IJAS Iranian Journal of Applied Animal Science

CSN1S1 Gene: Allele Frequency, and the Relationship with Milk Production Traits in Three Indigenous Cattle Breeds and Holstein

Research Article

S. Zakizadeh[1*], E.M. Prinzenberg[2], M. Reissmann[3], S.R. Miraei Ashtiani[4], P. Reinecke[3] and G. Erhardt[2]

[1] Department of Animal Science, Higher Educational Complex of Jihad-e-Agriculture, Kalantari Highway, Mashhad, Iran
[2] Institute of Animal Breeding and Genetics, Justus Liebig, University, 35390, Giessen, Germany
[3] Department for Crop and Animal Science, Faculty of Agriculture and Horticulture, Humboldt University Berlin, 10115, Berlin, Germany
[4] Department of Animal Science, Faculty of Agricultural Science, University of Tehran, Karaj, Iran

Received on: 20 Aug 2012
Revised on: 5 Dec 2012
Accepted on: 31 Dec 2012
Online Published on: Sep 2013

*Correspondence E-mail: sonia_zaki@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

CSN1S1 is one of the major genes encoding milk proteins of mammals. In this study we determined allele frequencies of *CSN1S1*-5` flanking region as well as exon 17 variants and their effects on milk traits in three indigenous cattle breeds Mazandarani, Golpaygani (*Bos indicus*) and Sarabi (*Bos taurus*) and Holstein cattle in Iran. *CSN1S1**B variant was nearly fixed in Holstein but ranged from 0.40 to 0.66 in indigenous breeds. *CSN1S1**C allele had higher frequency in indigenous breeds, especially in *Bos indicus*. Four genetic variants of the promoter were found in all breeds in different frequencies with allele 2 being the prevalent in all breeds (frequency 0.359 to 0.711) and allele 4 the least frequent (0.074 to 0.011). Allele B of the coding region was found in combination with all four promoter alleles. Allele 4 of the promoter was not found in any cow having the exon 17 allele C in all breeds except Mazandarani. BC / 23 genotype yielded the highest fat percentage ($P<0.05$) in Holstein but it had no significant effect in Golpaygani. There was not any homozygous *CSN1S1**CC cow, to investigate the influence of C variant for fat content. None of the genetic combinations had significant effect on fat yield, although variant '2' of promoter indicated a negative effect. No significant effect among various combined genotypes on milk yield was found, but *CSN1S1**B tended to higher milk production. Differences of allelic frequencies and milk production traits found among these breeds might be due to differences in origin of breeds or selection breeding programs.

KEY WORDS *CSN1S1*, Holstein, indigenous cattle, milk fat percent, PCR-SSCP.

INTRODUCTION

The major proteins of milk are αs1-, αs2-, β- and κ-caseins (CSN1S1, CSN1S2, CSN2 and CSN3), α-lactalbumin and β-lactoglobulin which are present in different balanced concentration in bovine milk. The *CSN1S1* gene has 22069 bp in length with 19 exons (Koczan *et al.* 1991). It has been reported that its position is located between the *FBN14* and *CSN3* markers, with 5.6 cM distance (Prinzenberg *et al.* 2003). So far, nine genetic variants (A, B, C, D, E, F, G, H and I) of *CSN1S1* in bovine milk have been identified. The 'B' allele is predominant in European cattle breeds, followed by *CSN1S1**C (Rando *et al.* 1998; Formaggioni, 1999; Farrell *et al.* 2004). For *CSN1S1* casein, Ayrshire cattle posses the 'B' variant only, Shorthorn, Guernsey, and Jersey cattle posses the 'B' and 'C' variants and Holstein cattle posses the 'A', 'B' and 'C' variants (Lin *et al.* 1986; Lien *et al.* 1999). The rare variants *CSN1S1* 'A' and 'D' were found in German Holstein and German and Polish Red Cattle with low frequency. Homozygous genotypes

were not found (Erhardt, 1993). The 'E' variant was reported only in *Bos grunniens*; F, G and I in German Black Pied (Erhardt, 1993), Italian Brown cows, Kuri Cattle in Chad, and in *Bos indicus* of Cameron, respectively (Caroli *et al.* 2008). The relationship between milk protein genotype, milk yield and physico-chemical properties of the milk were studied by DNA-based techniques and there is great interest to support genetic improvement. Lin *et al.* (1986) reported that *CSN1S1* had the strongest effects on first lactation yields compared to other protein loci. *CSN1S1**C variant was found to be associated with higher protein and higher casein contents in milk, while the B variant showed higher total milk production (Ng Kwai Hang, 1998). Unfortunately *CSN1S1**B is almost fixed in Holstein, a result of long-term selection for high milk production in dairy cattle (Lin *et al.* 1986; Ng Kwai Hang, 1998).

Four alleles were reported for *CSN1S1* promoter (*CSN1S1*-5') with the highest frequency of allele '2' (Prinzenberg *et al.* 2003; Sanders *et al.* 2006). An association with fat and protein yields with either *CSN1S1*-5' allele 2 or 3 in German Angeln cattle, and higher protein content in cows heterozygous for allele '4' was found (Prinzenberg *et al.* 2003). Grand-Daughter-Design of German Holstein revealed significant effects of *CSN1S1*-5` genotype on breeding values for dairy character, fore udder attachment, length of productive life and somatic cell scores (Prinzenberg *et al.* 2005).

The aim of this study was firstly to determine the allele frequencies of coding sequence and promoter of three indigenous Iranian breeds and Holstein cattle of Iran. Moreover, the impact of allelic differences on milk production traits in different breeds was also investigated.

MATERIALS AND METHODS

In this study, 406 genomic DNA samples from three indigenous Iranian cattle (97 Mazandarani and 112 Golpaygani as *Bos indicus*, 87 Sarabi as *Bos taurus*) and Holstein (110) were considered. Mazandarani samples were collected from 14 distinct villages related to 5 cities in the North of Iran. This breed is usually kept in villages and jungle for meat and work, and the milk produced is either consumed by calves or left for family daily use. Golpaygani breed is considered as a dairy breed and its samples were collected from two breeding stations located in two provinces in center of Iran. Sarabi is also a dairy indigenous breed which is spread in Northwest of Iran. Samples were collected from two indigenous breeding stations. Holstein samples were collected from 9 cities in Khorasan province. DNA was extracted from whole blood using the procedure of Miller *et al.* (1988).

Promoter analysis

A 216 bp fragment including length variation discriminating *CSN1S1*-5' alleles 1, 2 and 3 (Prinzenberg *et al.* 2003) was amplified by Cy5-labeled primers CSN1S1pro2f (5'TGC ATG TTC TCA TAA TAA CC3') and CSN1S1207r (5' TGG TTT CAG TTT AAC CAA CAG 3') DNA was amplified in a total volume of 20 µL containing 50 ng genomic DNA, 5 pmol/µL of forward primer, 10 pmol/µL of backward primer, 0.2 mM dNTP, 2 mM $MgCl_2$, 1X PCR buffer and 1 U Taq DNA polymerase (GENAXXON, Germany) and 1% of total volume of DMSO. PCR conditions were 94 ˚C for 2 minutes, 53 ˚C for 30 seconds and 72 ˚C for 40 seconds at the first round but for the next 35 cycles 94 ˚C was performed for 1 minute. Finally, fragments were elongated at 72 ˚C for 5 minutes. PCR products were prepared with denaturing sample buffer and internal length standards according to standard procedures, loaded on a denaturing polyacrylamid 16% gel and different allele sizes were separated on an ALF instrument (Amersham Biosciences). Alleles 1, 2 and 3= 4 were directly assigned with the AlleleLocator software. For final discrimination of alleles 3 and 4, SSCP was used as described by Prinzenberg *et al.* (2003).

Exon 17

Primers suggested by Prinzenberg *et al.* (2003) were used to amplify a 265 bp fragment of exon 17, encoding the mutation responsible for the Glu192Gly substitution in the mature protein of *CSN1S1**B and C, respectively. PCR amplification was the same as used for promoter except DMSO, 1.5 mM $MgCl_2$ and 10 pmol/µL of each primer. PCR fragments were subjected to SSCP analysis. 5 µL of PCR product with 5 µL loading dye heated to 95 ˚C for 3 min and chilled on ice immediately. 4.5 µL of denaturized samples were loaded on a 9% polyacrylamide gel containing 0.5% glycerol and separated at 500V-5 ˚C for 4 hours and silver stained following with stop solution. Alleles were designated manually compared to reference samples.

Statistical analysis

All frequencies for *CSN1S1* polymorphism in all breeds were determined by direct counting. Chi-square and Kruskal Wallis tests were used to compare allele frequencies of *CSN1S1* coding and promoter regions between breeds. Milk trait records were available only for Golpaygani and Holstein breeds, therefore not all genotypes in the dataset were included in this analysis (Golpaygani, n=40; Holstein, n=87). Variance analysis was performed using statistical package JMP (version 4.0.4).

Milk and fat production, and fat content records were adjusted for each breed separately according to independent factors such as season, days in milk and haplotype in both

breed and also herd in Holstein. Statistical linear model included:

$$Y_{ijkl} = M + S_i + H_j + CG_k + \alpha(DIM_{ijkl} - \overline{DIM}) + e_{ijkl}$$

Where:

Y_{ijkl} is the milk, fat production or fat percent measured on each of ijklth animal, M is the overall population mean, S_i is the effect of ith season, H_j is the effect of jth herd, CG_k is the fixed effect associated with the kth combined genotypes of promoter and coding region, α is the linear regression coefficient of days-in-milk on milk production, and e_{ijkl} is the random residual effect. Since Golpaygani samples were collected from one herd, its effect was ignored for this breed.

RESULTS AND DISCUSSION

Promoter

Genotyping revealed all four alleles in indigenous and Holstein breeds. Allele frequencies and the most frequent order of genotypes can be found for each breed in tables 1 and 2.

Table 1 Frequencies of *CSN1S1* promoter alleles in breeds

Breed	Allele			
	1	2	3	4
Mazandarani	0.247	0.359	0.288	0.106
Sarabi	0.240	0.582	0.089	0.089
Golpaygani	0.208	0.500	0.218	0.074
Holstein	0.021	0.711	0.184	0.084

Table 2 Order of genotypes and genotypes missing in different breeds

Breed	Pattern	Genotypes missing
Mazandarani	23 >12 > 22 > 13 > 33 > 34 > 14 > 11 > 24	44
Sarabi	12 > 22 > 23 > 24 > 14 > 11 > 13 >	33, 34, 44
Golpaygani	22 > 12 > 23 > 13 > 24 > 33 > 14 > 11 > 34	44
Holstein	22 > 23 > 24 > 33 > 34 > 12 > 13 > 14	11, 44

No homozygous cows for '44' genotype were found in all breeds, furthermore genotypes '33' and '34' were missing in Sarabi, and '11' was not found in Holstein.

Exon 17 polymorphism

The allele frequencies of B variant were 0.53, 0.66, 0.40 and 0.99 for Mazandarani, Sarabi, Golpaygani and Holstein, respectively. Table 3 shows the genotype and allele frequencies in all breeds. Differences of the genotype frequencies between each two breeds were calculated by Chi-Square test. Results showed significant differences between indigenous breeds and Holstein. There was also a significant difference between each two indigenous breeds (Table 4).

Intragenic haplotypes

By simultaneous consideration of *CSN1S1* promoter and exon 17 genotypes, combinations of BB / 11, BB / 13, CC / 13, CC / 33, and CC / 34 were absent in all four breeds.

Table 3 Allele and genotype frequencies of exon 17

	BB	BC	CC	Total	Allele B	Allele C
Mazandarani*	24 0.26	49 0.53	19 0.21	92	0.53	0.47
Sarabi*	35 0.42	39 0.47	9 0.11	83	0.66	0.34
Golpaygani*	17 0.15	55 0.5	38 0.35	110	0.4	0.6
Holstein*	96 0.98	2 0.02	0 0.00	98	0.99	0.01

* On the first line the absolute number is reported, and on the second line its frequency.

Table 4 Chi-Square and P-values for genotype frequency

	Holstein	Golpaygani	Sarabi
Mazandarani	105.43**	6.32*	6.31*
Sarabi	70.03**	23.53**	-
Golpaygani	142.29**	-	-

* Significant and ** Highly significant.

In Mazandarani cattle the haplotypes B-1, B-2, B-3, B-4, C-1, C-2 and C-3 were found, and two CC / 14 and CC / 24 genotypes also give indication that haplotype C-4 is present in this breed.

The haplotypes C-3 as well as C-4 in Sarabi and B-1 as well as C-4 in Golpaygani were not observed. Allele 'B' variant of exon 17 was found in combination with different alleles of promoter in Holstein, except B-1. One Holstein sample showed BC / 23 genotype resulting in C-2 or C-3 haplotype, while there was not any C-1 or C-4 haplotpyes. Due to missing genotypes, haplotypes containing *CSN1S1* promoter 33, 34 or 44 with any variants of exon 17 were missing in Sarabi indigenous breed. Furthermore, Holstein did not show any combination between 11 or 44 with coding region exon 17.

Association between different genotypes and milk production traits

In Table 5 an analysis of variances for milk and fat yield and fat content is displayed.

Combined genotypes of *CSN1S1* promoter and exon 17 had no significant effects on milk and fat yields in Golpaygani, but it was significant for fat percentage in Holstein ($P<0.05$). BC / 23 genotype yielded the highest fat content (3.6%).

Although combined genotypes had no significant effect on Holstein milk yields, the BB / 14, genotype tended to be associated with higher milk production (9395 kg; Table 6). Combined genotype had no significant effect on milk production traits in Golpaygani, but BC / 22 genotype tended to produce higher milk yield (2010 kg, not shown).

Polymorphisms

In our study the allele *CSN1S1**B was nearly fixed in Holstein cattle (0.99).

Table 5 Effect of combined genotype on milk production traits in Holstein and Golpaygani breeds

Variables	P-values		
	Milk yield	Fat yield	Fat, %t
Holstein:			
Season	0.0773^{ns}	0.1087^{ns}	0.6155^{ns}
Herd	0.1189^{ns}	0.0024^{**}	$< 0.0001^{**}$
Days in milk (d)	$< 0.0001^{**}$	$< 0.0001^{**}$	0.1763^{ns}
Combined genotype	0.5920^{ns}	0.5503^{ns}	0.0488^{*}
R^2	0.58	0.64	0.56
Golpaygani:			
Season	0.1805^{ns}	0.4536^{ns}	0.8529^{ns}
Days in milk (d)	0.0004^{**}	0.0774^{ns}	0.93^{ns}
Combined genotype	0.4776^{ns}	0.7072^{ns}	0.9449^{ns}
R^2	0.70	0.87	0.61

* Significant and ** Highly significant.
NS: non significant

Other researchers have also reported a near fixation of 'B' allele of *CSN1S1* in Holstein (Lin *et al.* 1986; Ng Kwai Hang, 1998; Prinzenberg *et al.* 2003), Finnish Holstein (0.872; Lien *et al.* 1999) and a high frequency in Carora breed (0.802; Caroli *et al.* 2008). Indigenous breeds had intermediate frequencies for *CSN1S1**B. That could be the result of missing selection by breeding programs. A small frequency of *CSN1S1**C (0.00 for 6 breeds to 0.33 for Icelandic cattle) has been reported in 22 old Nordic cattle breeds (Lien *et al.* 1999), which was less than observed in indigenous cattle in our study (0.34 to 0.60).

Indeed, the frequency of *CSN1S1**C in Finnish Holstein-Friesian was reported to be 0.13 (Lien *et al.* 1999), as well as 0.145 in Swedish Holstein (Lunden *et al.* 1997). It has also been reported, that *CSN1S1**C occurs with a high frequency in *Bos indicus* and *Bos grunniens* (Eigel *et al.* 1984). *CSN1S1**C was about 0.25 in the local Reggiana breed in Italy (Caroli *et al.* 2004) and was predominant (0.56 to 0.75) in zebu breeds in Africa (Ceriotti *et al.* 2004). In accordance with these findings, the two indigenous *Bos indicus* breeds (Mazandarani and Golpaygani) had higher frequency of *CSN1S1**C allele than Sarabi as *B. taurus*.

All four alleles of *CSN1S1* promoter were found in all four breeds investigated. Frequency of allele '2' was the highest in all breeds, but allele '4' frequency was very low, with the highest frequency in Mazandarani (0.106). Indeed, the predominance of allele '2' was reported in other studies (Prinzenberg, *et al.* 2003; Prinzenberg *et al.* 2005). This explains why no '44' homozygous cows were found.

The allele frequencies of the *CSN1S1* promoter in Holstein were in accordance with previous reports in German Holstein (Prinzenberg *et al.* 2003) and German Angeln dairy cattle (Sanders *et al.* 2006). Prinzenberg *et al.* 2005 determined promoter allele frequencies of *CSN1S1* in 14 European cattle ranging from 0.0 to 0.35 for allele '1', 0.08 to 0.91 for allele '2', 0.04 to 0.41 for allele '3', and 0.0 to 0.16 for allele '4'. Allele frequencies of flanking regions of promoters in our study were in the range of results reported by Prinzenberg *et al.* (2005).

The most frequent combined genotypes were BB / 22 and BB / 23 in Holstein. In our investigation, there was one sample with BC / 23 combinations, thus the probability of C-2 or C-3 haplotype may be possible. In case of double heterozygotes, unequivocal assignment of intragenic haplotypes is not possible. Allele '4' of *CSN1S1* promoter was not found in any cow having the allele 'C' in all breeds except Mazandarani. This breed had one 14 / CC and one 24 / CC genotype, which show the possibility of C / 4 haplotype.

Promoter genotypes 11, 44 (Prinzenberg *at al.* 2003) and 14 (Prinzenberg *et al.* 2005) were not reported in Holstein breed before. However, they found genotype 44 in low frequency in Angler (0.041) and Jersey (0.019). That was not consistent with our results, where the 11 promoter genotype was present with low frequency in all indigenous breeds, and promoter genotype 14 in all breeds. Therefore, the indigenous breeds clearly show a higher number of genotypes and haplotypes.

Associations

Our results show that the combined genotype BC / 23 had significant effect on higher fat content in Holstein. Only the combination with BC genotype suggests that the C variant produces an effect on fat content (BC / 23 vs. BB / 23). Homozygous CC for *CSN1S1* was not observed, and therefore it was not possible to ascertain if the C variant would be more advantageous for fat content.

Heterozygous BB / 23 cows showed higher fat content than BB / 22 or BB / 33. No other combination of '11', '44' and '13' with BB or BC were found, and therefore it was not possible to highlight whether variant '2' variant could produce higher fat content rather than '1', '3' or '4' variants of promoter.

None of the genetic combinations had significant effect on fat yield, although variant '2' of the promoter tended to have a negative effect on fat yield (BB / 22<BB / 23<BB / 33). Sanders *et al.* (2006) reported a significant negative substitution effect of allele '2' on fat yield in German Angler cattle.

No significant differences between various combined genotypes on milk yield were detected, but *CSN1S1**B tended to produce higher milk yields. Ng Kwai Hang (1998) reviewed that there was no significant difference in milk production between different phenotypes of *CSN1S1* for Ayrshire, Jersey and Brow Swiss in the course of three lactations.

Table 6 LSQ means (±SE) for milk and fat yield and fat percentage in milk of Holstein cows grouped according to their combined genotype

Trait	Combined genotype							
	BC / 23	BB / 14	BB / 24	BB / 23	BB / 33	BB / 22	BB / 12	BB / 34
Fat, %	3.61 ±0.34	3.43 ±0.33	3.35 ±0.19	2.98 ±0.14	2.95 ±0.33	2.94 ±0.11	2. 49 ±0.33	-
Fat, kg	245.46 ±49.92	325.30 ±49.26	237.40 ±28.76	255.18 ±21.40	271.63 ±48.11	239.19 ±16.22	237.35 ±47.29	-
Milk, kg	7259.70 ±1662.00	9395.40 ±1651.00	7156.40 ±895.00	8543.70 ±682.00	8860.80 ±1617.00	8054.80 ±539.00	9261.10 ±1590.00	8236.70 ±1774.00

Whenever a milk yield difference was observed, the B variant was associated with higher milk production. Prinzenberg *et al.* (2003) reported significant effects of *CSN1S1* promoter variants on milk production breeding value and protein content, with genotypes 12 and 24 showing the best results. They concluded these effects were probably caused by a linked locus. Differences of allelic frequencies and milk production traits found among Iranian breeds might be due to differences in origin of breeds or selection plans applied to Holstein population to improve milk production, and to non-selection program in indigenous breeds. In order to know more about indigenous breeds in Iran, and their potential milk producing features, it is suggested to improve registration and recording programs, which could be included in modern breeding programs, such as marker assisted selection, allowing then an accelerated genetic gain.

ACKNOWLEDGEMENT

The authors thank the TransMIT for contribution of material. We also thank Mrs. Peschel and Mrs. Wagner from laboratories of Humboldt University of Berlin and Justus-Liebig-University of Giessen for helpful cooperation in preparation of samples. Experiments were conducted in the Institute of Animal Breeding and Genetics Justus-Liebig-University of Giessen and Institute for Animal science of Agriculture Horticulture Faculty of Humboldt University of Berlin. Part of this study was kindly supported financially by the State Committee of Animal Science Research Institute of Iran, as well as the Institute of Animal Breeding and Genetics Justus-Liebig-University of Giessen.

REFERENCES

Caroli A., Chessa S., Bolla P. and Gandini G.C. (2004). Genetic structure of milk polymorphisms and effects on milk production traits in a local dairy cattle. *J. Anim. Genet*. **121,** 119-127.

Caroli A., Chessa S., Chatti F., Rignanese D., Melendez B., Rizzi R. and Ceriotti G. (2008). Short communication: carora cattle show high variability in αS1-casein. *J. Dairy Sci.* **91,** 354-359.

Ceriotti G., Marletta D., Caroli A. and Erhardt G. (2004). Milk protein loci polymorphism in taurine (*Bos taurus*) and zebu (*Bos indicus*) populations bred in hot climate. *J. Anim. Genet*. **121,** 404-415.

Eigel W.N., Rutler J.E., Ernstrom C.A., Farrell J.R.H.M., Harwalker V.R., Jenness R. and Whitney R.M. (1984). Nomenclature of protein of cow's milk: fifth revision. *J. Dairy Sci*. **67,** 1599-1631.

Erhardt G. (1993). A new αs1-casein allele in bovine milk and its occurrence in different breeds. *Anim. Genet*. **24,** 65-66.

Farrell H.M., Jimenez Flores Jr., Bleck R., Brown G.T., Butler E.M. and Creamer L.K., Hicks C.L., Hollar C.M., Ng-Kwai-Hang K.F. and Swaisgood H.E. (2004). Nomenclature of the Proteins of Cows' Milk-Sixth revision. *J. Dairy Sci*. **87(6),** 1641-1674.

Formaggioni P., Summer A., Malacarne M. and Mariani P. (1999). Milk protein polymorphism: Detection and diffusion of the genetic variants in *Bos genus*. *Ann. Fac. Med. Vet. Univ. Parma*. **19,** 127-165.

Koczan D., Hobom G. and Seyfert H.M. (1991). Genomic organization of the bovine alpha-S1 casein gene. *Nucl. Acid. Res*. **19 (20),** 5591-5596.

Lien S., Kantanen J., Olsaker I., Holm L.E., Eythorsdottir E., Sandberg K., Dalsgard B. and Adalsteinsson S. (1999). Comparision of milk protein allele frequencies in Nordic cattle breeds. *Anim. Genet*. **30,** 85-91.

Lin C.Y., Mc Allister A.J., Ng Kwai Hang K.F. and Hayes J.F. (1986). Effects of milk protein loci on first lactation production in dairy cattle. *J. Dairy Sci*. **69,** 704-712.

Lunden A., Nilsson M. and Janson L. (1997). Marked effect of β-lactoglobulin polymorphism on the ration of casein to total protein in milk. *J. Dairy Sci*. **80,** 2996-3005.

Miller S.A., Dykes D.D. and Polesky H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acid. Res*. **16(3),** 1215-219.

Ng Kwai Hang K.F. (1998). Genetic polymorphism of milk protein: relationships with production traits, milk composition and technological properties. *Can. J. Anim. Sci*. **78,** 131-147.

Prinzenberg E.M., Weimann C., Brandt H., Bennewitz J., Kalm E., Schwerin M. and Erhardt G. (2003). Polymorphism of the bovine $CSN1S_1$ promoter: linkage mapping, intragenic haplotypes, and effects on milk production traits. *J. Dairy Sci*. **86,** 2696- 2705.

Prinzenberg E.M., Brandt H., Bennewitz J., Kalm E. and Erhardt G. (2005). Allele frequencies for SNPs in the αS1-casein (*CSN1S1*) 5` flanking region in European cattle and association with economic traits in German Holstein. *Livest. prod.*

Sci. **98,** 155-160.

Rando A., Gregorio P.D., Raumunno L., Mariani P., Fiorella A., Senese C., Marletta D. and Masina P. (1998). Characterization of the $CSN1S_1^G$ allele of the bovine α_{s1}-casein locus by the insertion of a relict of a long interspersed element. *J. Dairy Sci*. **81,** 1735-1742.

Sanders K., Bennewitz J., Reinsch J., Thaller G., Prinzenberg E.M., Kuehn C. and Kalm E. (2006). Characterization of the DGAT1 mutations and the CSN1S1 promoter in the German Angeln dairy cattle population. *J. Dairy Sci*. **89,** 3164-3174.