype, the kind and composition of feeds used in the diet, the stage of lactation and other environmental factors. Recognition of that variability is difficult due to the complexity of factors influencing milk production.

The composition and physical properties of milk influence its biological value, technological properties, and quality of final consumer products. Thus, knowledge of gene expression in cows of different milk protein genotypes may elucidate the relationship between genotype and milk properties, and create new opportunities for nutrition and selection of dairy cows. Ng-Kwai-Hang *et al*. [1990], Walawski *et al.* [1994], Lundén *et al.* [1997], Kučerova *et al.* [2006] and others point out to a significant relationship between protein genotype of milk in cows and its physical and technological properties.

The results of feeding corn gluten [Wohlt *et al.* 1991, Schroeder 2003] or extracted soybean meal [Broderick 2003, Olmos Colmenero and Broderick 2006] demonstrated their profitable influence on milk yield, and milk fat and protein yield and content. In turn, Boddugari *et al.* [1999] and Gunderson *et al.* [1988] did not observe any changes in milk yield and composition. Such differences may be a result of differences in feed composition, but also be related to animal genotype response to feed supplements [Szulc *et al.* 2008].

To our knowledge, no prior studies have investigated the effect of an actual kappa-casein genes expression on composition and physical properties of cows' milk depending on a level of their feeding and type of feed mixtures. In the present study, it is hypothesised that there is an effect of kappa-casein genotypes (AA, AB and BB) on the utilization of additional protein introduced into the diet during the production of milk components, including caseins. Thus, the present study aimed at determining the influence of wheat gluten and extracted soybean meal diet supplementation (300 g of crude protein/head/day) in cows with different kappa-casein genotypes (AA, AB and BB) on milk composition and its physical properties.

Material and methods

The study was conducted on Polish Holstein-Friesian cows, in a herd with a mean annual yield of 8500 kg milk per cow, 3.99% fat and 3.40% protein. The kappa-casein genotype of all animals in the herd was determined with the PCR method [Ziemiński *et al.* 2002] with the distribution of AA, BB and AB genotypes of 51.2%; 4.5% and 43.5%, respectively. Based on that distribution, 53 cows (23 AA, 23 AB and 7 BB) were selected for the experiment. The small number of BB cows resulted from the very low occurrence of the B kappa-casein gene [Klauzińska *et al.* 2004]. Within the genotypes, animals were selected for the experiment on the basis of milk yield, lactation parity and lactation month. Cows in lactations 2-5 were selected on the basis of their similarity in milk yield in the last test milking. Cows from day 30 to day 100 of lactation took part in experiment I, whilst those from day 60 to 120 in experiment II.

Throughout the year the cows were fed *ad libitum* with the total mixed ration (TMR system) containing silages of maize, lucerne, and beet pulp together with hay and extracted rapeseed meal. The feed contained 45.52% dry matter (DM) including 12.43% ash, 15.75% crude protein and 18.75% crude fibre in DM. The quality of silages, according to Flieg-Zimmer's score, was 77. The basic ration was calculated for the production of 18 kg milk per day. Cows with a daily milk yield exceeding 18 kg received 1 kg concentrate containing 7.5 MJ of net energy for lactation (NET) and 185 g of crude protein for each 2 kg of additional milk yielded. During experiments I and II cows individually received the following supplements over a period of 12 days on the top of the TMR mixture:

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

Test milkings were performed before the beginning and after the 12th day of ration supplementation. Experiment II was conducted on the same cows, with the same basic TMR, 15 days after the termination of experiment I. Milk samples from the whole milking, collected with the use of Milkometr device, were transported within 2 hours in a car fridge to the laboratory. Then, the samples were analysed for crude protein, fat, lactose and total solids using the Milko-Scan 133B device (ASN FOSS–ELECTRIC, Denmark), whilst the casein content was determined according to Walker's method [Polish Standard – PN-68/A-86122, 1968]. Active acidity of milk was measured using a pH meter, potential acidity with the Soxhlet-Henkel's method [Polish Standard – PN-68/A-86122, 1968], thermostability with the alcohol test [Polish Standard – PN-68/A-86122, 1968], and mass density using a densitometer DMA 35 N (Anton Paar, Austria). Somatic cell count (SCC) was found with flow cytometry method using the Somacount 150 apparatus (BENTLEY, USA), total number of microorganisms was determined by flow cytometry using the Bactocount 70 device (BENTLEY, USA), urea content of milk using 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 Dramiński Company (Poland) that is designed for a determination of sub-clinical inflammatory state of an udder. The relative proportions of the casein protein fractions (alpha-casein, beta-casein and kappa-casein) were determined according to the electrophoretic method described by Laemml [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 Visking Tubes. Before the separation, protein included in samples was denaturated by an addition of 2% of SDS and incubated in 100°C for 5 min. In order to break disulphide bonds, reducing agent, *i.e*. 5% mercaphoethanol and 0.0625 M buffer of pH 6.75, were added to samples. To increase the density of samples glycerol (19%) was added, and bromphenol blue (0.25%) introduced to obtain a colour. Directly before putting on a gel, sample was centrifuged in order to remove all insoluble impurities. Qualitative protein separation was done according to Kim and Jimenez-Flores [1994]. Quantitative participation of analysed fractions on scanned electrophoretic picture, based on particles detection, was determined using Bio1D software (VIBER LOURMAT, France). Yield of casein fractions (g/l) in cows' milk was calculated as a quotient of their content determined in an electrophoretogram and casein content of milk.

The results were verified using analysis of variance with Duncan's test to discriminate the significance of differences between groups using Statistica 6.1 software (StatSoft Polska, Poland). The comparison of means within the genotypes of milk protein was done using Student's t-test for dependent samples, basing on considerably lower variation of differences among measurements than within the groups compared.

Results and discussion

Supplementary feeding with wheat gluten caused a significant (P≤0.05) increase in the crude protein, casein, beta-casein and kappa-casein content of milk (Tab. 1 and 2). The most significant changes were noted in the milk of cows with the AB kappa-casein genotype. This genotype was associated with significant increase in fat content (of 0.6 per cent points (pp), P≤0.05), crude protein content (0.29 pp, P≤0.01), casein content (0.22 pp, P≤0.01) as well as alpha-casein (0.9 g/l, P≤0.05), beta-casein (0.9 g/l, P≤0.01) and kappa-casein (0.4 g/l, P≤0.01) yield. It also resulted in an increase (P≤0.01) in total solids content (of 0.24 pp) and TBC, and decrease in thermostability (P≤0.05), alpha-casein content (1.2 g/l, P≤0.01) and electrical resistance. In cows of the AA kappa-casein genotype, there was an increase (P≤0.05) in crude protein (0.16 pp) and casein content (0.12 pp), kappa-casein yield (0.3 g/l) and total solids content (0.13 pp) as a result of supplementation with wheat gluten. Additionally, a decrease in bacterial count in milk and a reduction in the coagulation time were noted. In milk of cows with the BB kappa-casein genotype, the content of protein and casein decreased, but not significantly.

Table 1. Chemical composition and casein (CN) protein content of milk from cows of different kappa-casein genotypes before and after wheat gluten administration

Item	Determi-nation	Kappa-casein genotype						Total (n=53)	
		AA (n=23)		AB (n=23)		BB (n=7)			
		mean	SD	mean	SD	mean	SD	mean	SD
Daily milk yield (kg)	1	33.0	4.75	34.5	7.04	31.6	3.95	33.4	5.80
	2	32.9	4.76	34.1	6.63	30.9	3.21	33.2	5.55
Fat (%)	1	4.40	1.44	3.79*	0.89	4.82	1.56	4.18	1.27
	2	4.20	0.75	4.39*	0.95	3.91	1.18	4.25	0.90
Protein (%)	1	3.40*	0.45	3.36**	0.34	3.59	0.38	3.41*	0.40
	2	3.56*	0.56	3.65**	0.36	3.48	0.72	3.59*	0.50
Casein (%)	1	2.58*	0.34	2.58**	0.26	2.75	0.29	2.60*	0.30
	2	2.70*	0.45	2.80**	0.27	2.66	0.55	2.74*	0.39
Alpha-CN (% of CN)	1	56.8	2.8	56.5**	1.7	50.1	1.7	55.8	3.1
	2	56.1	2.6	55.3**	1.9	50.4	2.1	55.0	2.9
Alpha-CN (g/l)	1	14.7	2.2	14.6*	1.5	13.8	1.6	14.5	1.8
	2	15.2	2.6	15.5*	1.7	13.4	2.5	15.1	2.3
Beta-CN (% of CN)	1	29.6	2.5	28.0	2.0	31.2*	1.1	29.1	2.4
	2	29.8	2.1	29.0	1.8	30.6*	1.4	29.5	2.0
Beta-CN (g/l)	1	7.60	1.20	7.20**	0.90	8.6	0.90	7.60*	1.10
	2	8.10	1.50	8.10**	1.00	8.10	1.60	8.10*	1.30
Kappa-CN (% of CN)	1	13.6	1.9	15.5	1.2	18.7	1.0	15.1	2.3
	2	14.1	2.1	15.7	1.1	19.0	1.6	15.4	2.3
Kappa-CN (g/l)	1	3.50**	0.7	4.00**	0.4	5.2	0.7	3.90*	0.8
	2	3.80**	0.9	4.40**	0.4	5.1	1.5	4.20*	0.9
Lactose (%)	1	4.69	0.25	4.63	0.28	4.59	0.37	4.65	0.28
	2	4.70	0.19	4.62	0.28	4.53	0.52	4.64	0.29
Total solids (%)	1	13.1	1.58	12.4**	1.08	13.6*	1.69	12.9	1.43
	2	13.0	0.99	13.2**	1.17	12.5*	1.63	13.1	1.16
Solids-non-fat (%)	1	8.71*	0.35	8.60**	0.42	8.75	0.35	8.67	0.38
	2	8.84*	0.44	8.84**	0.36	8.59	0.83	8.81	0.47
Urea (mg/l)	1	246	73.2	275	107.5	188**	45.5	251	91.0
	2	264	50.3	275	69.0	260**	81.5	268	62.5

1 – before administration of wheat gluten; 2 – after administration of wheat gluten.
*,**Within each compound the difference between mean "before" and "after" gluten administration is significant at P≤0.05 and P≤0.01, respectively.
SD – standard deviation.

The addition of extracted soybean meal (Tab. 3 and 4) to the diet of cows with the AA and AB kappa-casein genotypes caused significant changes in composition and physical properties of milk These included increase (P≤0.05) in fat content (of 0.53 and 0.55 pp, respectively), and increase (P≤0.01) in crude protein (0.22 pp), casein (0.17 pp), total solids (0.7 pp), alpha-casein (0.11 pp) content and beta-casein (0.5 and 0.6 g/l) yield, milk acidity and thermostability. In cows of the BB kappa-casein genotype, ration supplementation with extracted soybean meal had no effect on milk composition or its physical properties. There was only a slight increase in the alpha-casein content of milk and an improvement in its thermostability.

Table 2. Physical properties of milk from cows with different kappa-casein genotypes before and after wheat gluten administration

Item	Determination	Kappa-casein genotype						Total (n=53)	
		AA (n=23)		AB (n=23)		BB (n=7)			
		mean	SD	mean	SD	mean	SD	mean	SD
TBC × 10^3	1	108	266	45**	63	95	94	79	181
	2	102	90	155**	239	77	46	122	171
SCC × 10^3	1	365*	511	306	512	544	565	362	514
	2	184*	214	369	417	659	947	328	469
Active acidity (pH)	1	6.67	0.07	6.70	0.04	6.72	0.09	6.69	0.06
	2	6.65	0.07	6.68	0.06	6.68	0.08	6.67	0.07
Potential acidity (°SH)	1	6.40	0.64	6.27	0.65	6.40	0.65	6.34	0.64
	2	6.37	0.70	6.13	0.73	6.06	1.02	6.22	0.76
Coagulability (min)	1	6.74**	3.57	6.04	3.61	8.57	4.76	6.67	3.76
	2	5.00**	0.00	5.83	2.41	7.14	3.93	5.65	2.18
Thermostability (cm^3)	1	2.30	0.72	2.60*	1.31	1.87	0.59	2.38	1.03
	2	2.19	0.53	2.08*	0.52	1.76	0.51	2.09	0.53
Resistance (Ω)	1	507	74.4	534**	91.9	527	118.1	521**	87.8
	2	491	36.2	478**	66.7	441	104.8	479**	63.0

1– before administration of wheat gluten; 2 – after administration of wheat gluten.
*,**Within each compound the difference between mean "before" and "after" gluten administration is significant at P≤0.05 and P≤0.01, respectively.
TBC – total bacteria count; SCC – somatic cell count; SD – standard deviation.

The addition of 300 g of crude protein in the form of wheat gluten to cows receiving 15.75% crude protein from a TMR caused some significant changes in the composition and physical properties of milk that were especially distinct in cows with the AA and AB kappa-casein genotypes. Wohlt *et al.* [1991] observed an increase in daily milk yield (4.5 kg) and fat content (of 0.11 pp), and a decrease in protein content (of 0.11 pp) during week 4 to 18 of lactation after supplementation the cows' ration with maize gluten. An increase in milk yield (of 0.9 kg), fat content (of 0.51 pp) and protein content of milk (of 0.07 pp) have been noted by Schroeder [2003], while Boddugari *et al.* [1999] observed a decrease in protein content. Conversely, Gunderson *et al.* [1988] did not find any changes in milk yield and composition after diet supplementation with corn gluten. It should be emphasized, however, that gluten addition has been found to cause a significant increase in the proportion and yield of kappa-casein of milk which could improve its physical and technological properties [Ng-Kwai-Hang *et al.* 1990, Walawski *et al.* 1994]. They also found a significant increase in crude protein yield and content of milk. Such high and significant increases in the protein production, including casein and casein fractions of the milk of cows with the AB kappa-casein genotype suggest that cows in question responded in an especially positive manner to protein supplementation. Their milk showed the greatest number of profitable changes in composition and physical properties. Cows with the other genotypes (AA and BB) did not exhibit such significant changes. Differences in genotype may play a part in explaining the discrepancies found in the literature described above.

Table 3. Chemical composition and casein protein content in milk from cows of different kappa-casein genotypes before and after soybean meal administration

Item	Determination	Kappa-casein genotype						Total (n=53)	
		AA (n=23)		AB (n=23)		BB (n=7)			
		mean	SD	mean	SD	mean	SD	mean	SD
Daily yield (kg)	1	32.8	4.4	33.8	5.9	30.5	2.9	32.9	5.0
	2	32.4	4.3	33.3	5.7	30.9	2.8	32.6	4.8
Fat (%)	1	3.95*	1.26	4.02*	1.08	4.44	0.94	4.04*	1.14
	2	4.48*	1.08	4.57*	1.04	4.20	0.70	4.48*	1.01
Protein (%)	1	3.63**	0.46	3.72**	0.40	3.73	0.31	3.68*	0.41
	2	3.85**	0.58	3.94**	0.46	3.75	0.15	3.88*	0.49
Casein (%)	1	2.78**	0.35	2.85**	0.31	2.85	0.24	2.82*	0.32
	2	2.95**	0.44	3.02**	0.35	2.88	0.12	2.97*	0.37
A-CN (% of CN)	1	56.9	2.6	56.3	1.6	49.9*	1.0	55.7	3.1
	2	57.3	2.2	56.4	1.3	50.3*	1.3	56.0	2.9
A-CN (g/l)	1	15.8**	2.0	16.0**	1.9	14.2	1.2	15.7*	2.0
	2	16.9**	2.7	17.1**	2.2	14.5	0.8	16.7*	2.4
B-CN (% of CN)	1	29.1	2.3	28.5	2.0	30.8	1.3	29.0	2.1
	2	29.2	2.1	28.7	1.6	30.7	1.5	29.2	1.9
B-CN (g/l)	1	8.10**	1.2	8.10**	0.9	8.8	0.9	8.20*	1.0
	2	8.60**	1.4	8.70**	1.0	8.8	0.7	8.70*	1.2
K-CN (% of CN)	1	14.0	2.4	15.2	1.4	19.4	1.2	15.3	2.5
	2	13.5	1.7	14.8	1.3	19.0	1.6	14.8	2.3
K-CN (g/l)	1	3.90	1.00	4.30	0.70	5.50	0.50	4.30	0.90
	2	4.00	0.80	4.50	0,.60	5.50	0.30	4.40	0.80
Lactose (%)	1	4.61	0.30	4.57	0.34	4.65	0.28	4.60	0.31
	2	4.55	0.25	4.49	0.30	4.53	0.26	4.52	0.27
Total solids (%)	1	12.8**	1.4	12.9*	1.2	13.4	1.0	12.9*	1.3
	2	13.5**	1.5	13.6*	1.3	13.0	0.6	13.5*	1.3
Solids-non-fat (%)	1	8.84	0.32	8.89	0.48	8.93	0.13	8.87	0.38
	2	8.90	0.58	9.02	0.48	8.79	0.25	8.94	0.51
Urea (mg/l)	1	230	56	206**	68	225	67	219	63
	2	239	44	218**	66	236	51	230	55

1– before administration of wheat gluten; 2 – after administration of wheat gluten.
*,**Within each compound the difference between mean "before" and "after" gluten administration is significant at P≤0.05 and P≤0.01, respectively.
α-CN – alpha-casein; β-CN – beta-casein; κ-CN – kappa-casein.

The supplementation of 300 g of crude protein in the form of extracted soybean meal per cow per day caused significant (P≤0.01) changes in most milk components in cows with the AA and AB kappa-casein genotypes. These changes included increase in protein and fat content, including casein, and also in alpha- and beta-casein yield that may influence the milk technological properties [Ng-Kwai-Hang *et al.* 1990, Walawski *et al.* 1994]. The effects of soybean meal on the composition and physical properties of milk were especially distinct in these genotypes. Despite the fact that Walawski *et al.* [1994] suggested that milk from BB cows has superior processing qualities, no significant changes of this range were observed in the present study. In experiments I and II, cows with the BB kappa-casein genotype did not response to supplementation with changes in milk composition or its physical properties.

Table 4. Physical properties of milk from cows with different kappa-casein genotypes before and after extracted soybean meal administration

Item	Determi-nation	Kappa-casein genotype						Total (n=53)	
		AA (n=23)		AB (n=23)		BB (n=7)			
		mean	SD	mean	SD	mean	SD	mean	SD
TBC × 10^3	1	173	312	267	476	134	135	209	377
	2	173	133	152	109	143	51	160	114
SCC × 10^3	1	399	660	632	1082	784	768	551	876
	2	501	655	463	500	804	850	524	619
Active acidity (pH)	1	6.61**	0.08	6.64**	0.07	6.70	0.14	6.63**	0.09
	2	6.75**	0.09	6.73**	0.09	6.69	0.07	6.73**	0.09
Potential acidity (°SH)	1	6.30	0.70	6.23	0.80	6.06	0.43	6.23	0.71
	2	6.16	0.66	6.12	0.62	6.06	0.63	6.13	0.63
Coagulability (min)	1	5.87	2.46	6.52	3.17	6.43	2.44	6.23	2.76
	2	5.87	2.46	5.87	1.94	7.14	2.67	6.04	2.27
Thermostability (cm^3)	1	2.48**	0.72	2.37**	0.59	2.37**	0.68	2.42**	0.65
	2	3.57**	1.75	3.75**	1.09	3.47**	0.93	3.64**	1.38
Resistance (Ω)	1	467**	58.7	460*	74.4	431	91.2	459**	69.9
	2	413**	60.1	413*	65.5	391	60.4	411**	61.8

1– before administration of wheat gluten; 2 – after administration of wheat gluten.
*,**Within each compound the difference between mean "before" and "after" gluten administration is significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.
TBC – total bacteria count; SCC – somatic cell count.

This suggests that the animals failed to show an increase in the expression of genes responsible for the production of these components. An increased share of crude protein in the ration resulted from extracted soybean meal supplementation, Olmos Colmenero and Broderick [2006] reported an increase in milk yield (of 2 kg), fat content (of 0.13 pp) and a constant level of protein content resulting from the addition of soybean meal to the ration for cows-in-milk. Similarly, Broderick [2003] observed an increase in milk yield (of 1.1 kg), in fat (of 0.15 pp) and protein (of 0.04 pp) content. In turn, Nakamura *et al.* [1992], and Atwal *et al.* [1995] have noted a decrease in fat and protein yield with an increase in fat and protein content.

The parameters of technological features of milk, like potential acidity, coagulability, thermostability and electric resistance showed significant and highly significant changes in analysed kappa-casein genotypes after an addition of wheat gluten or extracted soybean meal to the ration. These differences, however, were small and varied depending on an additive used, so that it is not possible to state whether they significantly affected the technological quality of milk. They point however, to some trends that might occur in that range.

The addition of wheat gluten to the diet of cows of the AB kappa-casein genotype led to an increase in fat, crude protein and casein, including alpha-casein, contribution in the milk, and to increases in beta-casein and kappa-casein yield and total solids content. It also resulted in a decrease in thermostability and an increase in electrical resistance. In the case of cows of the AA kappa-casein genotype, there was a slight increase in kappa-casein yield and in kappa-casein, fat and protein content in the milk. For cows of the BB kappa-casein genotype, no significant, desirable changes in milk composition were noted.

Dietary addition of extracted soybean meal to cows of the AA and AB kappa-casein genotypes caused significant changes in the composition and physical properties of milk. These included increases in the fat, crude protein, casein and dry matter content, in alpha-casein and beta-casein yield, and in the acidity and thermostability of milk. The addition of protein to cows of the BB kappa-casein genotype did not cause any significant beneficial changes in milk composition.

The results of this study suggest that the identification of milk kappa-casein genotypes may facilitate future research aimed at improving dairy cow feeding strategies. They may also explain why individuals of the BB kappa-casein genotype fall out of a population in which cattle selection is aimed at improving the yield of milk components.

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