Association Of Aggregate Milk Protein Genotypes In Cattle With Yoghurt Quality Traits

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Introduction

Many studies have been carried out to determine possible associations between milk protein gene polymorphism and economically important traits like milk production, milk composition and cheese - making properties (Caroli et al., 2004; Casandro et al., 2008; de Marchi et al., 2008; Heck et al., 2009). Nevertheless an effect of milk protein gene polymorphism on milk fermentation ability and the production of yoghurt has not been documented so far. The proteins in yoghurt are of excellent biological quality, as are those in milk, because the nutritional value of milk proteins is well-preserved during the fermentation process. The quality of the fermentation process and lactic acid production is represented by its actual acidity (pH values) or titratable acidity according to Soxhlet-Henkel (^{o}SH) and also by the lactic acid-producing bacteria (LAB) content of the finished yoghurt product. It must contain live LAB in amounts $\geq 10^8$ organisms/g at the time of manufacture (Chandan and O'Rell, 2006). The constituent composition of yoghurt is based on the composition of milk from which it is derived, which is affected by many factors, such as genetic and individual differences between cows, feed, stage of lactation and age, and environmental factors such as the season of the year (Adolfsson et al., 2004).

The objectives of this study were to examine association of the aggregate α_{S1}-, β- and κ-casein and β-lactoglobulin genotypes in Czech Fleckvieh Cattle with yoghurt quality traits such as pH, titratable acidity, and amount of lactic acid-producing bacteria. The following gene combinations were taken into account: *CSN1S1/CSN2*, *CSN2/CSN3*, *CSN3/LGB*, *CSN2/CSN3/LGB* and *CSN1S1/CSN2/CSN3*. The given genotype combinations were preferred due to their previously described effects either on milk composition, milk and protein production or on milk coagulation and technological properties (Comin et al., 2008; Hallén et al.; 2008, Heck et al., 2009; Joudu et al., 2007; Matějíček et al., 2008).

Material and methods

Sampling. A total of 338 healthy Czech Fleckvieh cows in their first lactation were included in the investigation. Milk samples for laboratory analysis were collected once between 60-140 days of an individual lactation.

Genotyping. Genetic polymorphisms of the *CSN1S1*, *CSN3* and *BLG* loci were analysed by PCR-RFLP as described previously (Kučerová et al., 2006), with slight modifications. The

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CSN2 locus was analysed with melting curve genotyping analysis after DNA amplification via PCR with a Light Cycler 1.5 instrument (Roche Diagnostics, France) according to Sztankóová et al. (2008).

Yoghurt Analysis. The fermentation ability (FA) of milk was tested with the thermophilic yoghurt culture **YC-180-40-FLEX**, which consists of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus delbrueckii* subsp. *Bulgaricus.* FA was expressed as the total count of the fermenting noble microorganisms (**FAM-TCM**) or as the count of *Lactobacilli* (**FAM-CL**) and *Streptococci* (**FAM-CS**) strains in $CFU.ml^{-1}$, after classical plate cultivation according to the international technical standard ISO 6610:1992. Beside this the following yoghurt parameters were measured: actual acidity (**Y-pH**) and titratable acidity (**Y-TA**) according to Soxhlet-Henkel. Results are presented in Table 1.

Statistical analysis. Genotype frequencies were obtained by gene counting. The association of milk protein genetic polymorphisms with the investigated yoghurt parameters was analysed using the general linear model (GLM) function in the statistical software program SAS (SAS Institute Inc., Cary, NC, USA). The linear model used to investigate the relationship between genotypes and observed parameters was as follows:

$y_{ijk} = \mu + HYS_i + G_j + bA_k + e_{ijk}$

Where: y = observed parameter, μ = population mean, HYS = effect of herd, year and season of calving, G = genotype effect of two or three gene combinations investigated, bA = effect of age at first calving of cow.

The statistical significance of the genotypes was evaluated via an F-test.

Results and discussion

Some of the investigated parameters were found to be significantly affected by the *CSN2-CSN3-LGB* genotype. The other milk protein aggregate genotypes had no effect on any of the investigated parameters.

A total of 49 different *CSN2-CSN3-LGB* genotypes, including 18 rare (<1%) genotypes, were identified. In this study, the genotypes A^1A^2ABAB (8.9%), A^1A^2AAAB and A^2A^2ABAB (both 8.3%) were the most prevalent and the sum of the rare aggregate genotypes was 7.4%. The *CSN2-CSN3-LGB* genotype affected yoghurt pH and decimal logarithm of the count of *Lactobacilli* in $CFU.ml^{-1}$ (at significance level: $0.05 \geq P > 0.01$) and statistical analysis confirmed the negative relationship between Y-pH and log FAM-CL. Larger numbers of *Lactobacilli* produce more lactic acid and consequently decrease the pH of the yoghurt. Observed Y-pH values (in the range of 4.8-5.1) were in general slightly higher than those preferred by the current consumer, which are in the range of 4.2-4.4 (Chandan and O'Rell, 2006). This likely reflects the fact that only the first lactation was taken into account in the present study; later lactations may yield lower pH values. Samples with A^2B or A^2A^2/AA/A- genotypes at the *CSN2/CSN3/LGB* loci reached the lowest Y-pH values (4.7-4.8) and highest log FAM-CL values (7.40-7.64). Samples with higher Y-pH and lower log FAM-CL tended to have the A^1 or A^2 allele at the *CSN2* locus, together with *AA* or *AB* genotypes, or alternatively the *E* allele at the *CSN3* locus and an almost equal occurrence of *A* and *B* allele at the *LGB* locus. The negative effect of *CSN3 E* allele on milk quality and colagulation and gelation properties was described also by some other authors (Comin et al., 2008; Hallén et

al., 2008; Matějíček et al., 2008). Nevertheless, it could be useful to perform a detailed analysis of milk protein gene interaction in a larger cattle population with a more sophisticated statistical model to confirm the present results.

Table 1: Analytical determination of yoghurt parameters

Yoghurt parameter		Units	Mean	SD
Y-pH	Actual acidity		4.87	0.18
Y-TA	Titratable acidity	^{o}SH	31.89	3.48
FAM-TCM	Total count of the fermenting microorganisms	cfu.ml^{-1}	6.8669×10^{8}	2.2977×10^{8}
Log FAM-TCM			8.82	0.15
FAM-CL	Total count of Lactobacilli	cfu.ml^{-1}	2.7455×10^{7}	1.5106×10^{7}
Log FAM-CL			7.36	0.26
FAM-CS	Total count of Streptococci	cfu.ml^{-1}	6.5960×10^{8}	2.2555×10^{8}
Log FAM-CS			8.79	0.15

n=338
cfu=colony forming units

Conclusion

The relationship of bovine milk gene polymorphism and milk production, composition and milk coagulation characteristics and cheese production is very well documented by many scientific studies. However, the results presented in this study are the first to show a possible effect of milk protein gene polymorphism on milk fermentation ability, souring capability and the production of yoghurt, which is another cow's milk product that is significant for human health and nutrition. Genetic polymorphism of the aggregate milk protein system CSN2-CSN3-LGB affected important yoghurt quality parameters such as pH and ability to support the growth of lactic-acid producing bacteria.

This study supported the hypothesis that milk protein genetic polymorphism probably contributes to the secondary and tertiary structure of the relevant protein molecule, including casein micelle formation and lactoglobulin molecule assembly. These protein structural characteristics, along with protein composition, are the main factors that influence the milk gelation and coagulation properties important for cheese and yoghurt production. We believe that further study of the aggregate genotypes of milk protein in cattle will have future implications for breeding selection and the production of milk with defined characteristics.

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