

Brief report

Effect of thyroglobulin gene polymorphism on the intramuscular fat content in cattle examined by x-ray computed tomography and Soxhlet methods

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Background

Intramuscular fat content or marbling represents a valuable beef quality trait and is an important determinant of palatability due to its contribution to juiciness and flavour. Meat which has little marbling may be dry and flavourless. Higher levels of intramuscular fat have been associated with increased tenderness, juiciness and flavour of beef (Thompson 2004). Whereas this feature is highly appreciated in some markets (by example Australia, China, Korea, Japan) breeders usually try to increase intramuscular fat deposition in cattle by nutrition. However, genetic factors have been shown to affect marbling in cattle. Thyroglobulin (TG) is a glycoprotein hormone synthesised in the thyroid gland and is the precursor of triiodothyronine (T3) and thyroxine (T4) hormones which influence lipid metabolism. T3 and T4 hormones have been associated with marbling in Wagyu cattle (Mears *et al.* 2001). The effect of a C/T single nucleotide polymorphism in the 5'-untranslated region of TG gene has been concluded to affect intramuscular fat content in cattle (Barendse 1999). High probability of positive association between the T allele of bovine TG gene and marbling was observed by Wood *et al.* (2006). Thyroglobulin mainly affects the fat content of *longissimus dorsi* muscle (LD) (Thaller *et al.* 2003).

X-ray computed tomography (CT) has been utilised in cattle breeding mainly for the estimation of entire carcass composition based on the CT measured tissue composition of three-ribs-joint (Holló *et al.* 2007). The non-invasive CT technique offers the possibility to analyse the whole muscle composition and to determine detailed marbling characteristics of muscles such as area, volume and distribution. The intramuscular fat level measured by CT is generally lower compared to values of the chemical percentage method, but the correlation between mentioned methods is significant (Holló *et al.* 2012).

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The objective of this study was to estimate the effect of the TG locus on the intramuscular fat content of meat in some cattle breeds in Hungary. Since the use of TG polymorphism in selection to increase intramuscular fat content in cattle is available in Hungary, it has been decided to confirm the obtained results by chemical percentage intramuscular fat (Soxhlet method) and CT as well.

Abbreviations: AX: Angus crossbred, CT: computed tomography, DNA: deoxyribonucleic acid, HH: Hungarian Holstein purebred, HS: Hungarian Simmental purebred, LD: *longissimus dorsi* muscle, PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism, T3: triiodothyronine, T4: thyroxine, TBE buffer: tris-borate-EDTA buffer, TG: thyroglobulin

Procedures

Altogether, 120 bulls of different genotypes [Angus crossbred (AX, n=40), Hungarian Holstein purebred (HH, n=40) and Hungarian Simmental purebred (HS, n=40)] were selected, kept in identical conditions and fed the same diet until reaching the age of 16-18 month. Animals were slaughtered at similar live weight (AX: 598.50+24.07 kg; HH: 569.65+21.83 kg; HS: 599.83+17.49 kg) under commercial conditions using Hungarian standard procedure. Blood samples have been collected during exsanguinations and were stored at -20 °C until DNA extraction. Genomic DNA was isolated from the whole blood (Zsolnai *et al.* 2003). Thyroglobulin polymorphism was detected by PCR-RFLP assay using the method described by Barendse (1999). Digested PCR products were separated in 4% Meta-Phor agarose gel (Rockland, ME, USA) in 1×TBE buffer and stained with ethidium bromide. About 10% of the samples were genotyped in duplicate and repeatability was in all cases perfect. Slaughter traits were recorded prior to slaughter and on the slaughter line. After 24 h chilling, right carcasses have been jointed and dissected: lean meat, fat and bone were separated and weighted. Rib samples have been taken from LD cut between the 11-13th rib. Computed Tomography-analysis of ribs joint was performed by Siemens Somatom Emotion 6 equipment according to the method described by Holló *et al.* (2012). The intramuscular fat level of LD in the whole ribs joint was evaluated on standard CT thresholds for fat on Hounsfield scale (interval: -200 ± 20). After removing surface fat, the lipid content of LD was determined gravimetrically by Soxhlet method using petroleum ether as solvent. Dataset was analysed with the SPSS 15.0 for Windows software (SPSS Inc., Chicago, IL, USA). Multivariate analysis of variance (general linear model, GLM TYPE III.) was applied to determine differences in beef characteristics; breed and genotype were included as fixed effects and fat percentage of LD as dependent variable. So the formula of GLM was as follows:

$$y_{ij} = \mu + \text{breed}_i + TG_j + \text{breed}_i \times TG_j + e_{ij} \quad (1)$$

where y is the measured value of the studied traits (*e.g.* IM fat %), μ is the general mean, *breed* represents the crossbred (AX) and purebred (HH, HS) animals included in the study, *TG* refers to the the TG polymorphism (CC, TC, TT), and e is the residual error. The significance of differences was tested by Tuckey's Post Hoc test. Moreover, Pearson correlation coefficients were calculated between results obtained by CT and analytical methods.

Results and discussion

Least square mean values of carcass fat (manual dissection), ribs joint fat as well as intramuscular fat content of different TG genotypes obtained by CT examinations and Soxhlet method are presented in Table 1.

The effect of TG genotype on slaughter weight was not significant. TT genotype was found only in AX bulls. TT animals showed the highest fat percentage/intramuscular fat percent values and a decreasing tendency was observed from genotype TT to CC. In case of intramuscular fat percent, contrasting TT genotype vs. CC, the difference proved to be significant. Hungarian Holstein TC bulls had more fat in carcass, but lower fat percentage within LD compared to CC animals. Similar results were obtained by both conventional (dissection and chemical percentage) and CT methods. On the contrary, in HS breed CC bulls had lower intramuscular fat but higher fat percentage in carcass than TC animals. The coefficients of the correlation between dissected and CT fat percentage, as well as between the chemical percentage intramuscular fat and CT fat in LD, were 0.96 and 0.71 ($P < 0.001$), respectively. Among genotypes, the closest coefficient of correlation for fat percentage in carcass ($r = 0.98$) and for intramuscular fat level in LD ($r = 0.79$) was detected in AX bulls.

Table 1

Least square means (LSM) and standard errors (SEM) for fat content of different genotypes in different Hungarian cattle breeds

TG genotype (n=120)	Fat % in carcass (manual dissection)	Fat % ribs joint (%) (CT technique)	IM fat % (Soxhlet-method)	IM fat % (CT technique)
	LSM ± SEM			
CC _{AX} (n=26)	17.14 ± 0.85	20.09 ± 1.28	5.17 ± 0.68 ^a	2.02 ± 0.34 ^a
CC _{HH} (n=24)	6.74 ± 0.41	9.29 ± 0.39	2.11 ± 0.28	0.92 ± 0.08
CC _{HS} (n=29)	6.30 ± 0.39	8.62 ± 0.40	1.54 ± 0.16	0.61 ± 0.06
TC _{AX} (n=11)	18.90 ± 1.97	21.61 ± 2.95	6.48 ± 0.81	2.68 ± 0.66
TC _{HH} (n=16)	7.45 ± 0.44	9.89 ± 0.41	2.07 ± 0.32	0.76 ± 0.08
TC _{HS} (n=11)	5.58 ± 0.71	8.28 ± 0.84	1.71 ± 0.56	0.62 ± 0.15
TT _{AX} (n=3)	19.40 ± 1.10	22.23 ± 1.21	8.93 ± 0.90 ^b	3.15 ± 0.81 ^b

^{a,b}Means with different letters indicate significant differences between TG genotypes ($P < 0.05$).

The presented chemical percentage intramuscular fat values for the bulls with different TG genotypes are lower than those obtained by Thaller *et al.* (2003) in German Holstein and Charolais breeds and are moderately lower than our previous results in different Hungarian cattle breeds (Anton *et al.* 2008). Thaller *et al.* (2003) found significantly higher intramuscular fat values for TT genotypes compared to CC genotypes and supposed a recessive effect of the T allele on marbling. Barendse (1999) came to the same conclusion, although in both cases the number of animals included in the study was low. All studies confirm the extremely low frequency of the TT genotype against other genotypes. Obviously, a marker assisted selection approach – such as selecting for TT genotypes at the TG locus – might be carried out if higher intramuscular fat content is preferred. Gene tests can offer possibilities for direct selection among variants suited to adequate breeding programs based on economic reasons.

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