Genet. Sel. Evol. (1989) 21, 547-554

Note

Different rates of synthesis of whey protein and casein by alleles of the β-lactoglobulin and α_{s1}-casein locus in cattle

R. Graml[1], G. Weiss[2], J. Buchberger[2] and F. Pirchner[1]

[1] *Lehrstuhl für Tierzucht der Technischen Universität München, D-8050 Freising-Weihenstephan;*

[2] *Institut für Chemie und Physik der Süddeutschen Versuchs- und Forschungsanstalt für Milchwirtschaft der Technischen Universität München, D-8050 Freising-Weihenstephan, FRG*

(received 22 March 1989; accepted 28 August 1989)

Summary – Quantities of α_{s1}-caseins and β-lactoglobulins were determined in milk of 2059 *Fleckvieh* cows and 1809 *Braunvieh* cows in Bavaria; 6353 milk samples were analysed for α_{s1}-casein and 5355 for β-lactoglobulin. α_{s1}-Cn^C homozygotes produced significantly more α_{s1}-casein than B homozygotes. The β-Lg^A allele showed greater expression both in heterozygotes and in homozygotes than the β-Lg^B allele. In heterozygotes, the β-Lg^A allele produced nearly 50% more whey protein than its homologue. During the spring–summer season α_{s1}-Cn^B appeared to synthesize more, relatively, α_{s1}-casein than α_{s1}-Cn^C. Possible causes for this may be a greater rate of expression of the allele or increased phosphorylation during spring–summer, producing proportionally more α_{s1}-casein.

cattle – milk protein genes – gene expression – α_{s1}-casein – β-lactoglobulin

Résumé – Synthèse protéique différentielle selon les variants de β-lactoglobuline et de la caséine α_{s1} chez les bovins. *Les quantités de caséine α_{s1} et de β-lactoglobuline ont été déterminées dans le lait de 2059 vaches de race* Fleckvieh *et de race* Braunvieh *de Bavière; 6353 échantillons de lait ont été analysés pour la caséine α_{s1} et 5355 pour la β-lactoglobuline. Les individus homozygotes α_{s1}-Cn^C produisent significativement plus de caséine que les individus homozygotes α_{s1}-Cn^B. L'expression de l'allèle β-Lg^A est supérieure à celle de l'allèle β-Lg^B chez les individus hétérozygotes ou homozygotes. Chez les hétérozygotes, l'allèle β-Lg^A a une production de protéine supérieure d'environ 50% à celle de son homologue. Durant la période printemps–été, l'allèle α_{s1}-Cn^B synthétise plus de caséine α_{s1} que l'allèle α_{s1}-Cn^C. Ceci pourrait provenir d'un taux d'expression supérieur de l'allèle α_{s1}-Cn^B ou à une augmentation de la phosphorylation pendant cette période produisant plus de caséine α_{s0}.*

bovins – gènes des protéines du lait – expression génique – α_{s1}-casein – β-lactoglobulin

INTRODUCTION

In cattle rather few loci have been identified and efforts to link them to quantitative traits have not been very successful. Milk protein genes, however, are associated with the quantitative variation of the proteins for which the codominant alleles are coding. Moustgaard *et al.* (1960), Golikova and Panin (1972), Michalak (1973), Cerbulis and Farrell (1975), Komatsu *et al.* (1977), Mariani *et al.* (1979), McLean *et al.* (1984), Ng-Kwai-Hang *et al.* (1987) and Aaltonen and Antila (1987) demonstrated that the β-Lg genotype AA produce more β-lactoglobulin than genotypes BB or AB. Also McLean *et al.* (1984) on cattle and Boulanger *et al.* (1984) and Grosclaude *et al.* (1987) for goats showed that α_{s1}-Cn genotypes influence the production of α_{s1}-casein.

Although β-Lg and α_{s1}-Cn genotypes show a different rate of protein synthesis, there is little known about the expression of the alleles in heterozygotes. However, the haemoglobin of sickle-cell heterozygote is composed of more than 60% haemoglobin A and less than 40% of haemoglobin S (Wellis and Itano, 1951; Wrightstone and Huisman, 1968). Such different rates of expression of globin genes appear to be even more marked in Hb-C heterozygotes (Boyer *et al.*, 1963; Itano, 1965) and in thallasemias (Na-Nakorn and Wasi, 1970; Huisman *et al.*, 1972). Here we report on differences in the concentration of α_{s1}-caseins and β-lactoglobulins coded by the different alleles of heterozygotes and homozygotes of the *Bavarian Simmental* and *Bavarian Brown Alpine* cattle.

MATERIALS AND METHODS

The data are based on casein resp. whey protein analysis of 6353 resp. 5355 milk samples from 2059 *Simmental* and 1809 *Brown Alpine* cows. *Simmental* cows were sampled twice, *Brown Alpine* cows once. The statistical analysis of *Simmental* data was based on a model with effects of herd, year-season, stage and number of lactation and cows; that of the *Brown Alpine* herd, year-season, stage and number of lactation, sire of the cow and genotypes at 3 loci (in the case of the α_{s1}-Cn expression, the β-Cn, κ-Cn and β-Lg locus; in the case of the β-Lg expression, the α_{s1}-Cn, β-Cn and κ-Cn locus). The different mean expression of the alleles of heterozygous genotypes was tested by a simple t-test; those of the homozygous genotypes by the Student–Newman–Keuls test.

In *Simmental* cows 2 samples were analysed from nearly every cow. This permitted estimation of the repeatability of the ratio of the proteins in the heterozygotes (α_{s1}-Cn^{B}/α_{s1}-Cn^{C} resp. β-Lg^{A}/β-Lg^{B}).

The milk protein content was measured by the amido-black method, the proportion of the α_{s1}-casein B resp. C and β-lactoglobulin A resp. B by quantitative photometric determination from cellogel electropherograms (Kirchmeier, 1975; personal communication, 1988), where the optical density of the bands was measured by a photodensitometer. The area under the respective peaks was recorded and the integral area computed. This corresponds to the relative quantity of the protein, provided that the specific affinity to bind the dye is taken into consideration.

β-lactoglobulin was isolated from whey proteins after removal of α-lactalbumin (Sluyterman and Elgersma, 1978). The separation of the two genetic variants

was achieved by chromatofocusing (Sluyterman and Wijdenes, 1978). Purity and homogeneity was checked by Page electrophoresis (Raymond and Weintraub, 1959).

For determination of the specific dye binding affinity, known quantities of β-lactoglobulins were electrophorized, the bands coloured by amido-black and measured densitometrically. In comparison with the standard β-lactoglobulin A, β-lactoglobulin B had a dye-binding activity of 1.05, similar to published results (Reimerdes and Mehrens, 1978; Krause, personal communication, 1988). The analogous coefficient for α_{s1}-casein B relative to α_{s1}-casein C was taken as 1.06, as published previously by McLean *et al.* (1982).

RESULTS

The average differences between the expression of α_{s1}-casein B and C alleles in heterozygotes were insignificant (Table I). However, homozygous α_{s1}-Cn^{CC} cows had a higher α_{s1}-casein content than the alternative BB homozygote. As shown in Figure 1, the degree of activity of the alleles in the heterozygote varied considerably and its distribution approached that of a normal curve.

Locus	*Kind of genotypes*	*Simmental*					*Brown Alpine*			
		n	*Allele/ Genotype*	*g/10⁴ ml*	*%*	*R*	*n*	*Allele/ Genotype*	*g/10⁴ ml*	*%*
α_{s1}-Cn	Hetero-	802	B	50.0±2.5 [a]	51.5	0.18±0.05	177	B	47.7±3.0 [a]	50.7
	zygotes		C	47.1±2.4	48.5			C	46.4±3.0	49.3
	Homo-	3675	BB	87.5±3.4 [b]	92.2 [1]		1616	BB	80.7±4.2 [b]	89.8 [1]
	zygotes	60	CC	102.3±3.9	107.8		16	CC	99.1±5.4	110.2
				94.9 [2]					89.9 [2]	
β-Lg	Hetero-	1932	A	38.9±2.4 [c]	61.0	0.43±0.03	699	A	33.2±1.2 [c]	60.0
	zygotes		B	24.9±1.9	39.0			B	22.1±1.1	40.0
	Homo-	925	AA	71.6±2.3 [c]	111.5 [1]		276	AA	59.3±1.5 [c]	109.0 [1]
	zygotes	1045	BB	56.8±2.3	88.5		478	BB	49.5±1.4	91.0
				64.2 [2]					54.4 [2]	

The two alleles of β-lactoglobulin heterozygote β-Lg^{AB} differed significantly in their activity. β-Lg^{A} produced about 50% more lactoglobulin A than β-Lg^{B} did lactoglobulin B. This difference is paralleled by the difference between alternative homozygotes. The distribution (Fig. 1) indicates considerable variability and a leptocurtosis.

In Figs. 2 to 4, the course over seasons in 2 years of the ratio between the proteins produced by the alleles of the respective α_{s1}-Cn and β-Lg heterozygotes and the expression of the alleles in homozygotes is shown. The difference between the whey proteins of the β-Lg heterozygotes remains nearly stable during the 2 years of the investigation (Fig. 4). In contrast, the B allele of the α_{s1}-Cn heterozygote shows

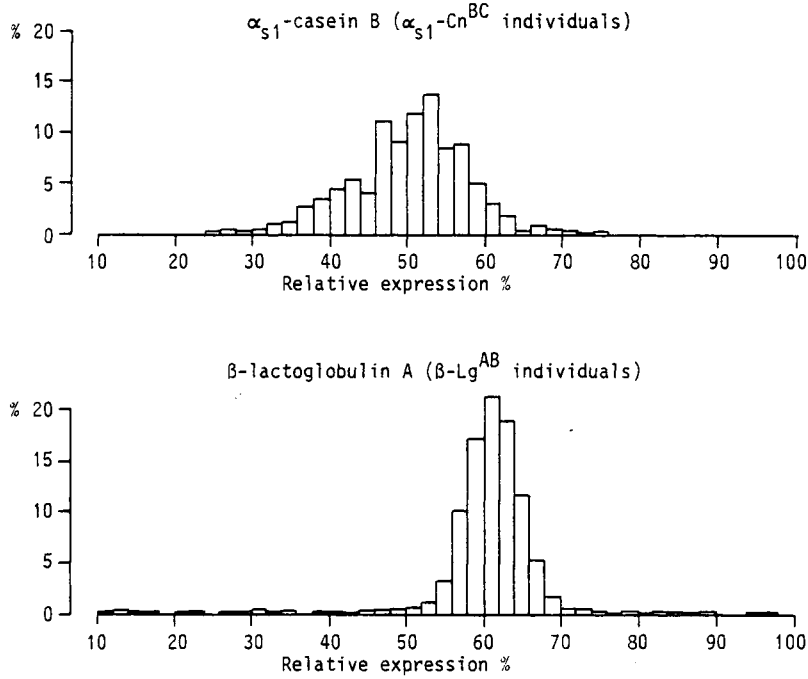

Fig. 1. Distribution of the relative expression of α_{s1}-casein and β-lactoglobulin genes from heterozygous genotypes (g/10^4 ml α_{s1}-casein B + g/10^4 ml α_{s1}-casein C = 100%; g/10^4 ml β-lactoglobulin A + g/10^4 ml β-lactoglobulin B = 100%).

significantly more synthetic activity during the spring– summer seasons than the C-allele (Fig. 2). Even in homozygous genotypes, α_{s1}-Cn^B shows more activity in this period (Fig. 3). In general, β-Lg^B and α_{s1}-Cn^C show a more constant expression in heterozygous genotypes than the resp. homologous alleles.

For the ratio of α_{s1}-caseins in heterozygotes, repeatability was estimated as 18%, and as about 50% for the β-lactoglobulins. This indicates that this ratio reflects to a considerable degree an innate property of cows which probably is inherited to a large extent. However, even for whey protein, a large proportion of the variability is due to factors not accounted for in the model. The lower repeatability of the ratio between the caseins may reflect *inter alia* the interaction between the allelic activity and seasonal influences.

DISCUSSION

The two breeds *Bavarian Simmental* and *Bavarian Brown Alpine* are located in different regions and the analysis of the milk samples was performed at different times. The differences between genotypes in both breeds are similar (Table I), as

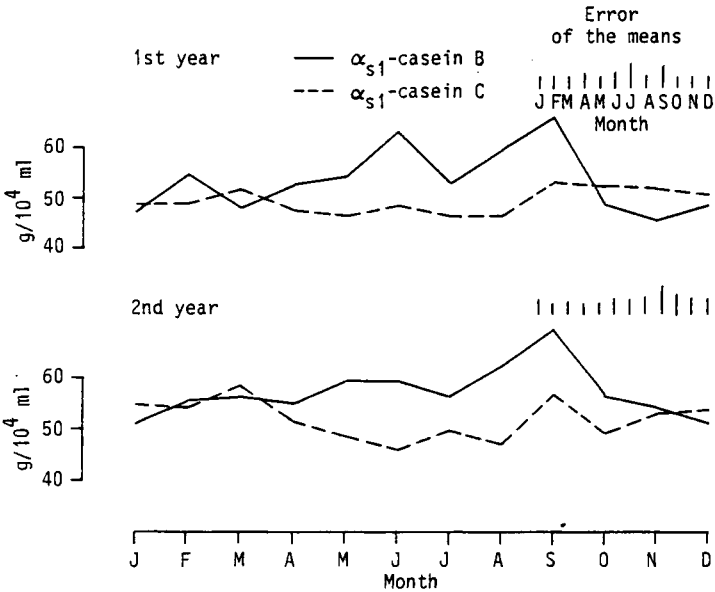

Fig. 2. Seasonal expression of α_{s1}-casein alleles B and C in heterozygous genotypes (allele B 1st and 2nd year $F_{25:393} = 6.27$, $P < 0.001$, allele C 1st and 2nd year $F_{25:393} = 1.90$, $P < 0.05$).

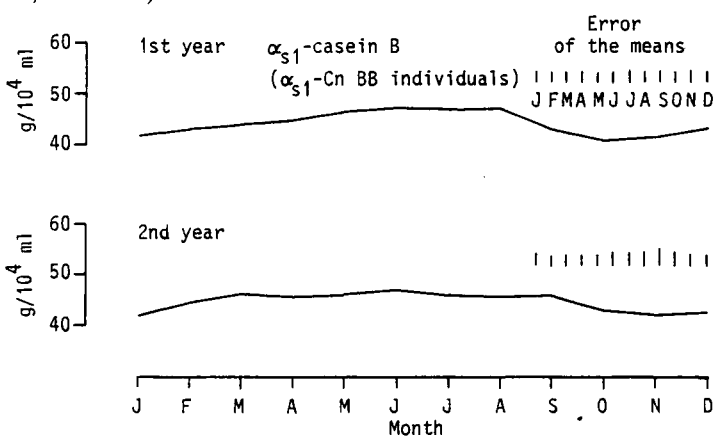

Fig. 3. Seasonal expression of α_{s1}-casein allele B in homozygous genotypes (allele B 1st and 2nd year $F_{25:1801} = 5.02$, $P < 0.001$).

are the distributions and the seasonal changes. As to seasonal effects on the ratio of caseins, we can only speculate at this time. During spring–summer seasons, cows are either on pasture or zero-grazing and receive fresh grass which contains steroids which, in turn, may activate the different alleles to different degrees.

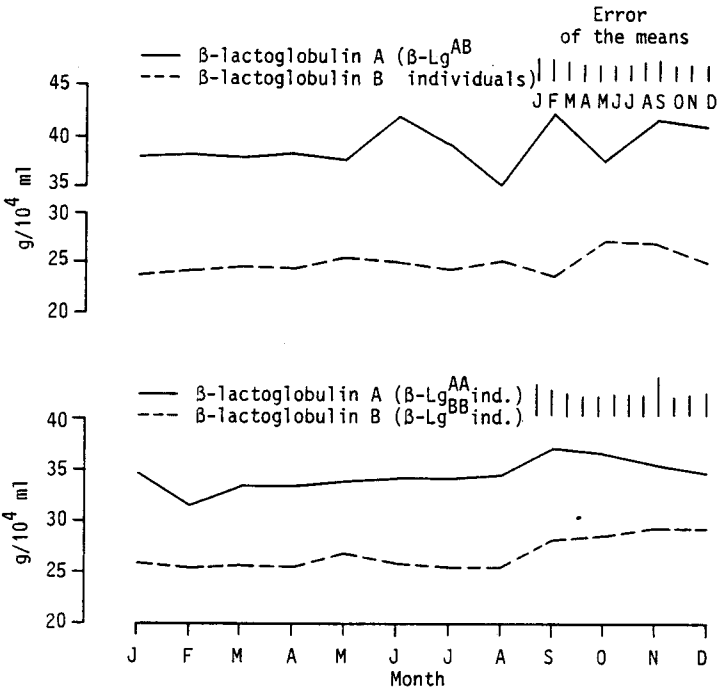

Fig. 4. Seasonal expression of ß-lactoglobulin alleles A and B in heterozygous and homozygous genotypes :

β-Lg^A (from AB) $F_{24;903} = 3.37$, $P < 0.001$;
β-Lg^B (from AB) $F_{24;903} = 1.86$, $P < 0.05$;
β-Lg^A (from AA) $F_{23;434} = 1.33$, n.s.;
β-Lg^B (from BB) $F_{23;457} = 1.87$, $P < 0.01$.

The above average expression of the B allele in α_{s1}-Cn heterozygotes could, to some degree, be a product of α_{s0}-caseins of C-α_{s1} protein co-migrating with the B-α_{s1} protein. For the B protein, the contribution of the α_{s0}-casein is evident in the electrophoregram and has been considered in estimating the B-fraction. Also the area in the case of homozygotes was corrected for where indeed CC genotypes produce significantly more casein than the BB genotypes. Therefore, the above average expression of the B alleles in heterozygotes during spring–summer could be influenced also by differences in phosphokinase activity. However, the significant increase in the expression of BB homozygotes in the spring–summer season cannot be accounted for by such an influence.

ACKNOWLEDGEMENT

The authors are grateful to the referees for constructive criticism. The investigation was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

Aaltonen M.L. & Antila V. (1987) Milk renneting properties and the genetic variants of proteins. *Milchwissenschaft* 42, 490-492

Boulanger A., Grosclaude F. & Mahé M.F. (1984) Polymorphisme des caséines α_{s1} et α_{s2} de la chèvre (*Capra hircus*). *Génét. Sél. Evol.* 16, 157-176

Boyer S.H., Hathaway P. & Garrick M.D. (1964) Modulation of protein synthesis in man: an *in vitro* study of haemoglobin synthesis by heterozygotes. *Cold Spring Harbor Symp. Quant. Biol.* 29, 333

Bunn H.F., Forget B.G. & Ranney H.M. (1977) *Human Hemoglobins.* W.B. Saunders, Philadelphia

Cerbulis J. & Farrell H.M. Jr. (1975) Composition of the milks of dairy cattle. I. – Protein, lactose, and fat contents and distribution of protein fraction. *J. Dairy Sci.* 58, 817-827

Golikova A.P. & Panin I.A. (1972) Ratios of protein fractions in milk of Swiss Brown cows in relation to protein polymorphism. *Trudy Vsesoyuznogo Sel'skokhozyaistvennogo Instituta Zaochnogo Obrazovaniya* 51, 57-58 (*Dairy Sci. Abstr.* 39, 116)

Grosclaude F., Mahé M.F., Brignon G., Di Stasio L. & Jeunet R. (1987) A Mendelian polymorphism underlying quantitative variations of goat α_{s1}-casein. *Génét. Sél. Evol.* 19, 399-412

Huisman T.H.J., Wrightstone R.N., Wilson J.B., Schroeder W.A. & Kendall A.G. (1972) Haemoglobin Kenya, the product of fusion of r and β polypeptide chains. *Arch. Biochem. Biophys.* 153, 850-853

Itano H.A. (1965) The synthesis and structure of normal and abnormal haemoglobins. *In: Abnormal Haemoglobins in Africa* (J.H.P. Jonxis ed). Blackwell, Oxford

Kirchmeier O. (1975) Transparenz elektrophoretischer Analysenergebnisse bei Anwendung verschiedener Methoden. Untersuchungen an Casein. *Z. Lebensm. Unters. Forsch.*, 157, 205-210

Komatsu M., Abe T. & Oishi T. (1977) Relationship between β-lactoglobulin types and the concentrations of β-lactoglobulin and α-lactalbumin in milk. *Jpn. J. Zootechn. Sci.* 48, 237-242

Mariani P., Morini D., Losi G., Castagnetti G.B., Fossa E. & Russo V. (1979) Ripartizione delle frazioni azotate del latte in vacche caratterizzate da genotipo diverso nel locus β-lattoglobulina. *Sci. Techni. Lattiero-Casearia* 30, 153-176

McLean D.M., Graham E.R.B. & McKenzie H.A. (1982) Estimation of casein composition by gel electrophoresis. *In: XXI Int. Dairy Congr., Moscow, USSR,* July 12-15, 1982, vol. 1, Book 1, Mir, Moscow, p. 221

McLean D.M., Graham E.R.B., Ponzoni R.W. & McKenzie H.A. (1984) Effects of milk protein genetic variants on milk yield and composition. *J. Dairy Res.* 51, 531-546

Michalak W., (1973) Research on the content of some milk constituents in cow's milk throughout lactation. IV. Milk protein composition during the lactation, with regard to β-lactoglobulin and κ-casein genotypes of cows. *Prace i Materialy Zootechniczne* 2, 31-58

Moustgaard J., Moller J. & Sorensen P.H. (1960) β-Lactoglobulintyper hos kvaeg. Aarsberetning. Institute for Sterilitetsforskning retning, *K. Vet. Landbohojskole*, Copenhagen, pp. 111-123

Na-Nakorn S. & Wasi P. (1970) Alpha-thalassaemia in Northern Thailand. *Am. J. Hum. Genet.* 22, 645-651

Ng-Kwai-Hang K.F., Hayes J.F., Moxley J.E. & Monardes H.G. (1987) Variation in milk protein concentrations associated with genetic polymorphism and environmental factors. *J. Dairy Sci.* 70, 563-570

Raymond S. & Weintraub L. (1959) Acrylamide gel as a supporting medium for zone electrophoresis. *Science* 130, 711

Reimerdes E.H. & Mehrens H.A. (1978) Die quantitative Bestimmung der genetischen Varianten von β-Lactoglobulin in Milch. *Milchwissenschaft* 33, 345-348

Sluyterman L.A.AE. & Elgersma O. (1978) Chromatofocusing: isoelectric focusing on ion-exchange columns. I. General principles. *J. Chromatogr.* 150, 17-30

Sluyterman L.A.AE. & Wijdenes J. (1978) Chromatofocusing: isoelectric focusing on ion-exchange columns. II. Experimental verification. *J. Chromatogr.* 150, 31-44

Wellis I.C. & Itano H.A. (1951) The ratio of sickle cell anemia haemoglobin to normal haemoglobin in the sicklemics. *J. Biol. Chem.* 188, 65-74

Wetherall D.J. & Clegg J.B. (1972) *The thalassaemia syndromes.* Blackwell, Oxford, 2nd edn

Wrightstone R.N. & Huisman T.H.J. (1968) Qualitative and quantitative studies of sickle cell hemoglobin in homozygotes and heterozygotes. *Clin. Chim. Acta* 22, 593-602