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EFFECT OF *TG* AND *DGAT1* POLYMORPHISMS ON BEEF CARCASS TRAITS AND FATTY ACID PROFILE

KELAVA NIKOLINA, KONJAČIĆ M and IVANKOVIĆ A

University of Zagreb, Faculty of Agriculture, Croatia

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The objectives of this study were to determine allele frequency and genotype count of thyroglobulin (TG) and diacylglycerol-0-acyltransferase-1 (DGAT1) genes encoding the TG and DGAT1 enzymes, and to determine the effect of TG and DGAT1 polymorphisms on fatty acid profile in beef carcass. All genotypes were determined by PC-RFLP assay. Polymorphism of TG and DGAT1 had no significant influence on total lipid content, backfat thickness, EUROP and carcass conformation score. The TT genotype of TG gene resulted in a lower total lipid content and backfat thickness, while KK genotype of DGAT1 gene showed a greater total lipid content and backfat thickness. The content of the majority fatty acids in MLD and subcutaneous fat tissue was not significantly affected by TG and DGAT1 polymorphism. The results confirm the lack of an association found by other studies. Further research should be carried out to validate the initial observation.

Key words: beef, DGAT1, fatty acids, lipids, TG

INTRODUCTION

Generally, beef meat is considered to be a valuable source of proteins, essential vitamins and minerals, and a major energy source (Williams, 2007). Beef carcass fat components, like the total lipid content, backfat thickness and fatty acid composition, affect beef meat quality and nutritive value. Although a source of n-3 and n-6 polyunsaturated fatty acids, beef meat is not regarded as a healthy component of the human diet (Garcia *et al.*, 2007). The dominant saturated fatty acids are considered to increase the risk of coronary heart disease and cancer, the leading diseases of the Western World. As beef quality and carcass traits are influenced by genetic and environmental factors, numerous researches have been done in order to change and improve the beef lipid profile predisposed by ruminal biohydrogenation and animal diet (Garcia *et al.*, 2007). Genetic researches suggested that the polymorphism of several candidate genes affected fat deposition and fat metabolism (Thaller *et al.*, 2003; Barendse, 1997).

Polymorphism of the thyroglobulin (*TG*) and diacylglycerol-0-acyltransferase (*DGAT1*) genes was proposed to affect intramuscular and backfat tissue deposition in beef carcasses (Barendse, 1997; Thaller, 2003; Anton *et al.*,

2010). The *DGAT1* gene encodes diacylglycerol-0-acyltransferase, the enzyme included in the final step of triglyceride synthesis (Thaller *et al.*, 2003; Chen and Farese, Jr., 2005). Lysine/alanine (K232A) substitution results in a lysine/lysine (KK) genotype of *DGAT1* gene that is proposed to result in a greater content of lipids in animal products (Winter *et al.*, 2002; Thaller *et al.*, 2003; Anton *et al.*, 2011). The *TG* gene controls the synthesis of thyroid hormones (T3 and T4) that regulate adipocyte differentiation and growth (McPeake, 2005; Ailhaud *et al.*, 1992), as well as intramuscular fat deposition (McPeake, 2005). The allele polymorphism of the *TG* gene results in a TT genotype that is proposed to affect greater lipid content (Thaller *et al*., 2003; Barendse, 1997).

Earlier researches have reported conflicting results regarding the effect of the *TG* and *DGAT1* genes on fat deposition and metabolism, offering no explanations of the *TG* and *DGAT1* polymorphism effect on fat structure i.e fatty acid profile. The objective of this study was to determine the effect of the *TG* and *DGAT1* genes on beef carcass trait and fatty acid profile in subcutaneous fat tissue and MLD. The study should provide additional information concerning this issue.

MATERIALS AND METHODS

The research included 175 steers non-related and randomly chosen from the Croatian beef cattle population (60 Simmental, 60 Hereford, 55 Charolais), kept in identical conditions and fed with the same diet consisting of corn silage (46%), wet corn (39%), wheat straw (4.63 %) and concentrate with 34% protein (10.37%) distributed to the steers as TMR (total mixed ration). Steers had *ad libitum* access to water during the whole fattening period. The average nutritional content of the feeds was approximately 7.6 MJ ME/kg dry matter and 950 g crude protein. Before slaughter, 175 blood samples (6 mL BD Vacutainer; 10.8 mg K2E anticoagulant) were collected and stored at -20°C until further analyses. The animals were slaughtered under controlled conditions in commercial abattoirs at an average age of 16.5 (±2.5) months. At 24 hours, *post-mortem* carcass EUROP score and subcutaneous fat thickness were determined. The subcutaneous fat thickness was measured with a caliper over the *longissimus dorsi*, between the 12^{th} and 13^{th} rib at a point three-fourths of the length of the ribeye from the split chine bone (USDA, 1997; Tatum, 2007). Approximately 20 g of subcutaneous fat tissue was taken to determine subcutaneous fatty acid composition.

To determine the total lipid content and fatty acid composition, approximately 100 g of *muscle longissimus dorsi* (MLD) was taken between the 12^{th} and 13^{th} rib. Muscle and fat samples were vacuum-packed individually and stored at -20°C until analyses.

Crude fat determination

The total lipid content was determined gravimetrically by the Soxhlet method (ISO 1443:2002). After preparation, approximately 5 grams of meat were ground and homogenized, the meat sample was placed into the Soxhlet extractor and the extraction was carried out with n-hexane for 6 hours with a heating that allows a solvent circulation of minimum 10 times per hour. After the extraction, the

solvent was distilled and the residue was dried at 98°C±2°C for 1 hour. It was left to cool down in an exiccator and weighed. Then it was dried again for further half an hour and left to cool down and weighed. This was repeated until a difference between the last two measurements was less than 1 mg.

Fatty acid methyl ester analysis

A sample quantity containing 1.0 g fat was digested with 20 cm^3 of hydrochloric acid (37%) for 1 hour in a hot water bath. After being cooled down, 7 cm^3 of ethanol was added. Lipids were extracted with 15 cm^3 diethylether and 15 cm^3 benzine (b.p. <60°C), and the organic layers were combined. From a portion of this solution containing 200 mg fat, the solvents were removed at 80°C under reduced pressure (a complete evaporation is not necessary). To the residue 4 cm^3 of 0.5 M sodium hydroxide methanol solution was added and boiled for 5 minutes so that all the fat droplets disappeared. Thereon 4 cm^3 of 14% boron trifluoride in methanol was added through the cooler, and boiled for 3 minutes. Finally 4 cm^3 of n-hexane was added, boiled for one minute and left to cool down. The organic phase was brought to the neck of the flask with saturated sodium-chloride solution. When phases were separated the samples for analyses were taken from the organic phase and dried on sodium sulfate.

Gas chromatography analysis

Fatty acid methyl esters were quantified using Shimadzu GC 2010 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column, CP-Sill 88 (100 m length, 0.25 mm wall coated open tubular-WCOT, 0.2 μm, Varian, USA). Analysis was performed using an initial temperature of 130°C for 0 minutes and then the temperature was increased at a rate of 4°C/min to 202°C. At an end followed an isothermic period of 202°C for 15 minutes. The injector and detector were both maintained at 270°C. Helium at 34.08 psi was used as the carrier gas. Sample injection split mode was 1:20. Fatty acids were identified by comparing relative FAME peak retention times of samples and fatty methyl ester standards from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich). The PUFA FAMEs that were not included in this standard were determined by mass spectrometric detector. The peaks were determined by GCMS solution software (Shimadzu, UK). The fatty acids were expressed as percentages of each individual fatty acid to the total of all fatty acids present in the sample (Rowe *et al*., 1999).

DNA extraction and genotyping

Extraction of DNA was carried out from the blood samples using Blood Genomic DNA kit (Sigma-Aldrich). Detection of the *TG* polymorphism included PCR amplification with *TG*5U2 (forward: 5'-GGG GAT GAC TAC GAG TAT GAC *TG*-3') and *TG*5U1 (reverse: 5'-GTG AAA ATC T*TG TG*G AGG C*TG* TA-3') primers, and subsequent digestion at 37°C for 3 hours using *Psu*I restriction endonuclease (Barendse, 1997). Digestion yielded CC genotype with fragments of 295, 178 and 75 bp, CT genotype with fragments of 473, 295, 178 and 75 bp, TT genotype with fragments of 473 and 75 bp. Amplification of *DGAT1* was carried out with forward

(5'-TCC G*TG* C*TG* GCC C*TG* A*TG* G-3') and reverse (5'-CCA TCT CCA GGA GTC GCC GC--3') primers, yielding 411 bp PCR product. Incubation (3 hour/37°C) using restriction endonuclease *Cfr*I resulted in two fragments of 411 bp (lysine) and 203/208 bp (alanine) (Thaller *et al.*, 2003). Digestion products were separated on 2.5 % agarose gel in 1 x TBE buffer and visualized by ethidium bromide.

Statistical analysis

The allele and genotype frequencies were determined and Hardy-Weinberg equilibrium was tested in Genepop (Raymond and Rousset, 1995). Statistical analysis of experimental data was performed by using the GLM procedure of SAS (1999). Significance level of the treatment was set at $p<0.05$. Dependent variables included in the model were total lipid content in MLD, subcutaneous fat thickness, EUROP score, carcass conformation score, fatty acid composition in MLD and subcutaneous fat tissue. Fixed effects included *DGAT1* and *TG* genotype, while breed and age at slaughter were included as linear covariates.

RESULTS

Genotype numbers and allele frequencies for the analyzed cattle breeds are presented in Table 1. The *TG* and *DGAT1* genes were polymorphic for all three analyzed populations, although the TT genotype of *TG* gene was not found in the Hereford population. Significantly different allele frequencies regarding *TG* gene were found between Hereford and Simmental ($p<0.01$) and Hereford and Charolais population ($p<0.0001$). Allele frequencies of *DGAT1* gene did not differ significantly between the analyzed cattle breeds. The calculated χ^2 values for the *TG* and *DGAT1* genotypes indicate Hardy-Weinberg equilibrium in the population ($p=0.977$ and 0.523 for *TG* and *DGAT1*, respectively).

Table 1. Genotype numbers and allele frequencies of *TG* and *DGAT1* loci for analyzed beef cattle population

Breed	No. of animals	*TG*					*DGAT1*				
		Genotype count			Allele frequency		Genotype count			Allele frequency	
		CC	CT	TT	C	T	AA	KA	KK	A	K
Simmental	60	39	20	1	0.82[b]	0.18	34	18	7	0.73	0.27
Hereford	60	52	8	0	0.93[a]	0.07	32	25	3	0.74	0.26
Charolais	55	27	25	3	0.72[b]	0.28	24	22	9	0.64	0.36

[a,b] Within a column means without a common superscript differ letter ($p<0.01$)

To determine the effect of the *TG* and *DGAT1* polymorphisms on beef carcass traits and fatty acid profile, data were analyzed according to the hypothesis of recessive inheritance of K allele of *DGAT1* gene and T allele of *TG* gene (Barendse, 1997; Thaller *et al.*, 2003). Last square means and standard

errors of the total lipid content in MLD, backfat thickness, EUROP score and carcass conformation score are presented in Table 2. Neither the *TG* nor *DGAT1* polymorphism showed significant influence on the analyzed traits. Thyroglobulin CC/CT and *DGAT1* KK genotypes resulted in a greater total lipid content and thicker backfat (2.11%; 0.50 cm and 2.30%; 0.53 cm). Greater values of EUROP and conformation scores were found for thyroglobulin TT than CC/CT genotypes (1.71; 3.39 vs. 1.62; 3.33). The AA/KA genotypes of *DGAT1* resulted in a greater (+0.23 %) EUROP score than KK genotype, while the conformation score showed almost identical values within *DGAT1* polymorphism.

Table 2. Carcass and meat traits according to analyzed loci

Item	*TG*			*DGAT1*		
	CC/CT	TT	*P*	AA/KA	KK	*P*
Total lipids in MLD[1], %	2.11±0.21	1.72±0.74	0.622	2.02±0.23	2.30±0.48	0.617
Backfat thickness, cm	0.50±0.05	0.41±0.18	0.648	0.49±0.06	0.53±0.12	0.787
EUROP score[2]	1.62±0.11	1.71±0.38	0.812	1.67±0.12	1.44±0.24	0.418
Conformation score[3]	3.33±0.11	3.39±0.38	0.866	3.33±0.12	3.34±0.24	0.988

[1]MLD=*musculus longissimus dorsi*; [2]EUROP score = 1, 2, 3, 4, 5. [3]Conformation score = 1, 2, 3, 4, 5.

In Table 3 are presented the results of *TG* and *DGAT1* polymorphism effect on MLD fatty acid profile (% of fatty acid methyl esters). Polymorphism of *TG* and *DGAT1* gene loci in MLD showed no significant effect on the content of the majority fatty acids. Significant influence of *TG* polymorphism was observed for C20:1 fatty acid, and TT genotype resulted in a lower value than CC/CT genotypes, respectively. The predominant saturated (C16:0) and monounsaturated (C18:1n-9) fatty acid showed greater values for CC/CT genotypes than TT genotype (26.29; 35.25 vs. 25.66; 33.48 %). Greater value of the predominant polyunsaturated fatty acid, C18:2n-6, was found for TT than for CC/CT genotypes (6.87 vs. 4.11%). The nutritionally valuable polyunsaturated fatty acids, C18:2c9,t11 and C18:3n-3, showed greater values for CC/CT and TT genotype, respectively (0.29;0.20 vs. 0.24;0.16%). Minimum differences were observed in SFA content between analyzed *TG* genotypes, while CC/CT genotype resulted in a greater (+1.98 %) content of MUFA. Greater PUFA, PUFA/SFA and n-6/n-3 values were observed for the TT than for CC/CT genotypes (9.69; 0.20; 15.75 vs. 6.40; 0.13; 12.19%).

In MLD AA/KA and KK genotypes resulted in nearly equal C16:0 values (26.24 vs 26.20%), while KK genotype resulted in a greater C18:0 value than AA/KA genotypes (19.08 vs 19.57%). Greater C18:1n-9c (+1.16 %) and lower C18:2n-6 (-1.08%) values were found for KK genotype. The value of C18:2c9,t11 and C18:3n-3 were greater for AA/KA genotypes than for KK genotype (0.30;0.17 vs. 0.25; 0.13%). The SFA, MUFA and n-6/n-3 ratio had greater values for KK

genotype (50.21; 41.95; 12.65 vs. 49.77; 41.18; 12.45%), while AA/KA genotypes resulted in greater PUFA and PUFA/SFA ratios (6.99; 0.14 vs. 5.46; 0.11%).

Table 3. Effect of *TG* and *DGAT1* polymorphism on the MLD (*musculus longisimus dorsi)* fatty acid composition

FAME[1]	*TG*			*DGAT1*		
	CC/CT	TT	*P*	AA/KA	KK	*P*
C10:0	0.05±0.00	0.04±0.01	0.233	0.05±0.00	0.06±0.01	0.270
C12:0	0.06±0.00	0.06±0.01	0.819	0.06±0.00	0.05±0.01	0.331
C14:0	2.67±0.13	2.57±0.45	0.844	2.67±0.14	2.62±0.29	0.884
C14:1	0.42±0.04	0.42±0.13	0.976	0.42±0.04	0.39±0.09	0.718
C15:0	0.41±0.01	0.43±0.05	0.726	0.42±0.02	0.39±0.03	0.505
C16:0	26.29±0.42	25.66±1.46	0.690	26.24±0.46	26.20±0.95	0.972
C16:1	3.19±0.14	3.07±0.49	0.818	3.19±0.16	3.14±0.32	0.876
C17:0	1.13±0.04	1.17±0.14	0.795	1.12±0.04	1.21±0.09	0.403
C18:0	19.19±0.48	19.15±1.67	0.985	19.08±0.52	19.57±1.08	0.700
C18:1n-9t	2.52±0.17	2.47±0.60	0.944	2.57±0.19	2.29±0.39	0.525
C18:1n-9c	35.25±0.73	33.48±2.54	0.515	34.86±0.80	36.02±1.65	0.546
C18:2n-6	4.11±0.45	6.87±1.58	0.113	4.56±0.52	3.48±1.07	0.393
C18:2c9,t11	0.29±0.01	0.24±0.04	0.246	0.30±0.01	0.25±0.03	0.107
C18:3n-3	0.16±0.01	0.20±0.03	0.155	0.17±0.001	0.13±0.02	0.061
C20:0	0.13±0.01	0.13±0.02	0.967	0.13±0.01	0.11±0.02	0.424
C20:1	0.13±0.01	0.08±0.02	0.022*	0.13±0.01	0.11±0.01	0.315
C20:2	0.07±0.01	0.09±0.05	0.851	0.09±0.01	0.03±0.01	0.107
C20:3n-6	0.45±0.06	0.50±0.20	0.803	0.46±0.06	0.42±0.13	0.766
C20:4n-6	1.10±0.15	1.53±0.52	0.446	1.20±0.16	0.91±0.34	0.468
C20:5n-3	0.11±0.02	0.13±0.05	0.749	0.11±0.02	0.10±0.03	0.805
C22:5n-3	0.18±0.02	0.23±0.07	0.525	0.19±0.02	0.17±0.05	0.663
SFA[2]	49.92±0.37	49.21±1.31	0.612	49.77±0.41	50.21±0.85	0.650
MUFA[3]	41.51±0.85	39.53±2.98	0.533	41.18±0.94	41.95±1.95	0.771
PUFA[4]	6.40±0.67	9.69±2.34	0.196	6.99±0.76	5.46±1.56	0.403
PUFA/SFA	0.13±0.01	0.20±0.05	0.199	0.14±0.02	0.11±0.03	0.369
n-6/n-3	12.19±0.66	15.75±2.30	0.155	12.45±0.76	12.65±1.57	0.912

[1]FAME = % total fatty acid methyl esters (LSMEAN±SD).
[2]SFA = saturated fatty acids, (C10:0+C12:0+C14:0+C16:0+C17:0+C18:0+C20:0).
[3]MUFA = monounsaturated fatty acids, (C14:1+C16:1+C18:1n-9t+C18:1n-9c+C20:1).
[4]PUFA = polyunsaturated fatty acids, (C18:2n6+C18:2c9t11+C18:3n3+C20:2+ C20:3n6+C20:4n6+C20:5n3+C22:5n3).
* significant difference

Table 4. Effect of *TG* and *DGAT1* polymorphism on the subcutaneous fatty acid composition

FAME[1]	*TG*			*DGAT1*		
	CC/CT	TT	*P*	AA/KA	KK	*P*
C10:0	0.05±0.00	0.05±0.01	0.868	0.05±0.00	0.04±0.01	0.492
C12:0	0.08±0.00	0.08±0.02	0.820	0.08±0.01	0.08±0.01	0.854
C14:0	3.64±0.13	3.94±0.46	0.551	3.70±0.15	3.54±0.30	0.651
C14:1	1.00±0.08	0.96±0.26	0.850	0.96±0.08	1.16±0.16	0.298
C15:0	0.60±0.02	0.65±0.08	0.561	0.59±0.03	0.66±0.05	0.286
C16:0	27.15±0.45	26.72±1.56	0.793	27.35±0.48	26.23±0.99	0.334
C16:1	4.99±0.17	4.72±0.60	0.675	4.90±0.19	5.21±0.39	0.499
C17:0	1.27±0.06	1.41±0.22	0.567	1.26±0.07	1.35±0.14	0.608
C18:0	15.02±0.57	15.94±1.99	0.662	15.09±0.63	15.11±1.30	0.990
C18:1n-9t	3.50±0.18	4.32±0.63	0.229	3.60±0.21	3.43±0.42	0.726
C18:1n-9c	39.49±0.72	38.02±2.50	0.580	39.18±0.79	40.07±1.62	0.637
C18:2n-6	2.10±0.09	2.18±0.32	0.827	2.14±0.10	2.01±0.21	0.592
C18:2c9,t11	0.14±0.01	0.17±0.02	0.308	0.14±0.01	0.14±0.02	0.993
C18:3n-3	0.17±0.01	0.13±0.05	0.424	0.17±0.02	0.15±0.03	0.662
C20:0	0.10±0.01	0.11±0.02	0.806	0.10±0.01	0.09±0.01	0.598
C20:1	0.10±0.00	0.09±0.01	0.533	0.10±0.00	0.10±0.01	0.386
C20:2	0.03±0.00	0.02±0.01	0.730	0.02±0.00	0.04±0.01	0.053
C21:0	0.47±0.03	0.43±0.11	0.697	0.47±0.03	0.47±0.07	0.967
C20:3n-6	0.05±0.00	0.04±0.02	0.473	0.05±0.01	0.06±0.01	0.354
C20:4n-6	0.04±0.01	0.04±0.02	0.905	0.03±0.01	0.07±0.01	0.034*
SFA[2]	48.38±0.84	49.32±2.91	0.762	48.69±0.91	47.57±1.88	0.556
MUFA[3]	49.09±0.82	48.11±2.85	0.812	48.75±0.89	49.98±1.83	0.582
PUFA[4]	2.53±0.10	2.58±0.36	0.903	2.55±0.11	2.47±0.23	0.606
PUFA/SFA	0.05±0.00	0.05±0.01	0.963	0.05±0.00	0.05±0.01	0.738
n-6/n-3	14.96±1.54	18.17±5.33	0.572	15.41±1.69	14.56±3.48	0.834

[1]FAME =% fatty acid methyl esters (LSMEAN±SD).
[2]SFA = saturated fatty acids, (C10:0+C12:0+C14:0+C16:0+C17:0+C18:0+C20:0).
[3]MUFA=monounsaturated fatty acids, (C14:1+C16:1+C18:1n-9t+C18:1n-9c+C20:1).
[4]PUFA = polyunsaturated fatty acids,
(C18:2n6+C18:2c9t11+C18:3n3+C20:2+ C20:3n6+C20:4n6+C20:5n3+C22:5n3).
* significant difference.

In Table 4 are presented the results of the *TG* and *DGAT1* polymorphism effect on subcutaneous fatty acid profile (% of fatty acid methyl esters). Analyses of subcutaneous fatty acid profile showed that predominant saturated fatty acid, C16:0, had a greater value for CC/CT genotypes (27.15 vs. 26.72%), while TT genotype resulted in a greater C18:0 value (+0.92%). A greater value of

predominant unsaturated fatty acid, C18:1n-9c, as well as of C18:3n-3, was found for CC/CT genotypes (39.49; 0.17 vs. 38.02; 0.13%). The TT genotype showed greater C18:2n-6 and C18:2c9,t11 values (2.18; 0.17 vs. 2.10; 0.17%). In subcutaneous fat tissue, TT genotype resulted in greater SFA and PUFA values than CC/CT genotypes (49.32; 2.58 vs. 48.38; 2.53%). A greater (+0.98%) MUFA value was found for CC/CT genotypes. The PUFA/SFA ratio had equal values for all three genotypes (0.05%), while n--6/n-3 in subcutaneous tissue had a greater value for TT genotype (18.17 vs. 14.96%).

Analyzing the effect of the *DGAT1* polymorphism on fatty acid profile of subcutaneous fat tissue, it was found that AA/KA genotypes resulted in a greater value of predominant saturated fatty acid, C16:0 (27.35 vs. 26.23%), while minimal differences were observed for C18:0 value (15.11 vs. 15.09%). A greater C18:1n9c value was found for KK genotype (40.07 vs. 39.18%), while AA/KA genotype showed a greater C18:2n-6, as well as C18:3n-3 value (2.14 and 0.17%). The C18:2c9,t11 had equal values for analyzed genotypes (0.14%). The SFA and PUFA values were greater for AA/KA genotypes, while PUFA/SFA ratio had equal values for all analyzed genotypes (0.05%). The KK genotype resulted in a greater (+1.23%) content of MUFA than AA/KA genotype. A greater n-6/n-3 ratio was found for AA/KA genotypes than for KK genotype (15.41 vs. 14.56%). Significantly greater values of C20:2 and C20:4n6 were found for KK than for AA/KA genotypes of *DGAT1* gene (0.04; 0.07 vs. 0.02; 0.03%).

DISCUSSION

Allele T of *TG* gene and allele K of *DGAT1* showed to be less frequent than C and A allele in analyzed beef cattle population, respectively. The obtained results correspond to those reported in earlier researches (Thaller *et al.*, 2003; Casas *et al.*, 2007; Kaplanova *et al.*, 2009; Pannier *et al.*, 2010). Analyzing *Bos indicus* population, Fortes *et al.* (2009) reported that A allele (*DGAT1*) in the analyzed populations was less frequent (<50 %) than the dominant K allele. The significant difference of Hereford *TG* allele frequencies from other analyzed breeds corresponds to the results of Pannier *et al.* (2010).

In the present study, no significant effect of the *TG* and *DGAT1* polymorphism on beef carcass fat traits was found. The CC/CT genotypes of *TG* gene resulted in a greater total lipid content in MLD (0.39 %). Greater lipid content in MLD was found for KK genotype of *DGAT1* gene (0.28 %; 0.04 cm). Although Pannier *et al*. (2010) found no association between *DGAT1* and *TG* genes and intramuscular fat level in the analyzed crossbred cattle population, as in the present study, KK (*DGAT1*) and CC (thyroglobulin) genotypes yielded a greater fat percentage in *longissimus thoracis et lumborum*. Anton *et al*. (2010) reported that AA genotype (*DGAT1*) and TT genotype (thyroglobulin) resulted in a significantly greater fat percentage in LD and ST than other genotypes. Also, Thaller *et al*. (2003) reported that TT genotype resulted in significantly greater lipid content in MLD, while KK genotype showed a significantly greater lipid content in *m. semitendinosus*.

No significant influence of the *TG* and *DGAT1* polymorphisms on backfat thickness was found in the present study. The CC/CT genotypes of *TG* gene and KK genotype of *DGAT1* resulted in a thicker backfat tissue (0.50 cm; 0.53 cm). The same results were reported by other authors analysing *Bos taurus* and *Bos indicus* cattle populations (Casas *et al.*, 2007; Gan *et al.*, 2008; Fortes *et al.*, 2009). Rincker *et al.* (2006), using GeneSTAR marbling marker, also reported no significant influence of *TG* polymorphism on backfat thickness. However, Casas *et al.* (2005) reported that TT genotype had a significantly greater fat thickness than other two *TG* genotypes. In the same study, *DGAT1* polymorphism showed no effect on backfat thickness.

The lack of association between analyzed traits and *TG* polymorphism Kaplanova *et al.* (2009) explained as a result of a low number of tested animals and chosen analyzed carcass traits. The *TG* and *DGAT1* polymorphisms showed no effect on analyzed traits in *Bos indicus* and *Bos indicus* x *Bos taurus* bovine populations (Fortes *et al.*, 2009). This could be due to insufficiency of gene markers developed for *Bos taurus* when used for *Bos indicus* (Fortes *et al.*, 2009). Although Casas *et al.* (2007) reported no significant association between analyzed traits and *TG* polymorphism, due to numerically greater trait values for TT genotype, there are indications that *TG* gene markers could be useful predictors of marbling performance.

To analize in more detail the effect of *TG* and *DGAT1* polymorphisms on meat fat characteristics, the effect of the *TG* and *DGAT1* polymorphisms on fatty acid composition in MLD and subcutaneous fat tissue was determined. As for analyzed carcass fat traits, no significant influence of the *TG* and *DGAT1* polymorphisms on the majority fatty acids content was found in the present study. In MLD CC/CT genotypes of *TG* gene showed significantly greater C20:1 value than TT genotype. The KK genotype of *DGAT1* gene in subcutaneous fat tissue resulted in significantly greater C20:2 and C20:4n6 values. The TT genotype of *TG* gene resulted in a lower value of the less favorable SFA and a greater value of the more favorable PUFA both in MLD and in subcutaneous fat tissue. Also, TT genotype resulted in a more favorable PUFA/SFA ratio, but a less favorable n-6/n-3 ratio in MLD than KK genotype.

In MLD, greater values of SFA and MUFA were found for the KK genotype of *DGAT1* gene than for the thyroglobulin TT gene (50.21; 41.95 vs. 49.21; 39.53%). The TT genotype of *TG* gene resulted in greater PUFA, PUFA/SFA and n-6/n-3 ratios than KK genotype of *DGAT1* gene (9.69; 0.20; 15.75 vs. 5.46; 0.11; 12.65%). In subcutaneous fat tissue, the TT genotype of *TG* gene showed greater SFA, PUFA and n-3/n-6 ratio than the KK genotype of *DGAT1* gene (49.32; 2.58; 18.17 vs. 47.57; 2.47; 14.56%). The MUFA content dominated in the KK genotype of *DGAT1*, being 1.87% greater than in the TT genotype of *TG* gene. Equal values of the PUFA/SFA ratio were found for the KK and TT genotypes.

The lack of significance between analyzed polymorphisms and traits in the present study can be due to a small number of analyzed animals displaying the TT (thyroglobulin) or KK (*DGAT1*) genotypes. Similar results were reported by Thaller *et al.* (2003). Casas *et al.* (2007) suggested that increasing the frequency of the T allele could result in establishing a statistical difference between cattle

populations. The same could be concluded for *DGAT1*, while only 17 KK genotypes were present in the analyzed population (Pannier *et al.*, 2010).

The present study showed no significant association between *TG* and *DGAT1* polymorphisms and analyzed traits. The results are consistent with earlier studies. However, as this was the first study aimed to determine the effect of *TG* and *DGAT1* polymorphisms on meat fat traits in the Croatian beef cattle population, further research should be done to validate the initial observations.

Address for correspondence:
Prof.dr.sc. Ante Ivanković
Department of animal production and technology
Faculty of Agriculture
Svetošimunska 25
10000 Zagreb, Croatia
E-mail: aivankovic@agr.hr

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UTJECAJ TG I DGAT1 POLIMORFIZMA NA SVOJSTVA GOVEĐIH TRUPOVA I PROFIL MASNIH KISELINA

KELAVA NIKOLINA, KONJAČIĆ M i IVANKOVIĆ A

SADRŽAJ

Ciljevi ove studije su bili utvrđivanje frekvencije alela i genotipova tiroglobulina (TG) i diacilglicerol-0-aciltranferaze-1 (DGAT1), gena koji kodiraju TG i DGAT1 enzime te determinacija efekta polimorfizma TG i DGAT1 gena na profil masnih kiselina u trupu goveda. Svi genotipovi su determinirani PC-RFLP metodom. Polimorfizam TG i DGAT1 gena nije imao značajan utjecaj na ukupni sadržaj lipida, debljinu potkožnog masnog tkiva, EUROP i konformacijsku ocjenu trupova. TT genotip TG gena povezan je s manjim ukupnim sadržajem lipida i manjom debljinom potkožnog masnog tkiva, dok je KK genotip DGAT1 gena povezan s većim ukupnim sadržajem lipida i debljim potkožnim masnim tkivom. Na sadržaj većine masnih kiselina u MLD i potkožnom masnom tkivu polimorfizam TG i DGAT1 gena nije značajno utjecao. Rezultati potvrđuju nedostatak istraživanih povezanosti zapaženih u drugim studijama. Daljnja istraživanja treba provesti radi provjere inicijalnih zapažanja.