Effects of genetic variants in milk protein on yield and composition of milk from Holstein-Friesian and Simmentaler cows

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Abstract

Milk samples from 237 Holstein-Friesian (HF) and 177 Simmentaler cows in six herds were typed for genetic variants of α_{S1}-, β- and κ-caseins (Cn) and β-lactoglobulin (-Lg) using ultrathin-layer polyacrylamide isoelectric focusing in the presence of carrier ampholytes (3-10 pH). The frequency distribution of genetic variants of all four milk protein systems was different in HF and Simmentaler. In addition, milk production traits of the dairy cows were compared according to the genetic variants of each milk protein fraction. Milk yield was significantly influenced by κ-Cn (genotype AA > genotype AB) and β-Lg (AB > BB > AA) in HF, while in the Simmentaler milk yield was significantly associated with β-Cn ($A^2A^2 > A^1B > A^2B > A^1A^2 > A^1A^1$) and κ-Cn (AB > AA > BB). In terms of protein content α_{S1}-Cn was significant (BC > BB) in HF, and α_{S1}-Cn (BC > BB), β-Cn ($A^1A^1 > A^2A^2 > A^1B > A^1A^2 > A^2B$), κ-Cn (AA > AB = BB) as well as β-Lg (BB > AA > AB) were significant in Simmentaler. The effects of milk protein genetic variants on milk fat content in HF were not significant, while in Simmentaler this trait was significantly affected by α_{S1}-Cn (BC > BB), β-Cn ($A^1A^1 > A^1B > A^2A^2 > A^1A^2 > A^2B$) and β-Lg (BB > AA > AB).

Keywords: α_{S1}-casein, β-casein, κ-casein, β-lactoglobulin, isoelectric focusing (IEF), polyacrylamide gel
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Introduction

In bovine milk, six major milk protein fractions (α_{S1}-casein, α_{S2}-casein, β-casein, κ-casein, α-lactalbumin, β-lactoglobulin), which are controlled by codominant autosomal genes according to Mendelian inheritance exist in different allelic forms. Since the initial discovery of polymorphism of the whey protein, β-lactoglobulin (-Lg) and a quantitative distribution of its variants (Aschaffenburg & Drewry, 1955; 1957) researchers have become interested in the genetic polymorphism of milk proteins. It is known today that there are at least 39 genetic variants of six milk protein fractions (Eigel *et al.*, 1984; Bouniol *et al*., 1993; Miranda *et al.*, 1993; Mariani *et al.*, 1995; Visser *et al.*, 1995; Godovac-Zimmermann *et al.*, 1996; Prinzenberg *et al.*, 1996; 1998; Han *et al.*, 2000). These genetic variants occur as a consequence of either substitution or deletion of amino acids within the polypeptide chain (Eigel *et al.*, 1984). The four caseins are encoded by four clustered autosomal genes localized on chromosome 6, in the order α_{S1}-, β-, α_{S2}- and κ-casein (-Cn) (Threadgill & Womack, 1990; Rijnkels *et al.*, 1997) while the α-lactalbumin (-La) gene is on chromosome 5 (Eggen & Fries, 1995) and the β-lactoglobulin (-Lg) gene is on chromosome 11 (Vaiman *et al.*, 1994).

In the past 20 years researchers from different countries have indicated that there are possible associations between certain genetic variants of milk protein and production traits, milk composition and overall manufacturing properties of milk (see reviews by Jakob & Puhan, 1992 and Ng-Kwai-Hang & Grosclaude, 1992). An association of milk protein genotype with milk composition and properties of milk could be exploited commercially by using these genotypes as an additional criterion in selecting bulls for artificial insemination. For example, increasing the frequency of a milk protein genotype associated with increased milk yield and protein concentration would increase cheese yields. Therefore, these findings have raised the interest among research groups around the world because of the potential of using milk protein genes as markers to aid in the selection for milk yield and quality.

The objective of the present study was to determine the milk protein polymorphism of Holstein-Friesian (HF) and Simmentaler breeds and to evaluate the effects of composite milk protein genotypes on the milk production traits as well as protein and fat percentages of milk.

Materials and Methods

Records of 237 Holstein-Friesian and 177 Simmentaler cows from registered dairy herds in South-West Germany were used in this study. The records included data related to each milk sample such as the date of milking, somatic cell count, daily milk yield, protein and fat concentrations as well as the age of the cow and stage of lactation. Milk samples were collected monthly from six herds. Individual milk samples (10 mL), which were preserved with sodium-acid (NaN_3), were centrifuged for 10 min at 4550 G at 4 °C. The skim milk phase was partitioned and stored at –80 °C.

The fat and protein concentrations of the milk were analyzed by the infrared method and the Fossomatic cell counter was used to determine the somatic cell count in the milk. The phenotyping of skim milk samples was carried out by isoelectric focusing (IEF) in 0.5 mm polyacrylamide gels (114 x 248 mm), using carrier ampholytes (pH 3.0-10.0) according to the method reported by Seibert *et al.* (1985) and Erhardt (1989). For the preparation of the gel solution 3.1 mL acrylamide stock solution (30% T and 3% C), 2 mL glycerin, 520 µL Pharmalyte® (pH 4.2-4.9), 130 µL Ampholine® (pH 4.0-6.0) (LKB Pharmacia, Freiburg, Germany), 260 µL Servalyte® (pH 3.0-10.0), 130 µL Servalyte® (pH 5.0-7.0) (Serva, Heidelberg, Germany) and 13.86 mL urea (11.53 M) were mixed. After degassing of the gel solution for about 5 min at room temperature**,** 10 µL TEMED and 100 µL APS (10% w/w) were added. Skim milk samples (10 µL) were diluted with a 50 µL sample preparation solution containing 8 M urea and 0.2% (v/v) 2-mercaptoethanol, and shaken for 1 h at room temperature. For isoelectric focusing 1 M H_3PO_4 anode and 1 M NaOH cathode solutions were prepared. After pre-focusing at a 2000 V limit and 20 mA constant current for 350 Vh at 9 °C, 5 µL of each milk sample was injected into the gel at a distance of 1 cm from the anode. Focusing was carried out up to 3500 Vh under the same conditions and final focusing at 2500 V for 500 Vh. The genetic variants were identified after staining with a solution containing 0.08% (w/v) Coomassie Brilliant Blue G-250, 40 mM H_2SO_4, 8.7% (v/v) 10 N NaOH and 12.25% (v/v) trichloracetic acid (100%).

To study the relationship between genetic variants of α_{S1}-, β- and κ-casein (Cn) as well as β-Lg and milk production traits and milk composition, a least squares analysis of variance was carried out using the General Linear Model (GLM) procedure of SAS (1993). The model fitted included genetic variant of milk protein encoding gene, herd-breed combination, stage of lactation, parity number, season and cow. Somatic cell count (SCC) was included as a linear-quadratic covariate in this model. There were three, five, eight and five subclasses for the effects of season, parity number, stage of lactation and SCC, respectively. The χ^2-test was carried out to determine whether the observed genotype frequencies deviated significantly from the Hardy-Weinberg-Equilibrium.

Results and Discussion

The distribution of genotypes for milk proteins obtained by isoelectric focusing (IEF) in polyacrylamide gel in the milk of HF and Simmentaler cows is summarized in Table 1. The genotypes were recorded directly from the codominant patterns of milk proteins. The results obtained from the study showed that polymorphism exists in both breeds for α_{S1}-Cn, β-Cn, κ-Cn and β-Lg. The AA and BB genotypes were observed for α_{S2}-Cn and α-La, respectively. In Simmentaler cows there was no significant difference ($P > 0.05$) between observed frequencies and those estimated from the Hardy-Weinberg-Equilibrium. On the other hand, in HF cows the observed genotype frequencies for β-Cn and κ-Cn differed significantly from the estimated ones. Although according to estimations, approximately 17 HF cows were expected to carry the κ-Cn BB genotype, it was observed that only three cows possessed this genotype. Bösze & Dohy (1993) reported similar results and they suggested that the deviation could be due to crossbreeding with HF sires from the United States.

The allele frequencies are presented in Table 2. The frequency of allele B at α_{S1}-Cn was the highest in both breeds. At β-Cn the alleles A^1 and A^2 appeared most frequently. In HF the frequency of allele A^2 was 2.3 times higher than that of A^1, while in the Simmentaler the frequencies of these alleles were nearly equal. In both breeds the allele A of κ-Cn occurred most frequently, while in the Simmentaler and HF cows the frequency of allele A was *ca*. 2- and 2.7-times higher than that of allele B, respectively. At β-Lg there were mainly two alleles in which the frequency levels were equal for the Simmentaler cows, while in the HF cows the frequency of allele B was *ca*. 1.6-times higher than that of allele A.

Table 1 Genotype distributions of α_{S1}-, β-, κ-casein (-Cn) and β-lactoglobulin for the Holstein-Friesian and Simmentaler cows

Locus	Genotype	Holstein- Friesian N = 237				Simmentaler N = 177			
		Observed	Expected[1)]	%	P[2)]	Observed	Expected[1)]	%	P[2)]
α_{S1}-Cn	BB	228	228.1	96.2		154	153.8	87.0	
	BC	9	8.8	3.8		22	22.4	12.4	
	CC	0	0.1	0		1	0.8	0.6	
					n.s.				n.s.
β-Cn	A^1A^1	14	20.6	5.9		25	26.4	14.1	
	A^1A^2	109	93.6	46.0		68	63.6	38.4	
	A^1A^3	0	0.9	0		1	1.2	0.6	
	A^1B	3	4.4	1.3		15	16.3	8.5	
	A^1C	0	0	0		3	3.1	1.7	
	A^2A^2	97	105.2	40.9		33	37.9	18.6	
	A^2A^3	2	2.0	0.8		2	1.4	1.1	
	A^2B	11	10.0	4.6		23	19.3	13.0	
	A^2C	0	0	0		5	3.7	2.8	
	A^3B	1	0.1	0.4		0	0.4	0	
	BB	0	0.2	0		2	2.4	1.1	
	BC	0	0	0		0	1.0	0	
	CC	0	0	0		0	0.1	0	
					*				n.s.
κ-Cn	AA	113	126.9	47.7		76	80.6	42.9	
	AB	121	93.2	51.0		87	77.8	49.2	
	BB	3	16.9	1.3		14	18.6	7.9	
					***				n.s.
β-Lg	AA	35	34.8	14.8		46	43.1	26.0	
	AB	112	112.4	47.2		82	87.3	46.3	
	AD	0	0	0		1	1.5	0.6	
	BB	90	89.9	38.0		46	43.6	26.0	
	BD	0	0	0		2	1.5	1.1	
	DD	0	0	0		0	0	0	
					n.s.				n.s.

[1)] Expected - calculated according to the Hardy-Weinberg law. [2)] P signifies overall level of significance between observed and expected genotype frequencies determined by the χ^2-test;
n.s. - not significant, *P < 0.05, ***P < 0.001

Table 2 Allele frequencies for α_{S1}-, β-, κ-casein (-Cn) and β-lactoglobulin (-Lg) for the Holstein-Friesian (HF) and Simmentaler cows

	---- α_{S1}-Cn ----		---------- β-Cn ----------					----- κ-Cn -----		---------- β-Lg ----------		
Alleles	B	C	A^1	A^2	A^3	B	C	A	B	A	B	D
Breed												
HF	0.981	0.019	0.295	0.667	0.006	0.032	0	0.732	0.268	0.384	0.616	0
Simmentaler	0.932	0.068	0.387	0.463	0.008	0.119	0.023	0.675	0.325	0.494	0.497	0.008

Comparing allele frequencies with other studies showed similar results. In HF cows the frequency of α_{S1}-Cn B was similar to that found by Aleandri *et al.* (1990) and Panicke *et al.* (1996), and it was higher and lower, respectively, than the frequencies obtained by McLean *et al.* (1984) and Erhardt (1993). In this study the frequencies of β-Cn A^1 were lower and A^2 higher than the results of McLean *et al.* (1984), Aleandri *et al.* (1990), Erhardt (1993) Panicke *et al.* (1996), while the frequency of β-Cn B was similar to the findings of some authors (McLean *et al.*, 1984; Aleandri *et al.*, 1990; Erhardt, 1993). At κ-Cn, the frequency of allele A

was in agreement with the results of Aleandri *et al.* (1990), while it was lower than the results of other researchers (Erhardt, 1993; Bobe *et al.*, 1999) and higher than reported by McLean *et al.* (1984). The frequencies of β-Lg A and B were nearly similar to the findings of Panicke *et al.* (1996) and McLean *et al.* (1984), but were lower than that reported by Erhardt (1993) and higher than the results of Bobe *et al.* (1999). For Simmentaler cows the frequency of α_{S1}-Cn B was higher and that of α_{S1}-Cn C lower than the results of Graml *et al.* (1984), Putz *et al.* (1991), Erhardt (1993), Ortner *et al.* (1995) and Falaki *et al.* (1997). The frequencies of β-Cn A^1 and B were higher and that of allele A^2 lower than the frequencies reported by other researchers (Putz *et al.*, 1991; Erhardt, 1993; Ortner *et al.*, 1995). The β-Cn C was less frequent than the values reported by Graml *et al.* (1984) and more frequent than that of Putz *et al.* (1991), Erhardt (1993), Ortner *et al.* (1995) and Falaki *et al.* (1997). The frequencies of κ-Cn A and B were similar to that of Graml *et al.* (1984) and Ortner *et al.* (1995), while the frequency of κ-Cn A was higher than that of Putz *et al.* (1991) and lower than that of Erhardt (1993) and Falaki *et al.* (1997). The frequencies of β-Lg A and B were equal, in agreement with the findings of Graml *et al.* (1984) and Putz *et al.* (1991). The frequency of β-Lg B was higher than that reported by Erhardt (1993) and Falaki *et al.* (1997) and lower than the result of Ortner *et al.* (1995).

Least square means and standard errors of test-day milk, protein and fat yields, protein and fat percentages for the different genetic variants α_{S1}-, β- and κ-Cn and β-Lg are presented in Table 3. It was found that in both breeds milk protein levels were affected ($P < 0.001$) by α_{S1}-Cn, while this locus had no effect ($P > 0.05$) on daily milk, protein and fat yields and fat concentration in HF cows. In Simmentaler cows the daily milk, protein and fat yields were not affected ($P > 0.05$) by α_{S1}-Cn, while fat concentration was affected ($P < 0.05$). Compared to genotype BB, both the HF and Simmentaler cows with BC at the α_{S1}-Cn locus had a 3.6% higher protein content in their milk. This result is supported by other researchers (Aleandri *et al.*, 1990; Bovenhuis *et al.*, 1992; Mao *et al.*, 1992; Ortner *et al.*, 1995; Lodes *et al.*, 1997).

In Simmentaler cows the genetic variants of β-Cn were associated with a higher ($P < 0.001$) milk yield, protein yield and protein concentration. Fat concentration was affected ($P < 0.05$) by the genetic variants of β-Cn, while fat yield was not affected ($P > 0.05$). In HF cows the β-Cn genetic variants showed no effect ($P > 0.05$) on the daily milk and protein yields or on protein and fat content, while only fat yield was affected ($P < 0.05$) by genetic variants of β-Cn. The HF cows possessing β-Cn A^1A^1 genotype had 130 g more test-day milk fat than the cows with the β-Cn A^2B genotype. In Simmentaler cows the daily milk, milk protein and milk fat yields of cows possessing the β-Cn A^2A^2 genotype were 4050 g, 130 g and 120 g higher than that of cows with the A^1A^1 genotype, respectively, while the cows with the β-Cn A^1A^1 genotype had 9.8% and 11% higher protein and fat concentrations in their milk, respectively, than cows possessing the A^2B genotype. Bech & Kristiansen (1990) also found that the A^2A^2 genotype of β-Cn was favourable for milk, protein and fat yields. Ortner *et al.* (1995) showed that Simmentaler cows possessing β-Cn A^1A^1 had significantly higher protein and fat levels in their milk than the other genotypes.

The results of this study showed that daily milk, protein and fat yields were affected by the genotypes of κ-Cn ($P < 0.001$) in HF cows, while in Simmentaler cows the κ-Cn genotypes had an effect on protein content ($P < 0.001$) and daily fat yield ($P < 0.01$). The HF cows with κ-Cn AA produced respectively 1560 g, 50 g and 80 g more daily milk, protein and fat than the other genotypes. Simmentaler cows possessing the κ-Cn AA genotype produced 2% more protein as well as 60 g and 100 g more protein and fat per day, respectively, than the cows with the BB genotype. The cows possessing the AB genotype at the κ-Cn locus produced 1390 g more milk on test-day than the other genotypes. The results for milk and fat yield of HF cows were in agreement with that of Bovenhuis *et al.* (1992).

In the HF cows the genotypes, β-Lg, had an effect ($P < 0.01$) on daily milk, protein and fat yields, while in Simmentaler cows fat concentration was affected by the β-Lg genotypes ($P < 0.01$). It was found that the daily milk, protein and fat yields of HF cows with the β-Lg AB genotype were 2170 g, 70 g and 100 g higher than that of cows possessing the β-Lg AA genotype. Simmentaler cows possessing β-Lg AB had 2170 g more milk on test-day than that of β-Lg AA cows. In addition, β-Lg AA cows produced 40 g more daily milk protein than cows with the β-Lg BB genotype, while the β-Lg BB genotype showed 2% and 4% more protein and fat levels than that of cows of the β-Lg AB genotype. The results are in agreement with those reported by McLean *et al.* (1984), Bovenhuis *et al.* (1992), Mao *et al.* (1992), Falaki *et al.* (1997).

Table 3 Associations between milk protein genetic variants and test-day milk yield, protein and fat yields, and protein and fat content in Holstein-Friesian and Simmentaler cows (LS-Means ± s.e.)

Locus[1)]	Genotype[2)]	Number of sampled	animal	Milk yield (kg/day)	Milk protein (%)	Milk protein (kg/day)	Milk fat (%)	Milk fat (kg/day)
Holstein-Friesian								
α_{S1}-Cn	BB	1155	228	28.53 ± 0.42	3.31^{a} ± 0.02	0.94 ± 0.01	4.22 ± 0.02	1.21 ± 0.02
	BC	38	9	27.69 ± 1.05	3.43^{b} ± 0.05	0.95 ± 0.03	4.31 ± 0.07	1.22 ± 0.05
	P[3)]			n.s.	**	n.s.	n.s.	n.s.
β-Cn	A^1A^1	68	14	28.94 ± 0.74	3.35 ± 0.03	0.96 ± 0.02	4.47^{a} ± 0.05	1.27^{a} ± 0.03
	A^1A^2	547	109	28.59 ± 0.46	3.30 ± 0.02	0.94 ± 0.01	4.20^{b} ± 0.02	1.21 ± 0.02
	A^2A^2	491	97	28.68 ± 0.50	3.31 ± 0.02	0.94 ± 0.02	4.23^{b} ± 0.05	1.22 ± 0.02
	A^2B	58	11	27.29 ± 0.67	3.32 ± 0.03	0.90 ± 0.02	4.13^{b} ± 0.05	1.14^{b} ± 0.03
	P[3)]			n.s.	n.s.	n.s.	n.s.	*
κ-Cn	AA	581	113	29.18^{a} ± 0.56	3.31 ± 0.02	0.96^{a} ± 0.02	4.25 ± 0.04	1.25^{a} ± 0.03
	AB	597	121	27.62^{b} ± 0.31	3.32 ± 0.01	0.91^{b} ± 0.01	4.22 ± 0.02	1.17^{b} ± 0.01
	P[3)]			***	n.s.	***	n.s.	***
β-Lg	AA	175	35	26.89 ± 0.45	3.36 ± 0.02	0.89^{a} ± 0.01	4.22 ± 0.04	1.13^{a} ± 0.02
	AB	550	112	29.06^{a} ± 0.52	3.31 ± 0.02	0.96^{b} ± 0.02	4.21 ± 0.03	1.23^{b} ± 0.02
	BB	468	90	28.10^{c} ± 0.42	3.32 ± 0.02	0.93^{a} ± 0.01	4.26 ± 0.03	1.20^{b} ± 0.02
	P[3)]			**	n.s.	**	n.s.	**
Simmentaler								
α_{S1}-Cn	BB	747	154	24.01 ± 0.22	3.54^{a} ± 0.01	0.84 ± 0.01	4.12 ± 0.03	0.99 ± 0.01
	BC	100	22	22.66 ± 0.68	3.67^{b} ± 0.04	0.83 ± 0.02	4.15 ± 0.09	0.94 ± 0.04
	P[3)]			n.s.	**	n.s.	*	n.s.
β-Cn	A^1A^1	124	25	21.36^{a} ± 0.41	3.68^{a} ± 0.02	0.77^{a} ± 0.01	4.39^{a} ± 0.06	0.94^{a} ± 0.02
	A^1A^2	328	68	23.42^{b} ± 0.29	3.50^{b} ± 0.02	0.81^{a} ± 0.01	4.14^{b} ± 0.04	0.97 ± 0.02
	A^1B	70	15	24.91^{bc} ± 0.61	3.54^{bc} ± 0.03	0.87^{bc} ± 0.02	4.21 ± 0.08	1.04^{b} ± 0.03
	A^2A^2	152	33	25.41^{c} ± 0.64	3.63^{ac} ± 0.03	0.90^{b} ± 0.02	4.15 ± 0.09	1.06^{b} ± 0.03
	A^2B	115	23	24.07^{bc} ± 0.55	3.35^{d} ± 0.03	0.80^{ac} ± 0.02	3.95^{b} ± 0.07	0.95 ± 0.03
	P[3)]			***	***	***	*	n.s.
κ-Cn	AA	364	76	24.25 ± 0.41	3.62^{a} ± 0.02	0.86^{a} ± 0.01	4.14 ± 0.06	1.00^{a} ± 0.02
	AB	421	87	24.30^{a} ± 0.25	3.54^{b} ± 0.01	0.85 ± 0.01	4.09 ± 0.03	0.99^{a} ± 0.01
	BB	66	14	22.91^{b} ± 0.49	3.54 ± 0.03	0.80^{b} ± 0.02	3.98 ± 0.07	0.90^{b} ± 0.03
	P[3)]			*	***	*	n.s.	**
β-Lg	AA	222	46	26.89 ± 0.39	3.55 ± 0.02	0.87^{a} ± 0.01	4.15 ± 0.05	1.03 ± 0.02
	AB	396	82	29.06^{a} ± 0.28	3.52^{a} ± 0.02	0.84 ± 0.01	4.07^{a} ± 0.04	0.98 ± 0.02
	BB	217	46	28.10^{c} ± 0.35	3.60^{b} ± 0.02	0.83^{b} ± 0.01	4.25^{b} ± 0.05	1.00 ± 0.02
	P[3)]			n.s.	*	n.s.	**	n.s.

[1)]α_{S1}-casein, β-casein, κ-casein (-Cn) and β-lactoglobulin (-Lg), [2)]Genotypes with ≤ 5 cows are not given, [3)]P signifies overall level of significance between each locus and yield as well as compositional traits of milk (F-Test),
n.s. - not significant, *P < 0.05, **P < 0.01, ***P < 0.001. Means within columns for each milk protein locus with different superscripts differ at P < 0.01

Conclusions

This study demonstrated the possible association between genetic variants of milk protein loci and daily milk, protein and fat yields, and protein and fat content of milk. The results showed that daily milk, protein and fat yields are significantly affected by the genetic variants of κ-Cn and β-Lg in HF cows, while protein content was affected (P < 0.05) by the α_{S1}-Cn locus. However, in Simmentaler cows the genotypes α_{S1}-Cn and β-Lg are significantly associated with protein and fat content, while β-Cn and κ-Cn have a significant effect on daily milk and protein yields as well as protein content. In addition, in Simmentaler cows, β-Cn and κ-Cn have a significant effect on fat content of milk and test-day fat yield, respectively. In both breeds the α_{S1}-Cn BC is desirable for the protein content of milk. In addition, in HF cows, the

favourable genetic variants of milk proteins are: β-Cn A^1A^1 for fat percent and yield, κ-Cn AA and β-Lg AB for daily milk yield as well as protein and fat content. In Simmentaler cows β-Cn A^2A^2, κ-Cn AB and β-Lg AA are favourable for daily milk yield. The other desirable genetic variants of milk proteins are: β-Cn A^1A^1 for protein and fat content as well as β-Cn A^2A^2 for protein and fat yields; κ-Cn AA for protein and fat content and milk fat yield; β-Lg AA for protein yield and β-Lg BB for protein and fat content. In this context, selection for above-mentioned genetic variants of milk protein loci would be more appropriate, due to the fact that milk from these cows is associated with higher content of protein and fat as well as a higher daily milk yield. However, because of close linkage between casein loci, attention should be paid to the effect of composite genotypes or haplotypes on milk yield and composition.

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