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Effects of milk protein loci on content of their proteins

Abstract

Allele specific milk proteins were measured in 4544 samples from 2054 Fleckvieh (FV) cows in two succeeding years and in one year from 1809 Braunvieh (BV) cows partly crossbreds with Brown Swiss. The cows were from 454 and 403 sires, and in 316 and 46 farms, respectively. The allele specific milk proteins were measured photometrically.

Gene action was mainly additive, but the αS1-CN BC was 2 to 4 % above the mean of the homozygotes and the heterozygotes at the CSN2 locus deviated from –2 to + 8 % from the means of the respective homozygotes. The extent of expression of the alleles varied. At CSN1S1 higher expression was shown by the B alleles in heterozygotes, and by the C alleles in homozygotes. At the CSN2 locus the statistically highly significant order of degree of expression was C>B>A.

At the LGB locus allele both breeds showed higher expression than allele B and the difference between the expression of the two alleles in heterozygotes is twice of that observed in homozygotes.

The CSN1S1 and CSN2 loci affected the synthesis of all caseins. The CSN3 locus shows statistically significant influence on κ-CN in FV. κ-CN appears to be influenced in particular by C2N2 alleles. The LGB locus shows much influence on the ß-LG content but little on caseins. The effects of CSN1S1 and CSN2 loci on the contents of αS2-CN and κ-CN indicate epistasis.

Polygenic influence accounted for one third to one half of the overall genetic variance of contents of Ca-sensitive caseins. κ-CN shows much greater polygenic influence (two thirds to four fifth of the genetic variance) and ß-LG less (1/6 and less). The action of LGB was specific with none or very little influence on the caseins. Moreover, ß-LG was also unaffected by the casein loci. The effects of the alleles on the contents were similar in the two breeds. Repeatabilities were 2/5 to almost 3/5, heritabilities 1/4 to 1/3 with the exception of αS2-CN and κ-CN where it was lower.

Key Words: lactoptoteins, caseins, whey protein, allelic expression, gene effects, Fleckvieh, Braunvieh

Zusammenfassung

Titel der Arbeit: **Wirkung von Milchproteingenen auf den Gehalt von ihnen kodierter Proteine**

Allelspezifische Milchproteine wurden in 4544 Proben von 2054 Fleckviehkühen (FV) in zwei aufeinander folgenden Jahren und einmalig von 1809 Braunviehkühen (BV, teilweise Brown Swiss Kreuzungen) bestimmt. Die Kühe stammten von 454 bzw. 403 Stieren und standen in 316 bzw. 46 Betrieben. Die Proteine wurden photometrisch bestimmt. Genwirkungen waren weitgehend additiv, Gehalt an αS1-CN BC war aber 2-4 % über dem Durchschnitt der Homozygoten und CSN2 Heterozygote von 2 unter bis 8 % darüber. Der Grad der Expression variierte zwischen Allelen und Loci. Am CSN1S1 Locus war Allel B in Heterozygoten stärker exprimiert, Allel C in Homozygoten. Bei CSN2 war die statistisch hochsignifikante Reihenfolge des Grades der Expression C>B>A. Bei ß-LG war die Syntheseleistung des B Allels höher als die des A Allels, wobei die Differenz in Heterozygoten mehr als das Doppelte der Differenz bei Homozygoten war. Die beiden Loci der Ca-empfindlichen Kaseine beeinflussen den Gehalt aller Kaseine, besonders auch den von κ-CN. Auf diesen wirkt sich der eigene Locus nur bei FV in statistisch signifikanter Weise aus. Der ß-LG Gehalt wird vom eigenen Locus sehr stark kontrolliert, dieser wirkt sich jedoch kaum auf Kaseingehalte aus. Die Wirkung der Allele auf den Milchproteingehalt ist bei beiden Rassen ähnlich.

Polygene Einflüsse sind für etwa 1/3 bis 1/2 der gesamten genetischen Varianz des Gehaltes Ca-empfindlicher Kaseine verantwortlich, für 2/3 bis 4/5 bei κ-CN und für nur etwa 1/6 bei ß-LG. Die Wiederholbarkeit reicht von 2/5 bis fast 3/5, die Heritabilität von 1/4 bis 1/3 mit Ausnahme von κ-CN und αS2-CN, bei welchen sie niedriger ist.

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Schlüsselwörter: Laktoproteine, Kaseine, Molkenprotein, Allelexpression, Genwirkungen, Fleckvieh, Braunvieh

Introduction

Quantitative effects on proteins are of at least twofold interest. First, the properties of milk proteins are of considerable importance for manufacturing, in particular of cheese, second, clarification of the kind of action which leads to quantitative differences could help to understand the way such differences come about. There is much interest in QTLs but in relatively few cases the acting genes are identified and/or their functions known. Yet quantitative differences between products of some candidate genes have been recognized for some time, e.g. the difference in quantity of hemoglobin produced by normal and sickle cell alleles.

GROSCLAUDE et al. (1987) reported quantitative differences between goat αS1-caseins synthesized by different alleles. McLEAN et al. (1984) and NG-KWAI-HANG et al. (1987) investigated effects of milk protein loci on fractions and proportions of proteins coded therefrom. VAN EENENHAM and MEDRANO (1991) reported ratios of κ-CN A and κ-CN B to vary from 1:1 to 1:18 in milk of heterozygous cows. GRAML et al. (1985, 1986) and BRAUNSCHWEIG et al. (2000) published effects of ß- and of κ-CN loci and of the ß-lactoglobulin locus on yield and milk composition of Bavarian Fleckvieh and Braunvieh and of Swiss Braunvieh. EHRMANN et al. (1997a, b) investigated non-coding regions of the LGB gene and effects of milk protein genes on content and yield of their respective proteins. RIJNKELS et al. (1995, 1998) investigated expression of bovine casein genes in transgenic mice.

Material and methods

Milk samples were collected in 1991/92 from 2054 Fleckvieh (FV) cows in 316 farms and from 1809 Braunvieh (BV) cows in 46 farms. The cows were sired by 454 and 403 bulls, respectively. Fleckvieh was purebred Bavarian FV, 70 % of the Braunvieh cows were crosses of varying degrees with Brown Swiss. From FV a second sample was collected in the succeeding year.

The quantity of the protein fractions was measured photometrically on cellogel electropherograms (KIRCHMEIER, 1975). The photodensitometer at the given wave length measures the optical density of the bands while the relative quantity of the various proteins is deduced from the integral area. The following protein fractions were measured: αS1-casein (αS1-CN), αS2-casein (αS2-CN), ß-casein (ß-CN), κ-casein (κ-CN), ß-lactoglobulin (ß-LG) and α-lactalbumin (α-LA). Likewise the milk protein loci for αS1-casein (CSN1S1), ß-casein (CSN2), κ-casein (CSN3) and ß-lactoglobulin (LGB) were typed. The difference in colour binding capacity between ß-Lg and α-La was corrected with coefficients given by TARASSUK et al. (1967), that between ß-Lg A and B by the procedure suggested by WEI (1988). The different dye binding capacities of casein genetic variants were corrected as indicated by McLEAN et al. (1982). As no correction factor was given for the ß-CN-C allele an approximate ß-CN mean value of 0.7 was used. No genetic polymorphism was identified at the CSN1S2 locus by our method but ERHARDT (1993) demonstrated one by isoelectric focussing. However, we measured αS2-CN.

Cellogel electrophoresis does not measure the CN-g fraction, the immunglobulin or the proteose-peptones in whey protein. Therefore estimates of contents of whey proteins will be slightly biased upwards. Also αS0-CN and γ-CN could not be differentiated from the caseins in the heterozygote. Therefore from αS1-CN B in heterozygotes 10% αS0-CN was subtracted (WALSTRA and JENNESS, 1984) and from ß-CN C 12% of γ-CN.

The specific content of each protein fraction was analysed by a statistical model including for both breeds, the fixed effects of year-season, lactation stage, lactation number, a random sire effect, fixed effects of the genotypes of the primary locus (e.g. CASAS1 for αS1-CN) and of the genotypes at the other milk protein loci (CSN2, CSN3, LGB) and a random residual effect. For FV a random cow effect was included but this was not possible for BV because only one sample was taken per cow. Herd effects were assumed to be random in FV and were treated by regression of the specific protein content on herd mean of milk protein in BV, where crossbred group effects were considered as fixed.

Repeatability was estimated from the FV data (2056 cows in 317 herds with 4544 casein samples and 3902 ß-LG samples from 1991 cows in the same herds). The statistical analysis was based on a model which included year-season, lactation-stage and lactation number as fixed effects, herd and cow within herd and residual as random.

Heritability was estimated both from daughter-dam regressions and from sire variance components. In the FV data 1034 daughter-dam pairs in 286 herds and 120 sires with 1481 daughters could be used for the estimation, in the BV data 359 daughter-dam pairs in 46 herds and 160 sires with 1242 daughters. The sire had to have at least five daughters to be included in the analysis. In addition to the fixed effects indicated above, the statistical model comprised a random sire, cow within sire, the residual effect for FV, and for BV also the effect of genetic groups. The daughter-dam regressions were estimated within herds. Since very few sires were connected with more than one dam-daughter pair the within-sire analysis was neglected.

Results and discussion

Contents of specific milk proteins are given in Table 1 for the various genotypes, e.g. αS1-CN content of the homozygote CSN1S1. The largely parallel values of the genotypic effects in the two breeds is noteworthy. However, there are noticeable differences between them in the contents of κ-CN which in BV is nearly 50% above that of FV. The reverse, though not that drastic, is the case for the contents of αS1-CN and of ß-LG where FV milk has higher concentrations.

McLEAN et al. (1987) report differences of variable sign, between Jerseys and Holstein-Friesian (HF), in proportions of specific proteins. While the κ-CN proportion is considerably higher in Jerseys those of the other caseins and of ß-LG are higher in HF.

In each breed the genes act in a largely additive fashion, dominance effects and thus heterosis are fairly small. The exceptions are αS1-CN where contents of the heterozygote BC are 2 to 4 % above the mean of the homozygotes, and ß-CN where

heterozygotes deviate from –2 to +8 % from the homozygotes. For κ-CN the contents of homozygotes only are given.
The contents of the various combinations of alleles, 45 genotypes in all for the three casein and the LGB locus, are given in the thesis of GRAML (1994).

Table 1
Contents of specific milk proteins

Locus	geno-types	n	Fleckvieh content gx10^{-4}/ml	s	heterosis	n	Braunvieh content gx10^{-4}/ml	s	heterosis
CSN1S1									
	BB	3675	87.5	3.4		1616	80.7	4.2	
	CC	60	102.3	3.9		16	99.1	5.4	
	BC	802	97.1	3.5	2.3=2.3%	177	93.8	4.2	3.9=4.3%
CSN2									
	AA	3479	120.8	4.7		1040	116.8	3.8	
	BB	25	128.4	6.0		89	127.6	4.3	
	CC	10	137.7	7.4		4	150.1	8.9	
	AB	621	128.1	3.0	3.5=2.8%	560	124.4	7.0	2;.2=1.8%
	AC	354	140.0	3.3	10.8=8.3%	88	136.1	6.0	2.7=2.0%
	BC	46	130.0	6.1	-3.0=-2.2%	28	135.7	7.4	-3.1=-2.2%
CSN3									
	AA	2199	16.5	1.7		404	24.9	2.3	
	BB	562	17.7	1.7		716	25.2	2.2	
LGB									
	AA	925	71.6	2.3		276	59.3	1.5	
	BB	1045	56.8	2.3		478	49.5	1.5	
	BC	1932	63.8	3.1;	-0.4=-0.6%	699	55.3	1.6	0.9=1.7%

In Table 2 the contents of the αS1-CN´s, expressed by the CSN1S1 alleles, are given. In heterozygotes both B and C caseins can be differentiated. For ease of comparison, half of the content of homozygotes is given in the tables. The higher αS1-CN C content in both FV and BV heterozygotes contrasts with a smaller but reverse difference in homozygotes. In both breeds heterozygotes have a higher content of the B variant but in homozygotes the C content is higher. EHRMANN et al. (1997b) reported a significantly higher proportion of αS1-CN in heterozygotes than in either homozygote but did not differentiate B and C caseins in heterozygotes. McLEAN et al. (loc. cit.) found CASAS1-BC to have a casein content 8 % higher than the homozygote mean but NG-KWAI-HANG et al.(loc. cit.) reported no heterozygote superiority.

Table 2
Synthesis of αS1-CN by CSN1Sl alleles (gx10^{-4}/ml)

Allele	Fleckvieh homo-zygotes	hetero-zygotes	difference in synthesis	Braunvieh homo-zygotes	hetero-zygotes	difference in synthesis	avarage difference
B	43.75	50.00	-6.25	40.35	47.70	-7.35	-6.80
C	51.15	47.10	4.05	49.55	46.40	3.15	3.60
B-C	-7.40	2.90		-9.20	1.30		-10.40

The expression of the CSN2 alleles is given in Table 3. Alleles A and C show in heterozygotes a higher expression of ß-CN´s than in homozygotes but for allele B this difference is smaller and not consistent between the two breeds.The expression of the three alleles in descending order was C>B>A. FREYER et al. (1999) differentiated

A1, A2 and A3 and found CSN2-A2 improved yields in comparison to A1 and vice versa in respect to contents. These were increased by the B allele in both Black and White strains. EHRMANN et al. (1997b) differentiated A1 and A2 and found allele B to increase contents but not the effect of allele A2 on yield. However, this referred to the average of eight breeds.

Table 3
Synthesis of ß-CN by CSN2 alleles (gx10^{-4}/ml)

Allele	Fleckvieh homo-zygotes	hetero-zygotes	difference in synthesis	Braunvieh homo-zygotes	hetero-zygotes	difference in synthesis	average difference
A	60.40	64.00	-3.60	58.40	62.90	-4.50	-4.05
B	64.20	64.60	-0.40	63.80	62.10	1.70	0.65
C	68.85	74.00	-5.15	70.05	70.50	-0.45	-2.80
difference in allele expression							
A-B	-3.80	-0.60	-2.20	-5.40	0.80	-2.30	-2.25
A-C	-8.45	-10.00	-9.20	-11.65	-7.60	-9.60	-9.40
B-C	-4.65	-9.40	-7.00	-6.25	-8.40	-7.30	-7.15

Table 4
Synthesis of ß-Lg by LGB alleles (gx10^{-4}/ml)

Allele	Fleckvieh homo-zygotes	hetero-zygotes	difference in synthesis	Braunvieh homo-zygotes	hetero-zygotes	difference in synthesis	average difference
A	35.80	38.90	-3.10	29.65	33.20	-3.55	-3.30
B	28.40	24.90	3.50	24.75	22.10	2.65	3.10
difference in allele expression							
A-B	7.40	14.00		4.90	11.10		

The contents of the allele specific whey proteins expressed by LGB are shown in Table 4. In both breeds allele A shows higher expression in heterozygotes, but allele B is more expressed in homozygotes. The differences in expression of alleles A and B in heterozygotes are about twice as large as in homozygotes. However, allele A has a larger expression than allele B as was also reported by EHRMANN et al. (1997b) from eight breeds.

EHRMANN et al. (1997) discovered 16 DNA variants in the 5`flanking region of LGB but 82% of the cows from several breeds shared three haplotypes of the LGB allele and the five sites in the flanking region. The differences among carriers of the 3 genotypes were highly significant for yield and % lactoglobulin. Every BLG allele was linked to five different sites. Therefore the differences in expression could be due to the non coding sites or to the alleles. However, the authors inferred that the performance differences were due to the noncoding region.

The effects of the four loci on contents of all five milk proteins was submitted to an analysis of variance (Table 5). With the FV data the effects of the loci on contents was tested against the “cow variance” which in turn was tested against the residual mean square (MSQR). In the analysis of the BV data, the test of the locus variance was against the MSQR. As mentioned above, two samples per cow in succeeding years were available for FV but only one for BV. As evident from Table 5, casein loci not only affect their own coded protein but the proteins coded by the other loci as well.

The exception is the BLG locus which is specific and affects only the ß-LG. This in turn is not influenced by the casein loci. Noticeable also is κ-CN which is strongly influenced by the other CN loci, but in BV the CSN3 locus itself shows no effect on the protein coded by it. Apart from some significant effects on αS2 CN this locus does not appear to influence other milk proteins in BV. This contrasts with the effects of CSN1S1 and CSN2 loci on all other caseins with the exception of αS2-CN.
The joint effect of the casein loci may reflect a common set of transacting factors influencing the casein promoters by RIJNKELS et al. (1998).

Table 5
Analysis if variance of contents of specific milk proteins (F-values and Residual Mean Squares)

Loci	d.f.	αS1-CN	αS2-CN	ß-CN	κ-CN	ß-LG
		Fleckvieh				
CSN1S1	2	98.3**	12.2**	16.1**	3.7*	1.6
CNS2	5	6.7**	1.6	30.5**	12.1**	0.8
CSN3	2	1.3	2.1	2.6	6.1**	1.6
LGB	2	4.8*	5.1*	3.3*	0.4	253.4**
Cow/genotype	2053/1991	1.4**	1.4**	1.4**	1.4**	1.4**
Regr. on herd	1	0.8	3.2	0	32.1**	0
RMSQ	2426/1857	161.4	84.9	248.2	33.2	69.2
		Braunvieh				
CSN1S1	2	65.0**	40.6**	11.1**	0.9	1.7
CSN2	5	1.8	0.8	27.8**	9.0**	1.0
CSN3	2	0.2	3.3*	1.7	0.4	2.1
LGB	2	2.9*	1.3	1.1	0.7	89.8**
Herds linear	1	3.9*	12.1**	0	0	0
quadr.	1	0	0	3.3**	4.2**	0.2
Cross groups	16	1.2	3.5**	2.6**	3.3**	1.2
Sires	541/463	1.3**	1.1	1.1	1.2*	1.0
RMSQ	1199/924	149.4	77.9	219.3	44.0	58.1

First number of d.f. refers to casein, second to ß-LG analysis

Table 6
Effects of genotypes on casein contents (deviations from reference genotype, in brackets) (gx10^{-4}/ml)

Locus	genotype	αS1-CN		αS2-CN		ß-CN		κ-CN	
		FV	BV	FV	BV	FV	BV	FV	BV
CSN1S	BC	9.5	13.4	-2.1	-7.8	-5.7	-6.6	-0.7	-0.5
(BB)	CC	14.8**	18.4**	-0.2**	-9.3**	-11.3**	7.7**	0.3*	1.0
CSN2	AB	-6.1	-1.7	1.3	0.6	7.2	7.6	-3.1	-1.8
(AA)	AC	-7.8	-3.5	-0.8	-0.5	19.3	19.2	-2.9	-4.4
	BB	-11.8	-2.7	2.5	-0.7	7.6	10.8	-4.3	-3.1
	BC	-14.9	-4.8	-2.0	1.0	9.2	18.9	-4.7	-5.4
	CC	-7.4**	-7.8	6.4	-7.0	4.1**	33.3**	-1.9**	-9.7**
CSN3	AB	-0.9	-0.3	-0.2	1.6	0.7	-2.2	1.4	0.4
(AA)	BB	-1.2	-0.7	-0.6	0.2*	1.2	-0.9	1.2**	0.3
LGB	AB	1.4	2.5	1.4	-0.8	-0.6	-1.9	-0.3	0.2
(AA)	AD	5.4	6.5	2.5	-4.3	6.2	-3.7	-0.5	2.5
	BB	3.7	3.8	2.5	0.5	2.2	-0.6	-0.4	0.3
	BD	7.0*	6.6*	-0.2*	-0.9	-3.2*	-12.4	1.1	7.1

*, ** p<.05, p<.01

In Table 6 the effects of the genotypes are given as deviations from the most common genotype of the respective locus, e.g. the effects of CSN1S1 genotypes BC and CC as deviations from BB. The effects of CSN1S1 and CSN2 on their coded proteins and those coded by the other loci are similar in FV and BV. However, differences exist in respect of the effects on both αS2-CN and κ-CN. Nevertheless the effects of CSN2

genotypes on κ-CN are consistent between the two breeds as are the effects of LGB genotypes. The differences in contents from the major genotype agree in sign and magnitude with the majority of the values published by McLEAN et al. (loc. cit.) and NG-KWAI-HANG et al. (loc. cit.) even though their date refer to Holsteins and Jerseys. The few exceptions involve κ-CN. There are somewhat more differences with the results of EHRMANN et al. (1997b) but as these from eight breeds, comparison is somewhat more problematic.

Table 7
Share of genotypic variance (%) due to lactoprotein loci and polygenes

	αS1-CN		ß-CN		κ-CN		ß-LG	
	FV	BV	FV	BV	FV	BV	FV	BV
Primary locus	32.6	31.6	35.4	33.9	3.7	0.2	77.5	69.0
Other loci	37.4	15.7	12.9	16.7	31.7	17.8	4.5	16.9
Polygenes	30.0	52.7	51.7	49.3	64.6	82.0	17.0	14.1
Original units								
V(G)	48.6	74.6	98.2	96.2	7.6	16.9	35.7	20.2
V(P)	184.1	201.2	307.8	275.7	34.6	60.5	104.9	77.0

Genetiv variance was approximated from sire variance components and daughter-dam V(G) genotypic variance, V (P) phenotypic variance

The proportion of the genetic variance of milk protein content owing to the four loci is given in Table 7. CSN1S1; CSN2 and LGB loci account for a major part of the variance of the proteins coded by them, e.g. more than 30% of the genetic variance of αS1-CN is due to the alleles at the CSNS1 locus. However, in FV CSN3 accounts for only 10% of the variance in the κ-CN content and in BV for even less. The variance of κ-CN is dominated by the influence of the CSN2 locus. For ß-LG most of the variance is due to the primary locus. Interaction among loci, i.e. epistasis, appears to be important for αS2-CN and κ-CN.

The large variability of allele effects is noteworthy. The spread extends from less than 10 % to more than 90%, e.g. for αS1-CN B in BC heterozygotes of FV, and somewhat but not much less in BV. Very variable ratios were reported by VAN EENEMAN and MEDRANO (loc. cit.) between A and B κ-CN in heterozygous cows. EHRMANN et al. (1997a) found the within breed spread of ß-LG in heterozygotes to extend over 6 standard deviations.

The overall genetic variance was derived from daughter-dam covariances and sire variance components. Of course these reflect mainly additive-genetic variance but they are treated in Table 7 as representing the whole genotypic variance. The distribution of the variance between primary loci is shown in Table 7, e.g. in the case of αS1-CN the variance due to CSN1S1, that due the remaining milk protein loci, i.e. CSN2 , CSN3 and LGB, and that of the polygenes. These loci cause about one third to a half of the genetic variance of αS1-CN, a half of ß-CN, but up to 80 % of the genetic variance of κ-CN. Polygenes account for less than 1/6 of the genetic variance of the ß-LG content, while the alleles at the LGB locus cause some 80 % of it. FREYER et al. (1998) estimated zero recombination between the LGB locus and a QTL for milk protein. Thus the LGB locus appears to be a megaphenic locus and could serve as a model of quantitatively acting genes.

EHRMANN et al. (1997a) have begun to explore the non-coding regions of this locus with respect to their influence on the expression of the alleles. The difference in the

degree of expression between their alleles in homozygotes - and in heterozygotes found in this investigation deserves attention.

Table 8
Genetic parameter estimates of milk protein contents

	Repeatability	Heritability	
	FV	FV	BV
αS1-CN	.39	.27	.37
αS2-CN	.37	.17	.17
ß-CN	.56	.32	.34
κ-CN	.48	.22	.28
ß-LG	.38	.35	.26

The repeatabilities and heritabilities of milk protein contents are given in Table 8. The heritability estimates derived by the two methods and from the two breeds, weighted by the inverse of the respective error variances as suggested by HILL and NICHOLAS (1975), were combined.

Repeatabilities are in the lower range of such estimates for milk contents and the same pertains to heritabilities (GRAML et al., 1987). The heritabilities for κ-CN and αS2-CN are nearly significantly lower than estimates for the other protein contents. EHRMANN et al. (1997a) reported considerably higher repeatabilities but they referred to short periods within lactations while those of this paper comprise records from two lactations.

The different structure of the variance of κ-CN may reflect the difference in structure and function between it and the Ca sensitive caseins. K-CN is integrated in the surface of the micelles and its structure is related to fibrinogen. The ß-LG gene is located on a different chromosome and the protein is in structure similar to the retinol binding protein.

The expression of the CSN1S1 alleles changed over the seasons. The content of αS1-CN B is much higher between May and October than is that of αS1-CN C in milk of heterozygous cows and this response was repeated in the second sampling year. It is likely to reflect a hormonal influence.

The difference in the transcription and translation regions between the various alleles of the loci should be of considerable interest as EHRMANN et al. (1997b) pointed out. The genes of the three Ca sensitive caseins, close to the TATA box, have specific structures that are missing in CSN3 and in the whey proteins (MERCIER, 1990). Hormone binding sites have been identified in Ca sensitive casein genes (COMPTON et al., 1983; YU-LEE et al., 1986). The 5´flanking region of CSN3 misses a sequence that exists in genes coding for Ca sensitive caseins (STEWART et al., 1984). Relatively little is known about enhancer elements of milk protein genes (MACKINLAY et al., 1989). Repetitive sequences have been identified in all casein genes (KOCZAN et al., 1991; BONSING et al., 1988; ALEXANDER et al., 1988).

It is probable that the quantitatively different expressivity of milk protein alleles resides primarily in their regulatory sites, as pointed out by EHRMANN et al. (1997a). Work directed at these regions would seem desirable not only for an understanding the differences in the contents of milk proteins, but possibly even more so as help to clarify the quantitative action of genes.

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