Czech Academy of Agricultural Sciences

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Czech Journal of Animal Science

High resolution melting as an alternative method to genotype diacylglycerol O-acyltransferase 1 (*DGAT1*) *K232A* polymorphism in cattle

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Czech J. Anim. Sci., 56 (2011): 370-376

[fulltext]

PCR-RFLP analysis is a common metho for genotyping the *DGAT1 K232A* polymorphism in cattle. Our purpose was to develop a high resolution melting (HRM) assay in order to genotype the

polymorphic alleles. Firstly, the PCR-RFLP method was used and the 411 bp products including the *DGAT1* polymorphism were digested by *Cfr*I enzyme. Direct sequencing was performed to confirm genotypes of the *K232A* polymorphism for 30 samples tha presented different PCR-RFLP patterns. was determined according to sequencing results that partial enzyme digestion had occurred for some samples. A 130 bp fragment including the polymorphism wa amplified for real time PCR. Then, the HRM analysis was carried out using two fluorescent dyes, SYBR Green I and EvaGreenTM. Although the HRM genotyping using SYBR Green I was contradicted by the sequencing results, three correct melting curves were obtained for the *K232A* polymorphism when EvaGreenTM was used. There were no false genotypes and all genotypes were in agreement with their sequencing results. The difference in the T_m between the two homozygous groups was about 0.5°C and the *AA* genotypes showed a higher T_m than the *KK* genotypes. The heterozygous genotypes

were obtained from different concentrations of EvaGreenTM in the reactions. All 206 DNA samples were genotyped using this fluorescent dye with estimated allele frequencies of 0.66 and 0.34 for the *A* and *K* alleles, respectively.