Association Study Between Milk Protein Variants And Milk Performance Traits In East Friesian Dairy Sheep

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Introduction

Detection and mapping of genes with an influence on economic important traits in dairy sheep is a prerequisite to improve dairy sheep production systems. In dairy cattle and goats genetic polymorphisms of milk proteins show associations to quantitative and qualitative parameters in milk (Martin *et al.*, 2002; Caroli *et al.*, 2009), while in sheep association studies are rare and the results are controversial (Amigo *et al.*, 2000; Barillet, 2007).

So far different alleles/phenotypes are identified for ovine α_{s1}-casein (α_{s1}-CN A-F, H, I) (Amigo *et al.*, 2000; Ceriotti *et al.*, 2005; Giambra *et al.*, 2010a, b), α_{s2}-casein (α_{s2}-CN A-D) (Picariello *et al.*, 2009; Giambra *et al.*, 2010c), ß-lactoglobulin (ß-LG A-C) (Bell and McKenzie, 1967; Erhardt, 1989a), and α-lactalbumin (α-LA A, B) (Schmidt and Ebner, 1972). Compared to bovine and caprine κ-casein (κ-CN) this fraction is monomorphic at protein level in sheep, while some single nucleotide polymorphisms without relation to protein variability were demonstrated (Ceriotti *et al.*, 2004; Feligini *et al.*, 2005).

Casein genes are organized as a tightly linked cluster on ovine chromosome 6 (Threadgill and Womack, 1990; Bevilacqua *et al.*, 2006) and therefore the estimation of associations between casein variants and milk production traits can be improved by considering the entire casein haplotype instead of the single genes (Sacchi *et al.*, 2005).

The aim of this study was to determine the allele frequencies for milk proteins by isoelectric focusing and to estimate genotype and haplotype effects on milk production traits within Dutch East Friesian Dairy sheep.

Material and methods

Sheep milk samples. Milk samples of 76 Dutch East Friesian Dairy sheep (OMS) were collected during routine milking.

Isoelectric focusing (IEF). Separation and identification of milk proteins were done by IEF of skimmed milk samples according to Erhardt (1989b) and Giambra *et al.* (2010c).

Milk performance traits. For all tested lactations (in detail 260 lactations) of the 76 sheep the Fokwaarde+ organisation provided milk recording data, in particular milk yield (milkkg240), fat and protein % with a mean of 479 kg milk, 5.49% fat and 5.14% protein in the standardised 240 days lactation.

Statistical analyses. Allele and genotype frequencies were calculated with program PopGene V 1.31 (Yeh *et al.*, 1997). A χ^2-test was performed to test the goodness of fit to Hardy-Weinberg equilibrium expectations for the distribution of genotypes.

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Haplotype frequencies for α_{s1}- and α_{s2}-CN and the occurrence of linkage disequilibrium were estimated with EH software (Xie and Ott, 1993), considering only alleles with frequencies > 0.05. For association studies data were analysed with the GLM procedure of SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). Dependent variables in the analysis were milk, fat, and protein yield as well as fat and protein percent derived from routine evaluation. Beside the separately analysed milk protein genotypes the number of lactation (1, 2, 3, ≥4) and the current class of season (year and month of lambing) were included as fixed effects in the model.

Results and discussion

Isoelectric focusing. IEF lead to the identification of α_{s1}-CN C and H, α_{s2}-CN A, B, and C, as well as ß-LG A and B, while κ-CN and α-LA were monomorph. Genotype and allele frequencies are shown in Table 1.

Table 1: Genotype and allele frequencies for α_{s1}-CN, α_{s2}-CN, α-LA, and ß-LG in Dutch East Friesian Dairy sheep determined by IEF (n = 76)

	Genotype							Allele			
	AA	AB	AC	BB	BC	CC	CH	A	B	C	H
α_{s1}-CN	-	-	-	-	-	0.84	0.16	-	-	0.92	0.08
α_{s2}-CN	0.16	0.58	0.01	0.22	0.03	-	-	0.45	0.53	0.02	-
α-LA	1.00	-	-	-	-	-	-	1.00	-	-	-
ß-LG	0.56	0.41	-	0.03	-	-	-	0.77	0.23	-	-

Milk protein allele frequencies are similar to those reported in Germany for OMS (Giambra *et al.*, 2010c). There was a good agreement between the observed genotype frequencies and those expected on the basis of Hardy-Weinberg equilibrium in all milk proteins.
Estimated α_{s1}-/α_{s2}-CN-haplotype frequencies are presented in Table 2, whereas χ^2-test showed a significant association (linkage disequilibrium) between α_{s1}-CN and α_{s2}-CN ($P \leq 0.05$), mainly caused by linkage between α_{s1}-CN H and α_{s2}-CN B.

Table 2: Frequency of α_{s1}-/α_{s2}-CN-haplotypes (n = 73) [a]

	Haplotype (α_{s1}-/α_{s2}-CN)			
Frequency	CA	CB	HA	HB
Expected[b]	0.43	0.49	0.04	0.04
Observed	0.46	0.46	0.00	0.08

[a] For haplotype evaluation, α_{s2}-CN C (allele frequency < 0.05) was not considered (n = 3).
[b] Expected haplotype frequency under independence hypothesis.

Association studies. For the association studies the genotypes α_{s2}-CN AC and BC, as well as ß-LG BB were excluded (frequency < 0.10). Due to the linkage between α_{s1}-CN H and α_{s2}-CN B for animals genotyped as α_{s1}-CN CH and α_{s2}-CN AB the α_{s1}-/α_{s2}-CN-haplotype CA/HB was assumed. For all other animals haplotypes could definitely be reconstructed.
Highly significant effects of number of lactation on yield traits ($P \leq 0.01$) and of class of season on all traits ($P \leq 0.001$) were identified and confirm environmental influences on milk production traits in sheep (Ruiz *et al.*, 2000).

Genotype and haplotype effects of milk protein polymorphisms on milk production traits are demonstrated in Table 3. The results show a highly significant positive association between α_{s1}-CN CH and fat percent and yield, while in different Italian sheep breeds (Amigo *et al.*, 2000) the predominant α_{s1}-CN CC was associated with higher fat, protein, and casein content. We assume that α_{s1}-CN H did not occur in these Italian sheep breeds, as they used comparable techniques and conditions. Missing of α_{s1}-CN alleles A and D in the typed OMS seems to be caused by indirect selection, due to the advantage of α_{s1}-CN C and H. Furthermore α_{s1}-CN CH showed a tendency to higher milk yield also described by Wessels *et al.* (2004).

Within α_{s2}-CN we identified a significant positive association between α_{s2}-CN AB and protein yield, not described before. However similar studies with East Friesian Dairy sheep kept on different farms in Germany found significant relationships between α_{s2}-CN genotypes and milk yield (Horstick *et al.*, 2002) or fat kg (Wessels *et al.*, 2004).

Table 3: Estimates of genotype and haplotype (α_{s1}-/α_{s2}-CN) effects on milk production traits (least square means and standard errors) in Dutch East Friesian Dairy sheep

Genotype/Haplotype		Trait				
		milkkg240	fat %	protein %	fat kg	protein kg
α_{s1}-CN	Sign.[a]	n.s.	***	n.s.	**	n.s.
(260 lactations)	CC	499±5	5.50±0.03	5.17±0.02	27.4±0.3	25.8±0.3
	CH	516±14	5.83±0.09	5.16±0.06	30.0±08	26.6±0.7
α_{s2}-CN	Sign.	n.s.	n.s	n.s.	n.s.	**
(253 lactations)	AA	478±10	5.58±0.07	5.12±0.04	26.6±0.6	24.4±0.5
	AB	505±6	5.52±0.04	5.19±0.03	27.9±0.4	26.2±0.3
	BB	503±9	5.51±0.06	5.18±0.04	27.7±0.5	26.0±0.5
ß-LG	Sign.	n.s.	**	**	n.s.	n.s.
(252 lactations)	AA	498±7	5.61±0.04	5.23±0.03	27.9±0.4	26.0±0.3
	AB	503±7	5.46±0.04	5.11±0.03	27.5±0.4	25.7±0.3
α_{s1}-/α_{s2}-CN	Sign.	n.s.	**	**	**	**
(253 lactations)	CA/CA	478±10	5.58±0.06	5.12±0.04	26.7±0.6	24.5±0.5
	CA/CB	505±6	5.51±0.04	5.21±0.03	27.8±0.4	26.3±0.3
	CA/HB	509±17	5.74±0.11	5.05±0.07	29.0±1.0	25.7±0.9
	CB/CB	499±10	5.41±0.06	5.14±0.04	27.0±0.6	25.6±0.5
	CB/HB	523±22	6.00±0.14	5.37±0.09	31.6±1.3	27.9±1.1

[a]Sign. = Significance level: n.s. = $P > 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

The identified significant positive effects of ß-LG AA on fat and protein % confirm results of Rampilli *et al.* (1997). On the other side the described positive effects of ß-LG BB on milk yield (Amigo *et al.*, 2000) could not be approved as the frequency of this genotype was low within our population.

As shown in Table 3 significant haplotype effects could be identified on fat %, protein %, fat and protein yield. In detail haplotype CB/HB showed association with highest fat and protein percent and yield respectively. These are to our knowledge the first association studies including ovine casein haplotypes while in cattle numerous studies are already available (Caroli *et al.*, 2009).

The possibility to identify genotypes with α_{s1}-CN allele H by IEF (Giambra *et al.*, 2010c) and by DNA-tests (Giambra *et al.*, 2010b) opens the chance to screen further dairy sheep breeds for the occurrence of this allele and to confirm its positive effects. As the caseins are organized within a cluster (Threadgill and Womack, 1990; Bevilacqua *et al.*, 2006) these effects should be estimated using casein haplotypes or composite casein genotypes instead of separate genotypes. In the case of consistent results this will open the possibility to consider the casein locus as marker in breeding programs like in goat (Sanchez *et al.*, 2005).

Conclusion

The observation of significant effects of casein genotypes and haplotypes in Dutch East Friesian Dairy sheep should be confirmed in other dairy breeds. The haplotype that improved fat and protein percentage was also associated with positive effects on yield of fat and protein. If the observed effects are real, the casein complex offers the use in marker assisted selection in dairy sheep.

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