

## THE EFFECT OF ADENIN/GUANIN-TRANSITION AT NUCLEOTIDE POSITION 169 OF THE MITOCHONDRIAL GENOME ON MILK PRODUCTION TRAITS IN GERMAN HOLSTEINS

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### INTRODUCTION

The strictly maternally inherited (Kaneda *et al.*, 1995) mammalian mitochondrial DNA (mtDNA) molecule is a likely source of maternal genetic effects on production traits. In dairy cattle breeding, mtDNA effects would have an impact on the accuracy of genetic evaluations (Boettcher *et al.*, 1996a), and could be important with regard to advanced reproductive technologies such as cloning by nuclear transfer (Hiendleder *et al.*, 1999). Estimates of mtDNA effects in dairy cattle that relied solely on statistical analyses of herd book recorded lineages have been contradictory, showing evidence or lack of evidence for cytoplasmic genetic effects, sometimes depending on the models used (Bell *et al.*, 1985 ; Huizinga *et al.*, 1986 ; Kennedy, 1986 ; Reed and Van Vleck, 1987 ; Faust *et al.*, 1990 ; Schutz *et al.*, 1992 ; Boettcher *et al.*, 1996b). More recently, identification and verification of maternal lineages by molecular genetic analysis of mtDNA haplotype and subsequent statistical evaluation of cytoplasmic genetic effects in animal models showed evidence for significant effects on milk fat % and fat yield (Schutz *et al.*, 1993 ; Boettcher *et al.*, 1996c). An adenin/guanin-transition at nucleotide position 169 (Anderson *et al.*, 1982) in particular, located in the regulatory mitochondrial DNA control region, was reported to have significant effects on milk production traits of North American Holstein cows (Schutz *et al.*, 1993). This study relied on sequence analysis of the complete mitochondrial control region to identify maternal lineages. However, mtDNA of only one or two cows within the data set of 36 maternal Holstein lineages were analysed. Other animals identified by herd book data were assumed to be identical in mtDNA haplotype

### MATERIAL AND METHODS

A total of 2249 registered German Holstein cows representing 19 herds from two breeding regions (Thüringen n=1597; Hessen n=652) were typed by PCR-RFLP for nucleotide position 169 polymorphism to investigate the effect on yield traits in German Holsteins. DNA was extracted from blood by standard procedures. Amplifications and restriction enzyme digestions were performed as described by Döring (1999). For all 2249 cows first lactation records for milk yield and fat content were available. For 2208 animals the protein content in first lactation was available. For the second and third lactation these numbers reduced to 1345 animals with milk and fat, 1305 with protein and 757 cows with milk and fat and 739 with protein, respectively. The means and standard deviations for all traits within lactation are summarised in table 1. Using ANOVA- (SPSS Version 7.5.2) and MTDFREML-analysis (Boldman *et al.*, 1993) with a model including the fixed effects of herd, year of calving, month of calving and variant (A/G) of nucleotide position 169 was used for all production traits. Furthermore the covariates number of days in milk, age at first calving (1<sup>st</sup> lactation) and calving interval (2<sup>nd</sup> and 3<sup>rd</sup> lactation) was included in the model. In the MTDFREML-analysis, the random additive

effect of the animal was also in the model. Analyses were carried out separately for each lactation.

**Table 1. Means (m) and standard deviations (sd) for traits for all 19 herds**

Trait	First lactation		Second lactation		Third lactation	
	m	sd	m	sd	m	sd
Milk in kg	5895	1426	6838	1680	6987	1812
Fat in %	4.22	0.53	4.34	0.59	4.35	0.62
Fat in kg	247.5	60.0	291.8	69.0	300.9	77.5
Protein in %	3.35	0.24	3.42	0.24	3.38	0.24
Protein in kg	198.2	47.2	234.1	53.4	236.7	57.9

## RESULTS

The resulting nucleotide frequencies were 0.642 for position 169 G and 0.358 for position 169 A. Identical frequencies were observed for the two breeding regions with 0,346 and 0,345 for position 169 A and 0.654 and 0.655 for position 169 G in Thüringen and Hessen, respectively. Between herds, frequency for position 169 A varied from 0.048 to 0.650 and for position 169 G from 0.952 and 0.350. In the larger herds frequencies were more evenly distributed.

**Table 2. Estimated effect of adenin/guanin-transition at nucleotide position 169 of the mitochondrial genome on milk production traits from ANOVA-analysis (standard errors of estimates in brackets) (\* =  $p < 5\%$  ° =  $p < 10\%$ )**

Herds Trait	19	19	19	5
	1 <sup>st</sup> lactation	2 <sup>nd</sup> lactation	3 <sup>rd</sup> lactation	3 <sup>rd</sup> lactation
Milk in kg	13.6 (41.2)	29.1 (65.5)	204.2* (81.8)	64.8 (96.3)
Fat in %	0.024 (0.019)	0.015 (.028)	0.031 (0.037)	0.006 (0.042)
Fat in kg	0.68 (1.62)	1.56 (2.66)	6.93° (3.66)	2.63 (4.17)
Protein in %	0.005 (0.008)	0.004 (0.012)	0.009 (0.015)	0.019 (0.018)
Protein in kg	0.58 (1.26)	0.42 (2.04)	6.62* (2.58)	1.96 (3.06)

ANOVA- and MTDFREML-analysis failed to show any significant effects of nucleotide position 169 polymorphism on milk production traits for first and second lactation. For third lactation records of milk yield, fat-kg and protein-kg the mtDNA-polymorphism showed significant effects ( $p < 5\%$ ). However, these significant effects disappeared when the data set was reduced to the five largest herds with a more even distribution of animals with each mtDNA variant. The estimated effects of the nucleotide position 169 on all traits investigated by ANOVA- and MTDFREML-analysis are summarised in tables 2 and 3, respectively. All

estimates from ANOVA- and MTDFREML-analysis show very similar results. Only the estimates for the second lactation are smaller when derived from the model including the additive genetic effects. The results for the reduced data set (only 5 herds) for the third lactation are in between the estimates from all herds and those for the first or second lactation.

**Table 3. Estimated effect of adenin/guanin-transition at nucleotide position 169 of the mitochondrial genome on milk production traits from MTDFREML-analysis (standard errors of estimates in brackets) (\* =  $p < 5\%$  ° =  $p < 10\%$ )**

Herds	19	19	19	5
Trait	1 <sup>st</sup> lactation	2 <sup>nd</sup> lactation	3 <sup>rd</sup> lactation	3 <sup>rd</sup> lactation
Milk in kg	12.3 (42.8)	11.6 (67.0)	208.8* (87.1)	74.0 (100.6)
Fat in %	0.025 (.020)	0.014 (0.029)	0.036 (0.039)	0.005 (0.044)
Fat in kg	0.67 (1.69)	0.90 (2.76)	6.74° (3.85)	3.18 (4.34)
Protein in %	0.008 (0.009)	0.002 (0.013)	0.009 (0.016)	0.018 (0.019)
Protein in kg	0.27 (1.31)	0.17 (2.11)	6.70* (2.74)	2.35 (3.17)

## DISCUSSION

It can be assumed that results obtained in the present study are more likely to reflect the direct effects of nucleotide position 169 on milk production traits than the data published by Schutz *et al.* (1993) which were derived from a maternal lineage analysis. The large differences of 0.16 % in fat content and 39 kg of fat yield between the two nucleotide position 169 variants found by Schutz *et al.* (1993) are not confirmed in the present study. The differing results could be explained by other mtDNA polymorphisms specific to the mtDNA lineages studied by Schutz *et al.* (1993) and linked by chance to specific nucleotide position 169 variants. The results by Schutz *et al.* (1993) were derived from data of an experimental herd with animals kept under very similar conditions, while our data involved field records. It is also known that the mutation rate for mitochondrial DNA, especially the non coding control region, is much higher than for nuclear DNA (Brown *et al.*, 1979). The significant effects of nucleotide position 169 on fat and protein kg of the third lactation in the present study is mainly caused by higher milk yield and not a higher fat or protein content. The relatively large effect of nucleotide position 169 on milk yield in the third lactation is an effect of the low number of animals in 14 (11 herds with less than 20 animals) of 19 herds analysed in total. This leads to considerable differences in frequencies for the position 169 A and G variants between herds, resulting in a partial confounding of herd effect and effect of nucleotide position 169. Results obtained with the reduced material which contains only the 5 largest herds show much lower estimates, although they are still higher than estimates from first or second lactation.

The results of a further analysis with a larger data set comprising additional dams and grand-dams which had not been typed, but were assumed to have the same mtDNA variant at nucleotide position 169 as their typed daughters, did not differ from initial results. The data set

was thereby expanded to n=3927 for first and n=2909 for second lactation records. For the third lactation, the number of animals increased from 757 to 2087 for all herds and from 543 to 1611 for the 5 largest herds. Estimates for milk yield, fat and protein kg of effects of nucleotide position 169 was reduced to 36.6, 2.73 and 2.75 kg, respectively. For the 5 largest herds these estimates further declined to 12.9 kg for milk yield, 1.42 for fat kg and 1.40 for protein kg. These estimates are very similar to estimates for first and second lactation records and indicate a lack of evidence for mtDNA effects on third lactation traits. This suggests that initial results for third lactation records are overestimated because of structural problems in the data set.

## CONCLUSION

Contrary to previous observations, effects of mitochondrial DNA nucleotide position 169 polymorphism on yield traits were not significant in German Holstein cattle.

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