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POLYMORPHISMS IN CANDIDATE GENES FOR BEEF QUALITY IN PINZGAU CATTLE

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ABSTRACT

The aim of present study was to identify the polymorphisms in genes encoding calpastatin (*CASTUoG*), calpain (*CAPN1*, *CAPN2*), diacylglycerol O-acyltransferase (*DGAT1*), thyroglobulin (*TG5*), and Stearoyl-CoA Desaturase (*SCD*) in order to analyse genetic structure of Pinzgau cattle. The genomic DNA for genotyping was obtained from in total 56 blood samples of Pinzgau bulls. After extraction, the concentration of DNA was controlled by the spectrophotometry measurement. The genotyping of each individual was carried out by using PCR-RFLP methods. The average value of observed (0.37±0.05) and expected heterozygosity (0.39±0.06) clearly indicated the prevalence of homozygous individuals. Observed Wright's fixation indexes showed positive values across all loci (0.03±0.06), which confirmed slight deficiency of heterozygote animals compared to the Hardy-Weinberg equilibrium expectations. The Hardy-Weinberg equilibrium was found in population, which signalizes only slight impact of factors such as selection, migration or inbreeding. The effectiveness of loci allele impact in populations has been described also by effective allele numbers (1.68±0.13) that expressed the decrease of allele activity in population. The loss of heterozygosity in analysed population was found across all of genetic markers. Each of the evaluated indicators clearly points to the need of genetic diversity monitoring. Moreover, the analyses of single nucleotide polymorphisms in genes significantly involved in control of economically important production traits are still very usable methods for identification of genetic markers that can be used in marker assisted selection of cattle.

Keywords: *cattle, genetic markers, meat quality, SNPs genotyping.*

INTRODUCTION

Improvement for carcass traits related to beef quality is the key concern in beef production. The application of traditional selection to these traits is difficult, since they are expensive and difficult to measure because it requires the slaughter of the animals. Along with traditional selection, marker assisted selection can help

improve economically important traits earlier in the breeding cycle. Knowledge of genetic variation and the search for candidate genes or genomic segments that influence production traits in the population of interest are essential for the establishment of a molecular criteria for selection (Tizioto et al., 2012; Magalhães et al., 2016).

Carcass traits related to beef quality are normally controlled or regulated by a number of genes and single nucleotide polymorphisms (SNPs) in the genes may be significant markers for improved cattle performance. Beef quality and carcass traits usually have low or moderate heritability and are often recorded post-slaughter, therefore, SNPs can be used as markers for indirectly improving beef quality traits instead of direct measurements (Liu et al., 2015). Development of molecular-genetic tools allow the identification of single nucleotide polymorphisms associated with large-effect genes that influence these traits, providing a better biological understanding of the trait and a list of candidate genes for fine mapping (Magalhães et al., 2016).

The meat tenderness is one of the most important beef traits mainly with respect to the consumer satisfaction. However, tenderness is a complex trait for breeding programs, because evaluation also depends on how animals are slaughtered. Thus, molecular marker information can be of great usefulness for identification of animals with high genetic value for tenderness and the selection process can be conducted on younger animals, even before birth (Pinto et al., 2010). Up to now, several genetic markers associated with differences in beef tenderness have been reported. These markers target two genes corresponding to the most important proteolytic system of skeletal muscle, the calcium-activated neutral protease gene (*CAPN1*) encoding the large subunit of μ-calpain and the calpastatin gene (*CAST*) encoding a specific inhibitor of the calpains (Corva et al., 2007). Moreover, the causative mutations in the *CAPN* and *CAST* genes have been shown to affect significantly not only beef tenderness but also marbling score (Casas et al., 2006; Morris et al., 2006; Lisa and Di Stasio, 2009; Pinto et al., 2010).

The key parameter of beef nutritional quality is intramuscular fat content (IMF). Nowadays people become more and more aware of what they eat and there is more interest in meat containing less fat. However, it may be at the expense of flavour and tenderness. It was shown that both flavour and tenderness scores markedly increased with increasing IMF content (Thomson, 2004). Previous studies have indicated that single nucleotide polymorphisms in the diacylglycerol-O-acyltransferase1 (*DGAT1*), thyroglobulin (*TG*) and Stearoyl-CoA desaturase (*SCD*) genes are significantly associated with intramuscular fat levels as well as marbling scores in beef (Pannier et al., 2010; Wu et al., 2012; Zhang et al., 2015).

The objective of our study was to genotype the single nucleotide polymorphisms in six genes previously reported as candidate for beef quality in order to determine the genetic structure of Pinzgau bulls population. The Pinzgau cattle is one of the most important dual-purpose cattle in Slovakia bred mainly in mountain regions of northern Slovakia due to its excellent longevity, fertility, health, and grazing ability.

MATERIALS AND METHODS

The biological samples were collected from in total of 56 Slovak Pinzgau bulls. The genomic DNA for genotyping was extracted from blood samples according to protocol of Miller et al. (1988). Subsequently, the concentration and purity of genomic DNA were tested based on the spectrophotometry measurements by the optical density at wave length of 260 nm. The single nucleotide polymorphisms in six genes encoding calpastatin (*CASTUoG*), calpain (*CAPN1*, *CAPN2*), diacylglycerol O-acyltransferase (*DGAT1*), thyroglobulin (*TG5*), and Stearoyl-CoA Desaturase (*SCD*) were analysed according to Gábor (2009) using RFLP methods. The products of PCR reaction and restriction fragments have been separated and visualised using horizontal electrophoresis in 2 % agarose gels (130 V for 50 min) and stained with day GelRed. The genetic structure of analysed population as well as population genetic indices have been analysed by using Genalex version 6.1 (Peakall and Smouse, 2012). The significance of differences between observed and expected genotype frequencies were tested by Chi-square test to assess the deviation from Hardy-Weinberg equilibrium. The genetic diversity indices derived from the frequency of alleles including observed (H_o) and expected heterozygosity (H_e), effective allele number (N_e), Shannon's information index (I) and molecular inbreeding coefficient noted as Wright's fixation index (F_{IS}) have been calculated using Genalex version 6.1 (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

In analysed population of Slovak Pinzgau bulls each of selected loci was successfully genotyped. Except SNP in *DGAT1* gene, for all loci have been identified three genotyped. Only two genotype (AA and AK) was identified in case of *DGAT1* gene, the KK genotype was not observed in analysed population. The distribution of allele frequency within each locus is shown on Figure 1. The frequency of genotypes as well as the level of genetic diversity within population derived from commonly used population genetic indices are listed in Table 1. For SNPs in *CASTUoG*, *DGAT1*, and *SCD* genes the predominance of homozygote genotypes was found, whereas for *CAPN1*, *CAPN2*, and *TG5* the prevalence of heterozygote animals was detected. The significant differences between observed and expected genotype frequencies was found only for SNP in *CAPN1* gene ($P < 0.05$). Overall, the observed Hardy-Weinberg equilibrium across loci signalize only slight impact of factors such as selection, migration or inbreeding in analysed population. But, the average value of observed (0.37±0.05) and expected heterozygosity (0.39±0.06) signalized the decrease of genetic variability due to the increase of population homozygosity. Similarly, observed Wright's fixation indexes showed in average positive value (0.03±0.06), which confirmed slight deficiency of heterozygote animals compared to the Hardy-Weinberg equilibrium expectations. The effectiveness of loci allele impact in populations has been expressed also by the effective allele numbers. Comparison of N_e showed higher effective allele numbers across populations for *CAPN2* gene and indicated good level of genetic variability in analysed population at the considered locus.

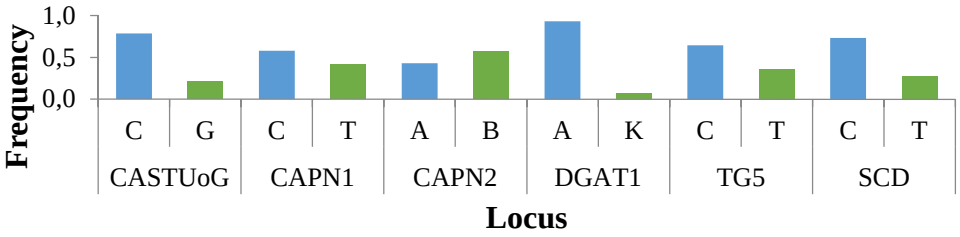

Figure 1. Frequency of alleles for each analysed locus in Pinzgau bulls population

Table 1. Summary of genetic structure and diversity indices evaluated in population

Locus	Genotypes frequency			Alleles frequency		χ^2 test	H_o	H_e	N_e	I	F_{IS}
CASTUoG	**CC** 34.571	**CG** 18.857	**GG** 2.571	**C** 0.786	**G** 0.214	ns	0.321	0.337	1.508	0.520	0.045
CAPN1	**CC** 18.862	**CT** 27.277	**TT** 9.862	**C** 0.580	**T** 0.420	*	0.339	0.487	1.950	0.680	0.303
CAPN2	**AA** 10.286	**AB** 27.429	**BB** 18.286	**A** 0.429	**B** 0.571	ns	0.500	0.490	1.960	0.683	-0.021
DGAT1	**AA** 48.286	**AK** 7.429	**KK** 0.286	**A** 0.929	**K** 0.071	ns	0.143	0.133	1.153	0.257	-0.077
TG5	**CC** 23.143	**CT** 25.714	**TT** 7.143	**C** 0.643	**T** 0.357	ns	0.500	0.459	1.849	0.652	-0.089
SCD	**CC** 30.018	**CT** 21.964	**TT** 4.018	**C** 0.732	**T** 0.268	ns	0.393	0.392	1.645	0.581	-0.002

ns – not significant; * $P < 0.05$

The obtained knowledge about genetic structure of analysed population can be used in the future for selection of animals with favourable genotypes to increase mainly economic important traits associated with muscle formation and level of intramuscular fat. The results are very perspective for breeders that use analysed bulls in mating programs. The *CAST* and *CAPN1* genes belong to the important genetic markers for beef quality. The calpastatin proteolytic axis has been identified as an important process to established meat tenderness. The μ-calpain (*CAPN1*) is a component of the calpastatin proteolytic axis (Casas and Kehrli, 2016). An association between meat tenderness and both genetic markers in the calpastatin and calpain genes has been confirmed in various studies (e.g. Casas et al., 2006; Barendse et al., 2007). The *DGAT1* gene genetic variants was primarily associated with milk production (Thaller et al., 2003), but several later studies reported the association between SNPs in *DGAT1* gene and marbling and fat thickness (Wu et al., 2012; Tait et al., 2014). Like the *DGAT1* gene, the genes encoding thyroglobulin and stearoyl-CoA desaturase are considered to be a perspective genetic marker for intramuscular fat levels as well as marbling scores in beef (Wu et al., 2012; Zhang et al., 2015; Bennett et al., 2013).

CONCLUSIONS

The loss of heterozygosity in analysed population was found across all of genetic markers. Each of the evaluated indicators clearly points to the need of genetic diversity monitoring. The analyses of single nucleotide polymorphisms in genes significantly involved in control of economically important production traits are still very usable methods for identification of genetic markers that can be used in marker assisted selection of cattle.

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