

Short communication

## Comparison of growth hormone and kappa-casein gene polymorphism in Polish Red and German Red cattle breeds

Jindřich ČÍTEK<sup>1</sup>, Andrzej FILISTOWICZ<sup>2</sup>, Václav ŘEHOUT<sup>1</sup>, Věra NEUBAUEROVÁ<sup>1</sup>

<sup>1</sup> University of South Bohemia, Agricultural Faculty, Department of Animal Breeding, České Budějovice, Czech Republic,

<sup>2</sup> Agricultural University, Faculty of Biology and Animal Breeding, Department of Genetics and Animal Breeding, Wrocław, Poland

**Abstract:** The polymorphism of kappa-casein (alleles A, B and E) and growth hormone (alleles L and V) genes was studied using PCR/RFLP method with the aim to evaluate the degree of genetic diversity of Polish Red and German Red cattle, which are endangered with extinction. At the kappa-casein locus, a very low frequency of allele B in German Red cattle was found. In both the breeds, allele E was absent. There was a relatively high frequency of allele L in the growth hormone locus, especially for German Red cattle. The results were compared with frequencies and diversities of other red cattle breeds.

**Key words:** kappa-casein, growth hormone, Polish Red cattle, German Red cattle, genetic diversity.

The polymorphism of kappa-casein and growth hormone genes has been of interest for decades due to the assumed associations with different aspects of production traits. In the kappa-casein locus, alleles A and B are the most frequent. Additionally, alleles C, D, E, F, G and H have also been described (ERHARDT 1996, SEIBERT et al. 1987). Out of the authors who have studied the kappa-casein locus polymorphism in relation to milk production, LIEN, ROGNE (1993) can be mentioned.

Received: March 2000.

Correspondence: A. FILISTOWICZ, Agricultural University, Faculty of Biology and Animal Breeding, Department of Genetics and Animal Breeding, ul. Skłodowskiej-Curie 42, 50-369 Wrocław, Poland.

In the growth hormone locus, the polymorphism causing substitution at position 127 (Leu - Val) was often studied. SABOUR et al. (1997) reported the frequency of allele V in Holstein cattle (0.09), Ayrshire bulls from Canadian artificial/insemination centres (0.29), and Jersey bulls (0.24). Other authors (LUCY et al. 1993, SCHLEE et al. 1994a) studied polymorphism at this locus and its associations to milk and meat production.

Preservation of biological diversity has become an important problem in populations of farm animals, especially in developed countries. Local breeds are dying out and being replaced with high-productive breeds, and thus the genetic diversity of small populations is reduced by inbreeding and genetic drift.

This paper refers to the polymorphism of growth hormone and kappa-casein genes in Polish Red cattle and German Red cattle breeds. Both breeds are under preservation programmes, since they are endangered with extinction. These dual-purpose breeds are classed with the Central-European group of brachycerous red cattle. One of the members of this group is also Czech Red cattle, an original Czech breed which is endangered with extinction. German Red and Polish Red breeds are also endangered, because their populations are very small. There are about 100 thousand cows in Poland (crossbreeds and purebreeds), but under milk recording there are only 1756 cows. The preservation programme covers less than 50 Polish Red cows. Many herds have been crossed with Angler cattle, which is related to the breeds mentioned, so the original Polish genotypes are dwindling.

The aim of this paper was to evaluate the genetic diversity of kappa-casein and growth hormone loci of Polish Red and German Red cattle populations. The study was carried out on 65 Polish Red cows, the offspring of 39 sires coming from two herds in Poland, and 28 German Red cows and bulls coming from four herds in Hessen. As mentioned above, both the breeds are endangered. Especially the population of German Red cattle is very small, therefore the group was composed of partially related animals, the offspring of twelve dams and nine sires; for nine animals the sire was unknown.

DNA was extracted from whole blood according to previously reported procedure of GEMMELL, AKYIAMA (1996) and amplified by PCR kappa-casein locus primers according to SCHLEE et al. (1992): 5' - CACGTCACCCACACCC ACATTTATC - 3' and 5' - TAATTAGCCCATTTTCGCCTTCTCTGT - 3', which were used at the annealing temperature 56°C. To distinguish alleles A and B, the PCR product was digested by HindIII, to distinguish alleles A and E, the PCR product of allele A carriers was digested by HaeIII endonuclease. For the growth hormone gene, primers according to SCHLEE et al. (1994b): 5' - GCTGCTCCT GAGGGCCCTTCG - 3' and 5' - GCGGCGGCACTTCATGACCCT - 3' were used at the annealing temperature 60°C, and the PCR product was digested by AluI. After digestion, the fragments were electrophoresed on 3.5% agarose gels and stained with ethidium bromide.

Allele frequencies and PIC (Polymorphism Information Content) values were calculated. To test the gene and genotype frequencies between the breeds, test of difference of relative values and  $\chi^2$  test were used.

The results are shown in Table 1. Neither in Polish Red nor in German Red cattle, allele E was found in the kappa-casein locus. Although its frequency in most of breeds is very low, the lack of this allele in 65 Polish Red animals may be considered surprising. However the number of German Red animals in the study was very small, and they were more or less related, which can be a possible explanation for the absence of this sporadic allele. In our previous study (ČÍTEK et al. 1997) the E allele frequency in Czech Red cattle was 0.058.

The very low frequency of allele B in German Red cattle was not expected, because dual-purpose cattle breeds show usually much higher values than dairy cattle, like Holstein Friesian (VAŠÍČEK et al. 1995). In our previous observations the frequency of this allele was 0.22 in Czech Black-and-White cattle (ČÍTEK et al. 1997). Even in Czech Red cattle, (ČÍTEK et al. 1997), the frequency of allele B was higher (0.48) than that of allele A (0.46) and consequently, Czech Red cattle had a significantly higher frequency of allele B than both Polish and German Red cattle. In this report, the allele and genotype frequencies at kappa-casein locus in Polish and German Red breeds differed significantly from each other. The frequencies in German Red cattle showed the negligible genetic diversity. The PIC (Polymorphism Information Content) in German Red cattle was only 0.123, in Polish Red cattle 0.339, whereas in Czech Red cattle it was 0.452. In both studied breeds, the allele and genotype frequencies revealed that the Hardy-Weinberg equilibrium maintained.

At the growth hormone locus (Table 1), the frequency of allele L in German Red was significantly higher than in Polish Red, but the genotype frequencies did not differ significantly between these breeds. The share of allele L in both breeds was remarkably higher than in Czech Red cattle (ČÍTEK et al. 1998), where the frequency of allele L was 0.51, and the genotype frequencies were 0.146 (LL), 0.729 (LV) and 0.125 (VV), respectively. Both allele and genotype frequencies in Czech Red differed statistically from Polish and German Red, except for the VV genotype frequency between Czech and Polish Red. The allele frequencies in our study are similar to those reported by LUCY et al. (1993) for Holstein Friesian and Guernsey breeds. The authors give an example of the frequency of allele L in Jersey cattle of 51%, and explain the variation by reference to different wild ancestors, *Bos longifrons* (allele V) for Jersey, and *B. primigenius* (allele L) for Holstein. As mentioned above, the red breeds are members of the brachycerous group, thus so a high frequency of allele V is expected. Nevertheless, we hint again at the restricted number of both populations, which could have changed the original allele and genotype distribution by genetic drift and/or inbreeding.

The reduction of genetic diversity, measured by the PIC value, could also be brought about by inbreeding and genetic drift. In Polish Red and German Red cattle the PIC values were: 0.292 and 0.173, respectively. The genetic disequilibrium

**Table 1.** Observed frequencies at loci of  $\kappa$ -casein and growth hormone (GH)

Polish Red cattle	n	Genotypes for $\kappa$ -casein			$\chi^2$ test	n	Genotypes for GH			$\chi^2$ test
		AA	BB	AB			VV	LV	LL	
frequency	30	6	29	0.110	frequency	9	12	44	23.034	
	0.46	0.09	0.45			0.14	0.18	0.68		
	n	Alleles			$\chi^2$ test	n	Alleles			$\chi^2$ test
		A		B			V		L	
		89	41	30			100			
frequency	0.68	0.32	frequency	0.23	0.77					
German Red cattle	n	Genotypes for $\kappa$ -casein			$\chi^2$ test	n	Genotypes for GH			$\chi^2$ test
		AA	BB	AB			VV	LV	LL	
frequency	24	0	4	0.592	frequency	0	6	22	1.441	
	0.86**	0 <sup>nc</sup>	0.14**			0 <sup>nc</sup>	0.21 <sup>-</sup>	0.79 <sup>-</sup>		
	n	Alleles			$\chi^2$ test	n	Alleles			$\chi^2$ test
		A		B			V		L	
		52	4	6			50			
frequency	0.93**	0.07	frequency	0.11	0.89*					

\*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , – = not significant, nc = not calculated due to zero frequency (for differences between breeds), n = number of individuals.

in Polish Red cattle, decreasing the frequency of heterozygous genotypes in relation to the values expected theoretically, may have been caused accidentally, since in the previous study of Czech Red cattle (ČÍTEK et al. 1998) we found the disequilibrium in the opposite direction.

In conclusion the genetic diversity in Polish and especially in German Red cattle seems to be restricted. It seems that further studies, covering larger populations and including more loci, are necessary to evaluate genetic variability in both breeds.

**Acknowledgements.** This research was supported by grants NAZV EP0960006215 and CEZ J 06/98 1222 0004.

## REFERENCES

- ČÍTEK J., ŘEHOUT V., HAJIČ F., KOŠVANEC K., ŠOCH M. (1997). Genetic polymorphism of kappa-casein locus in Czech Pied and Black Pied Cattle. *Živ. Vyr.* 42: 1-4.
- ČÍTEK J., PANICKE L., FREYER G., ŘEHOUT V., MAŠKOVÁ J. (1998). Polymorfismus genu pro růstový hormon u některých plemen skotu. *Czech J. Anim. Sci.* 43: 101-104.
- ERHARDT G. (1996). Detection of a new  $\kappa$ -CN variant in milk of Pinzgauer cattle. *Anim. Genet.* 27: 105-107.

- GEMMELL N.J., AKIYAMA S. (1996). An efficient method for the extraction of DNA from vertebrate tissues. *Trends Genet.* 12: 338-339.
- LIEN S., ROGNE S. (1993). Bovine casein haplotypes: number, frequencies and applicability as genetic markers. *Anim. Genet.* 24: 373-376.
- LUCY M.C., HAUSER S.D., EPPARD P.J., KRIVI G.G., CLARK J.H., BAUMAN D.E., COLLIER R.J. (1993). Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. *Dom. Anim. Endocrinol.* 10(4): 325-333.
- SABOUR M.P., LIN C.Y., SMITH C. (1997). Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle. *J. Anim. Breed. Genet.* 114: 435-442.
- SCHLEE P., ROTTMANN O., BUCHBERGER J., GRAML R., AUMANN J., BINSER R., PIRCHNER F. (1992). Die Milchproteingene des Fleckviehbulle „Hax1“ und dessen Einfluss auf die Allelfrequenzen. *Zuechtungskunde* 64: 312-322.
- SCHLEE P., GRAML R., ROTTMANN O., PIRCHNER F. (1994a). Influence of growth-hormone genotypes on breeding values of Simmental bulls. *J. Anim. Breed. Genet.* 111: 253-256.
- SCHLEE P., GRAML R., SCHALLENBERGER E., SCHAMS D., ROTTMANN O., OLBRICH-BLUDAU A., PIRCHNER F. (1994b). Growth hormone and insulin-like growth factor I concentrations in bulls of various growth hormone genotypes. *Theor. Appl. Genet.* 88: 497-500.
- SEIBERT B., ERHARDT G., SENFT B. (1987). Detection of a new  $\kappa$ -casein variant in cow's milk. *Anim. Genet.* 18: 269-272.
- VAŠÍČEK D., UHRÍN P., CHRENEK P., BAUEROVÁ M., OBERFRANC M., BULLA J. (1995). Genotyping of kappa-casein in different cattle breeds in Slovakia. *Živ. Vyr.* 40: 241-244.