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Technical note

Development of real-time PCR assay for genotyping SNP rs41255693 in cattle *SCD* gene

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Abstract: Increased content of monounsaturated fatty acids with low melting points in cattle milk and meat known to strongly affect their taste and nutritional value. Steroyl-CoA-desaturase (SCD) is a key enzyme in the biosynthesis of monounsaturated fatty acids. The gene encoding SCD in cattle is located on chromosome 26 and consists of 6 exons and 5 introns and is expressed in the cells of cow udder, adipose, muscles and other tissues. To date, several SNPs in cattle *SCD* gene have been found. For SNP rs41255693 (g.10329T > C), a correlation was shown between the content of monounsaturated fatty acids in cattle milk/meat and milk productivity. This article describes the development of a real-time PCR assay for genotyping SNP rs41255693 in cattle *SCD* gene with allele-specific TaqMan probes. The use of this assay allows to significantly reduce the time needed for cattle genotyping compared to the commonly used PCR-RFLP method.

Keywords: SCD; rs41255693; cattle; genotype; real-time PCR

1. Introduction

The content of fatty acids in milk and meat of dairy cattle strongly affects their taste and nutritional value. Fatty acids with low melting points are preferable [1,2].

Steroyl-CoA-desaturase (SCD) catalyzes the formation of a double bond in the cis-configuration between C9 and C10 carbon atoms in a number of fatty acids containing 12 to 19 carbon atoms [3–6]. In animals, SCD enzyme catalyzes desaturation of palmitic and stearic acids, which results in the formation of monounsaturated palmitoleic and oleic acids [6,7].

In cattle, the *SCD* gene is located in the 26q21 region [8]. The bovine *SCD* gene has a length of about 17 kb and includes six exons and five introns. The open reading frame consists of 1080 nucleotides encoding a 359-amino acid protein [9]. The udder and adipose tissue were shown to have a single *SCD* transcript of about 5 kb, which indicates an unusually long 3'-noncoding region [8,10].

Percentages of fatty acids in milk and meat were found to differ in various breeds that were on the same diet [11–13]. Associations of *SCD* gene polymorphisms with the composition of fatty acids have been demonstrated in many studies, e.g. [14–17]. A correlation has also been shown between the composition of fatty acids and the reproductive capacity of dairy cattle [18].

The SNP rs41255693 (c.878C > T, p.Ala293Val.) in the *SCD* gene results in an alanine (Ala) replacement with valine (Val). Fatty acids in animals with Ala/Ala genotype are characterized by lower melting temperature due to the higher content of oleic acid [19,20].

Despite the availability of significant data regarding the effects of the SNP rs41255693 on the fatty acid composition of cattle milk and meat, no effective, easy-to-use assay for genotyping SNP rs41255693 has been developed so far. Current patents connect the gene alleles with the fatty acid composition of meat using PCR-RFLP (patents JP3619833B2 and KR20070066332), which is neither easy nor cost-effective.

The goal of the study was to develop a method for detection rs41255693 SNP in cattle *SCD* gene using real-time PCR with allele-specific TaqMan probes.

2. Materials and methods

2.1. DNA isolation and oligonucleotide design

DNA was isolated from 89 whole blood samples obtained from black-and-white Holstein-cross cattle using an M-sorb kit (Syntol, Russia).

Nucleotide sequences of the primers and probes were designed using GeneRunner (http://www.generunner.net/) and Multiple primer analyzer software (https://www.thermofisher.com/). The primers and probes were synthesized by DNA-synthesis LLC (Moscow, Russia).

2.2. Real-time PCR

The amplification reaction was carried out in 10 μL of the PCR mixture containing 5 μL of LightCycler® 480 Probes Master (Roche, Switzerland), 1 μM of each the forward primer SCD-F: 5'-CCCTTATGACAAGACCATCAACC-3' and the reverse primer SCD-R: 5'-GACGTGGTCTTGCTGTGGACT-3' (10 μM), 0.4 μM of allele-specific probes SCD-T: 5'-FAM-CTTACCCACAGCTCCCA-BHQ1-3' and SCD-C: 5'-VIC-TACCCGCAGCTCCC-3-BHQ1, 10 ng of DNA. PCR was carried out using a LightCycler® 96 System (Roche, Switzerland) under optimized conditions (preliminary denaturation 95 ℃, 10 min; 40 cycles: 95 ℃, 20 sec; 55 ℃, 30 sec; 72 ℃, 20 sec). Fluorescence detection was carried out at the elongation stage using FAM and VIC channels. The software supplied with the LightCycler® 96, version SW1.1 amplifier (Roche, Switzerland) was used for the data analysis.

2.3. Method validation—PCR-RFLP

Validation was performed based on PCR-RFLP assay [21]. For PCR, we used 0.2 μM of the primers SCD-293R1: 5'-GGAAGAGAACAGCCCAAAGG-3' and SCD-293F1: 5'-GCCACCTTATTCCGTTATGCCC-3', Phusion Hot Start II High-Fidelity DNA polymerase (Thermo Scientific, USA) test kit (according to the manufacturer's instructions), and 30 ng of genomic DNA. PCR was carried out using T100 amplifier (Bio-Rad, USA) under the following conditions: preliminary denaturation at 98 ℃ for 30 sec, 40 amplification cycles: 98 ℃, 10 sec; 60 ℃, 20 sec; 72 ℃, 30 sec. Restriction was carried out in 15 μL of a mixture containing 1.5 μL of 10x buffer, 1 U of Fau I endonuclease (Sibenzyme, Russia) and the amplicons obtained for 16 hours at 55 ℃. Restriction results were assessed using 1.2% agarose gel electrophoresis.

3. Results and discussion

To date, one of the most common methods for identification of allelic variants of genes is PCR-RFLP analysis. The PCR-RFLP is a multistep method consisting of i) a genomic DNA isolation; ii) amplification of the polymorphic region of a gene; iii) restriction of the obtained amplicon by the endonuclease (restrictase); iv) electrophoretic separation and analysis of the obtained DNA fragments. The main disadvantages of the PCR-RFLP method include long duration, labor requirements and low productivity of the analysis, as well as unreliable results in cases of non-optimal DNA : restriction enzyme ration and duration of the restriction reaction [22,23].

In this study, we developed a real-time PCR assay for detection of allelic variants of the SNP rs41255693 in cattle *SCD* gene. We used two primers that were common for both *SCD* gene alleles and two allele-specific TaqMan probes labeled with FAM and VIC dyes. Using SCD-D and SCD-R primers, a 90 bp fragment of the *SCD* gene is amplified. Identification of T and C alleles of SNP rs41255693 substitution is based on comparison of fluorescence intensity of FAM and VIC dyes, respectively. The genotyping assay was performed using software supplied with LightCycler® 96 amplifier (version SW1.1) (Figure 1).

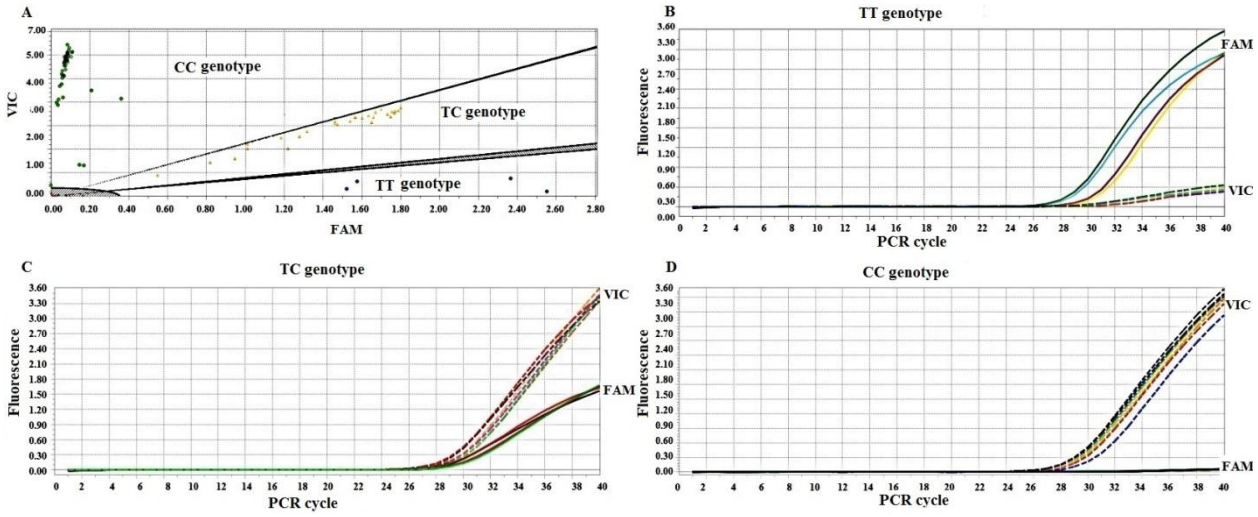

Figure 1. An example of detection of T and C alleles of cattle SNP rs41255693 in the *SCD* gene using real-time PCR. Fluorescence decay curves (B–D) and genotype distribution (A) are presented.

For cows with the TT genotype, there was an increase in the fluorescent signal in the FAM channel (Figure 1B). For cows with the CC genotype, the fluorescent signal was registered in the VIC channel (Figure 1D). For heterozygous cows (TC genotype), the signals were detected in both FAM and VIC channels (Figure 1C). Thus, the findings of real-time PCR using allele-specific TaqMan probes demonstrate the presence of each allele of the SNP rs41255693 in the DNA sample tested and, therefore, shows the animal's genotype.

Our real-time PCR assay was validated in 89 DNA samples obtained from black-and-white Holstein-cross cattle. The results of genotyping showed that 33.7% of animals had both alleles (CT genotype), 2.2% of cows were homozygous for the T allele (TT genotype) and 64.1% were homozygous for the C allele (CC genotype). Thus, the frequency of the T allele associated with the presence of fats with low melting points in milk and meat was 19% in the cattle population studied.

The real-time PCR assay developed by us was validated with PCR-RFLP analysis as previously described [21]. The results of both methods of genotyping coincided; however, our method helps significantly (up to 1 h) reduce the time needed for the assay in comparison with PCR-RFLP (18 hours). Also, the PCR-RFLP method requires the use of expensive restriction enzyme and gel electrophoresis, which increases the cost of its use. Other methods for identifying the genotype of one substitution (such as Sanger sequencing, pyrosequencing, allele-specific PCR, high-resolution melting, etc.) require more time and financial investment.

4. Conclusions

We have developed an effective and reliable real-time PCR assay using allele-specific fluorescently-labeled TaqMan probes for genotyping SNP rs41255693 in cattle *SCD* gene. This assay allows to carry out genotyping of a large number of animals (depending on the amplifier model) in a short time using a minimal number of laboratory procedures and, therefore, may be used for the selection of cows with high contents of fats with low melting points (linoleic acid) in milk and meat.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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