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Short communication

Effect of leptin, DGAT1 and TG gene polymorphisms on the intramuscular fat of Angus cattle in Hungary

I. Anton [a], K. Kovács [a], G. Holló [b], V. Farkas [c], L. Lehel [a], Z. Hajda [a], A. Zsolnai [a,b,*]

[a] Research Institute for Animal Breeding and Nutrition, Gesztenyés u. 1., 2053 Herceghalom, Hungary
[b] University of Kaposvár, Guba Sándor u. 40., 7400 Kaposvár, Hungary
[c] University of Pannonia, Georgikon Faculty of Agriculture, Deák F. u. 16., 8360 Keszthely, Hungary

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ABSTRACT

The objective of this study was to estimate the effect of leptin, thyroglobulin (TG) and acylCoA-diacylglycerol-acyltransferase 1 (DGAT1) loci and linoleic acid supplemented diet on the marbling of meat in the Hungarian Angus population. All genotypes were determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) assay. At leptin and TG loci TT bulls showed the highest fat percentage values in the *musculus longissimus dorsi* (*LD*) and *musculus semitendinosus* (*ST*), the difference between CC and TT genotypes was significant ($p<0.05$). DGAT1 AA/AA bulls had significantly higher ($p<0.05$) intramuscular fat content values than other genotypes. The sunflower supplemented group of bulls presented significantly higher ($p<0.05$) fat percentage values for LD, than the control group.

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1. Introduction

Intramuscular fat content, also known as marbling of meat, represents a valuable beef quality trait. Leptin is the hormone product of the obese gene synthesized and secreted predominantly by white adipocytes. This protein is supposed to be involved in the regulation of body weight by transmission of a lipostatic signal from adipocytes to the leptin receptor in hypothalamus resulting in appetite suppression and increased thermogenesis (Zhang et al., 1994; Ji et al., 1998). The leptin gene has been mapped to bovine chromosome 4 (Stone et al., 1996). Polymorphisms in the leptin gene have been associated with serum leptin concentration, feed intake, milk yield (Liefers et al., 2002) and body fatness (Buchanan et al., 2002; Nkrumah et al., 2004).

Animals with TT genotype of a cytosine/thymine (C/T) substitution detected at position 528 in the bovine leptin promoter region (GenBank accession no. AB070368) showed 13% and 9% increase in marbling score compared with CC and CT genotypes, respectively (Nkrumah et al., 2005).

The polymorphism in the 5′-untranslated region of TG gene – which product is the precursor of hormones that influence lipid metabolism – affects intramuscular fat content in cattle (Barendse, 1999). TG mainly affects the fat content of LD (Thaller et al., 2003).

A lysine/alanine (K232A) polymorphism in DGAT1 – a microsomal enzyme that catalyzes the final step of triglyceride synthesis – has been shown to affect intramuscular fat of ST and milk fat content (Grisart et al., 2002; Winter et al., 2002; Thaller et al., 2003).

DGAT1 and TG genes physically map to the centromeric region of bovine chromosome 14 (Coppieters et al., 1998; Winter et al., 2002). DGAT1 and TG genes are separated by about (Thaller et al., 2003; Moore et al., 2003).

Human health aspects have been highlighted in animal production worldwide. Conjugated linoleic acids (CLA)

* Corresponding author. Research Institute for Animal Breeding and Nutrition, Gesztenyés u. 1., 2053 Herceghalom, Hungary. Tel./fax: +36 23 319133.
E-mail address: attila.zsolnai@gmail.com (A. Zsolnai).

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present in meat and milk from ruminant animals, have potential health benefits, is likely that exert inhibitory properties in carcinogenesis (Belury, 2002). CLA are formed through isomerization of linoleic acid by ruminal bacteria and it may be possible to increase the content of CLA in fat and muscle from beef animals through increased dietary availability of the substrate, linoleic acid (Hristov et al., 2004). Most studies attribute the beneficial effects associated to the consumption of CLA to the reduction of risk factors for the development of cancer and cardiovascular diseases, such as the reduction of plasmatic triacylglycerols and cholesterol (Funck et al., 2006).

It was found that dietary supplementation of sunflower seed in cattle increases the CLA content in milk and intramuscular fat which is supposed to reduce the risk of cardiovascular diseases (atherogenicity and thrombogenicity) in humans (Hernández et al., 2007).

The objective of this study was to estimate the effect of leptin, TG and DGAT1 loci and of the linoleic acid supplemented diet on the marbling of meat in the Hungarian Angus population. Since the results for the possible use of the mentioned polymorphisms in selection to improve beef quality traits are few in number and rather contradictory, it has been decided to carry out studies in the existing Hungarian Angus population with the aim to provide additional data to this particular subject.

2. Materials and methods

173 blood samples from private herds were collected. The availability of the studied alleles and their frequencies in the randomly selected Red Angus bulls have been checked by PCR-RFLP assays (see Statistical analysis section as well).

Blood samples were stored at −20 °C until DNA extraction. Genomic DNA was isolated from whole blood (Zsolnai et al., 2003).

Primer design for the leptin promoter polymorphism (C/T substitution at position 528 according to GeneBank accession no. AB070368) was based on the 5′ nuclease assay by Nkrumah et al. (2004). The forward and reverse primers were: 5′-CAT TGC GTG CAA GCT TCT CAC T-3′ and 5′-$(T)_{24}$CGA GCC CAA GCT CCA GAG CCT-3′, respectively. The underlined base (position 536) in reverse primer was introduced to facilitate the specific cleavage of the C allele by *AlwNI* restriction endonuclease. T allele was not cut by *AlwNI*. The lengths of diagnostic fragments were 130 bp (T allele), 96 and 34 bp (C allele). TG polymorphism was detected using the method described by Barendse (1999). At DGAT1 locus, the forward and reverse primers were: 5′-$(T)_{30}$CGC TTG CTC GTA GCT TTG G-3′ and 5′-CAC CGC GGT AGG TCA GGT TGT C-3′ respectively, generating 121 bp PCR products. *Cfr1* restriction endonuclease (Winter et al., 2002) was used to differentiate the alanine (GCG) and lysine (AAG) encoding codons (SNPs are located in position 10433 and 10434 of the sequence under GenBank accession no. AJ318490). The lengths of diagnostic fragments were 121 bp (GC allele), 62 and 59 bp (AA allele). Poly T tails were incorporated in primers above, in order to improve the resolution of digested fragments. Digested PCR products were separated in 4% Meta-Phor agarose gel (Rockland, ME, USA) in 1× TBE buffer and stained with ethidium bromide.

Bulls were randomly allocated to two groups (control and sunflower supplemented) contemporary test groups consisting of 86 and 87 bulls, respectively. The experimental group of bulls was originated from 56 different sires. Animals were kept in identical conditions and fed a backgrounding diet of sugar beet silage (33%), grain silage (32%), extracted rape (3%), wet corn (28%) and grain (4%). After reaching 550 kg liveweight at approximately similar age, this diet was supplemented with 1 kg sunflower seed per animal for 90 days only for bulls belonging to the sunflower supplemented group.

Following slaughter, beef quality trait data were collected. Statistical analyses were carried out to find association between the individual genotypes and intramuscular fat deposition. After removing surface fat, lipid content of LD and ST were determined gravimetrically by the Soxhlet method, using petroleum ether as solvent. The analyses were executed in three repetitions and standard error of the mean values in repetitions was under 5%.

2.1. Statistical analysis

Allele frequencies were calculated and deviations from Hardy–Weinberg equilibrium were checked by chi-square test.

The dataset was analyzed with the SPSS 15.0 for Windows OS. Multivariate analysis of variance (general linear model, GLM) was applied to determine differences in beef characteristics in case of all polymorphisms, where genotype and sunflower supplemented diet were included as fixed effects and fat percentage of LD and ST as dependent variable.

The formula of General Linear Model included the following:

$$y_{ijkl} = \mu + Lep_i + TG_j + DGAT_k + TG_j{}^{*}DGAT_k + diet_l + e_{ijkl}$$

Additional models were also tested such as:

$$y_{ij} = \mu + Lep_i + diet_l + Lep_i{}^{*}diet_l + e_{il}$$
$$y_{ij} = \mu + TG_i + diet_l + TG_i{}^{*}diet_l + e_{il}$$
$$y_{ij} = \mu + DGAT_i + diet_l + DGAT_i{}^{*}diet_l + e_{il}$$

where y is the phenotypic record of the studied traits (e.g. fat % of LD or ST), μ is the general mean, *Lep* is the leptin hormone genotype (CC, TC, TT), *TG* refers to the TG polymorphism (CC, TC, TT), *DGAT* represents the effect of DGAT1 genotypes (AA/AA, AA/GC, GC/GC), *diet* indicates the impact of the sunflower supplemented diet and e is the residual error.

Dominance effects were estimated as the deviation of mean values of the studied traits in heterozygotes from the mean of homozygotes, using the least square means. Additive effect was calculated as the half of the difference between the two homozygotes. Significant level of these factors was detected by the method of LSD (least square difference).

3. Results and discussion

Concerning the herd genetic structure analyses, differences between the observed and expected genotype frequency values were not significant (Table 1). The calculated χ^2

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