Acta Veterinaria (Beograd), Vol. 62, No. 1, 91-100, 2012.

DOI: 10.2298/AVB1201091K UDK 636.23.:575.22:665.223:612.057

THE EFFECT OF SEX AND DGAT1 GENE POLYMORPHISM ON FAT DEPOSITION TRAITS IN SIMMENTAL BEEF CATTLE

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(Received 1st September 2011)

This study investigated diacylglycerol O-acyltransferase 1 (DGAT1) gene K232A mutation in Simmental cattle and its effects on fat deposition traits. The sample (n=26) consisted of yearling bulls and beef heifers from an intensive rearing system in Croatia. Carcass fatness was assessed by total dissection method, whereas intramuscular fat (m. longissimus dorsi) content was determined using Soxhlet extraction with hydrolysis. Intramuscular fatty acid composition was determined by gas liquid chromatography using in situ transesterification. The muscle DNA was extracted and Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) analysis of the 411 bp fragment of DGAT1 gene was applied. The DGAT1 K allele was less frequent in heifers than in bulls, with the overall allelic frequency of 17% K allele. Only KA and AA genotypes were obtained, without deviation from the Hardy-Weinberg equilibrium. Heifers showed a higher degree of carcass and muscle fattening with more unsaturated intramuscular fat than bulls; however, there was no interaction between sex and DGAT1 gene. Generally, no significant difference between DGAT1 AA and KA animals was observed for any of the examined traits, except the slightly higher carcass share of the fattest beef category and higher intramuscular C14:0 desaturation index in KA heterozygous.

Key words: cattle, DGAT1, fat deposition, Simmental breed

INTRODUCTION

One of the genes proposed to affect fat metabolism in cattle is diacylglycerol O-acyltransferase 1 (DGAT1), which catalyses the final step of triglyceride synthesis (Cases *et al*., 1998). A non conservative AA/GC dinucleotide substitution causing a K (lysine) to A (alanine) amino acid substitution (K232A) in the bovine DGAT1 gene, previously mapped to the centromeric region of chromosome 14 (BTA14), was first proposed to be the causative variation affecting milk fat traits (Grisart *et al*., 2002; Winter *et al*., 2002). Afterwards, Thaller *et al*. (2003) found significant effects of K/A polymorphism of DGAT1 on fat content of *m. semitendinosus* in German Holstain cattle, where the lysine allele showed to be the more efficient version of the enzyme with regard to triglyceride synthesis. Studies that followed on beef cattle showed that the K allele of DGAT1 has also a positive effect on subcutaneous fat thickness in Nerole cattle (Curi *et al*., 2011), sirloin fat depth in Aberdeen Angus-sired beef cattle (Gill *et al*., 2009), and intramuscular fat (IMF) content in Hungarian Angus bulls (Anton *et al*., 2011). In contrast, several other studies failed to observe any significant association of the DGAT1 polymorphism with carcass or muscle fattening in commercial lines or purebreds of *Bos Taurus* (Moore *et al*., 2003; Pannier *et al*., 2010) or *Bos Indicus* beef breeds (Fortes *et al*., 2009; Souza *et al*., 2010).

Previous studies on Simmental cattle from several countries have shown the absence (or almost complete absence) of variability at the DGAT1 locus, with a generally very low frequency of lysine allele (Kaupe *et al*., 2004; Pannier *et al*., 2010; Scotti *et al*., 2010). The aim of the present study was to estimate the allele and genotype frequencies of single nucleotide polymorphisms of the DGAT1 gene and to associate genotypes with carcass and muscle fat deposition traits in the Croatian population of Simmental cattle. The effect of sex and possible interactions were investigated, as well.

MATERIAL AND METHODS

Animals

The experiment was conducted on 26 Simmental beef cattle (13 bulls and 13 heifers) in compliance with national legislation on animal protection (Narodne Novine, 2006). All animals were of domestic origin and were raised under similar conditions of housing (indoor boxes with fully concrete floor) and feeding (total mixture ratio composed from maize grain and stalk silage, super-concentrate and hay). At the time of slaughter all animals were about one year old, with mean live weight of bulls and heifers about 510 kg and 455 kg, respectively.

Carcass measurements

Animals were humanely slaughtered at a commercial slaughterhouse using the standard procedure consisting of captive-bolt stunning, vertical exsanguination, evisceration and final washing. Hot carcass weight was measured without removing the subcutaneous fat and maintaining the kidney and pelvic fat. Dressing-out percentage was calculated as: (hot carcass weight / live weight before slaughter) x 100. The excessive cover fat on round and groin area and internal fat depots (kidney and pelvic fat) were trimmed and weighed together to obtain the trimmed fat value. After cooling for 48 h at 4°C, the carcass tissue composition was assessed by total dissection of right halves according to the scheme in Figure 1 (DLG method, Scheper and Scholz, 1985).

Each carcass part was weighed and dissected into the muscle, bone, fat and connective tissue. The total weight of separated tissues was used as the denominator for calculating proportions of particular tissue in the carcass. The shares of beef retail categories in the carcasses and tissue composition within each category were also calculated.

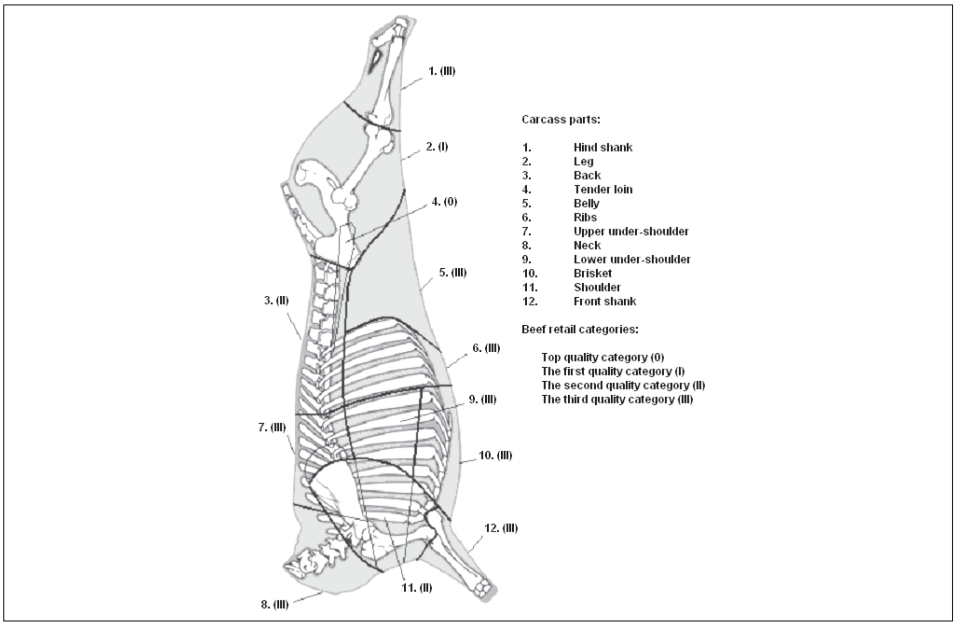

Figure 1. Carcass parts and corresponding beef retail categories

Tissue analysis

At 48 h post mortem, samples of *m. longissimus dorsi* (MLD) were taken from the right halves at the level of the 8^{th} rib and stored frozen (-20°C) until analyses of total lipid content and fatty acid (FA) composition. The IMF content was determined using Soxhlet extraction with hydrolysis (SIST ISO, 2001). The FA composition was determined by gas liquid chromatography using *in situ* transesterification method (Park and Goins, 1994). The content of FA methyl esters (FAME) was determined using Agilent Technologies 6890 N (USA) gas chromatograph equipped with a flame ionisation detector and capillary column Supelco Omegawax^{TM} 320 (length 30 m, internal diameter 0.32 mm and film thickness 0.25 μm) for FAME separation. Separated FAMEs were identified by comparison with the retention times of the FAMEs in a standard mixture (Nu-Check Prep, Inc, Elysian, USA). The same mixture was used to determine the response factor (RF) for each FA. The mass portion of each FA in the sample was determined using the RF and the factor of conversion of FA content from the FAME content. The FA desaturation indices were calculated as: C14 index = C14:1 / (C14:0 + C14:1) × 100; C16 index = C16:1 / (C16:0 + C16:1) × 100 and C18 index = C18:1 / (C18:0 + C18:1) × 100.

DGAT1 Genotyping

Tissue (MLD) samples of each carcass were collected and stored at -80°C. DNA was extracted by DNeasy Blood & Tissue Kit (Qiagen GmbH, Germany) according to manufacturer's recommendations. Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) analysis was done according to Winter *et al*. (2002). Thus, 411-bp fragment was amplified (of DGAT1 gene) using the forward: (5'- GCACCATCCTCTTCCTCAAG - 3') and reverse: 5' - GGAAGCGCTTTCGGATG - 3' (Invitrogen) primers and then conducted to restriction analysis by CfrI (Fermentas). The PCR protocol was performed in a 50 μL reaction mix containing approximately 50 ng of total DNA, 0.2 μM of each forward and reverse primer and Qiagen HotStarTaq®Plus Master mix Kit (Qiagen, GmbH, Germany). The PCR was carried out in a iCycler (Biorad, USA) and consisted of: an initial denaturation step at 95°C for 15 min followed by 38 cycles at 95°C, for 60 s, annealing at 6°C for 60 s, and elongation at 72°C for 60 s with a final elongation step of 7 min at 72°C. Restriction analysis was done in a mixture 18 μL of PCR product, 2 μL of buffer and 0.8 μL of restriction enzyme CfrI (Fermentas), 37°C for one hour.

Statistical analysis

Genotype frequencies of DGAT1 polymorphism were tested for deviations from Hardy-Weinberg equilibrium (HWE) by Chi-square ($\chi 2$) test. Differences between effects were analysed by the general linear model (GLM) procedure of SAS 9.1 (SAS, 2002). The model included sex and DGAT1 genotype as main effects and also their interaction, which was always non-significant and therefore removed from the final analyses. When any of the main effects was significant ($p \leq 0.05$), the differences between groups were analyzed by the Tukey's test. Least-square means and standard errors are reported in Tables 2 and 3.

RESULTS

Genotype and allele frequencies of DGAT1 gene are shown in Table 1. The K allele of the DGAT1 was less frequent in heifers (8%) than in bulls (27%), with the overall allelic frequency of 17% K allele. For the DGAT1 gene no combination of the two lysine alleles was identified after sequencing and cloning. Genotypes were assigned as defined in the literature: AA, KA and KK (Winter *et al*., 2002). The genotype distributions for bulls, heifers and overall did not deviate ($p > 0.05$) from HWE expectations ($\chi 2$ = 1.49, 0.12 and 1.028, respectively).

Table 1. Genotype and allele frequencies of DGAT1 polymorphism in Simmental beef cattle

Gene/Sex	Genotype and frequency[a]			Allele and allele frequency	
DGAT1	KK	KA	AA	K	A
Bulls	0	6	5	0.27	0.73
Heifers	0	2	10	0.08	0.92
All animals	0	8	15	0.17	0.83

[a]Allele "K" and "A" of DGAT1 represent lysine and alanine, respectively.
Analysis of three samples failed.

Table 2. The effect of sex and DGAT1 gene polymorphism on carcass fatness traits of Simmental beef cattle

Variable	Sex		p	DGAT1		p
	Bulls (n=13)	Heifers (n=13)		AA (n=15)	KA (n=8)	
Carcass weight (kg)	278.1 ±6.7	244.9±7.3	*	265.4 ±5.9	257.6 ±8.2	ns
Trimmed fat (%)	4.02 ±0.28	6.21±0.30	*	5.07 ±0.25	5.16 ±0.34	ns
Fat tissue (%) in:						
Hind shank	6.22 ±0.51	6.35 ±0.56	ns	6.16 ±0.46	6.41 ±0.63	ns
Leg	4.37 ±0.45	5.95 ±0.49	*	5.31 ±0.40	5.00 ±0.55	ns
Back	4.67 ±0.74	6.42 ±0.81	ns	5.62 ±0.66	5.47 ±0.91	ns
Belly	16.67 ±1.65	22.21 ±1.80	*	20.97 ±1.47	17.91 ±2.02	ns
Ribs	18.77 ±1.90	24.90 ±2.07	*	20.37 ±1.69	23.29 ±2.33	ns
Upper und.shoulder	6.11 ±0.61	7.94 ±0.66	t	7.67 ±0.54	6.37 ±0.74	ns
Neck	4.36 ±0.66	7.69 ±0.72	*	6.33 ±0.59	5.71 ±0.81	ns
Lower und.shoulder	15.13 ±1.31	23.80 ±1.43	*	19.28 ±1.17	19.65 ±1.61	ns
Brisket	16.97 ±1.34	22.77 ±1.46	*	19.15 ±1.19	20.59 ±1.64	ns
Shoulder	7.24 ±0.71	10.66 ±0.77	*	9.25 ±0.63	8.66 ±0.87	ns
Front shank	1.45 ±0.14	1.51 ±0.15	ns	1.56 ±0.12	1.39 ±0.17	ns
Carcass composition (%):						
Muscle tissue	70.38 ±0.58	66.93 ±0.63	*	68.65 ±0.51	68.67 ±0.70	ns
Fat tissue	7.53 ±0.62	10.79 ±0.67	*	9.23 ±0.55	9.10 ±0.76	ns
Bone tissue	16.33 ±0.24	15.97 ±0.26	ns	16.39 ±0.22	15.92 ±0.30	ns
Connective tissue	5.73 ±0.32	6.37 ±0.35	ns	5.75 ±0.29	6.34 ±0.40	ns
Retail beef category (%):						
0 (tender loin)	2.03 ±0.06	2.05 ±0.06	ns	2.04 ±0.05	2.04 ±0.07	ns
I (leg)	30.35 ±0.27	30.55 ±0.30	ns	30.45 ±0.25	30.45 ±0.34	ns
II (back, shoulder)	23.61 ±0.26	22.29 ±0.28	*	23.48 ±0.23	22.42 ±0.32	*
III (other parts)	44.00 ±0.26	45.12 ±0.29	*	44.03 ±0.23	45.08 ±0.32	*

ns = $p>0.05$; t = $p\leq0.10$; * = $p\leq0.05$

Fat deposition traits of bulls and heifers and different genotypes of DGAT1 polymorphisms are shown in Table 2. As expected, the bulls exhibited higher ($p\leq0.05$) carcass weight than heifers. The percentage of trimmed carcass fat was higher ($p\leq0.05$) in heifers, which also had a higher ($p\leq0.05$) share of dissected fat tissue in the leg, shoulder, lower under-shoulder, ribs, briskets, neck and belly part. The fat proportion in the back, front and hind shank was similar ($p>0.05$) between sexes, while in upper under-shoulder it tended ($p<0.10$) to be higher in heifers. Considering the whole carcass composition, dissection showed higher ($p \leq 0.05$) proportion of muscle tissue in bulls and fat tissue in heifers, while the bone and connective tissue proportion did not differ between sexes. The share of most valuable retail beef category was similar ($p > 0.05$) in both bulls and heifers. The share of second beef category was higher ($p \leq 0.05$) in bulls, while the share of third beef category was higher ($p \leq 0.05$) in heifers. Generally, there were no interactions between effects of sex and DGAT1 locus. The K allelic frequency of DGAT1 was too low in the studied population to produce sufficient numbers of KK genotype and comparison was only done for the remaining KA vs. AA genotype (Table 2). No significant difference was observed for any of the studied fat deposition traits between animals with the DGAT1 AA and KA genotype, except for the shares of the II and III category of beef, which in KA animals showed respectively lower and higher values ($p \leq 0.05$).

The effect of sex and DGAT1 gene polymorphism on IMF content and FA profile of MLD is shown in Table 3. Heifers had the greater ($p \leq 0.05$) IMF content, together with the higher ($p \leq 0.05$) total and individual monounsaturated FA (MUFA) proportions and higher ($p \leq 0.05$) indices of C14, C16 and C18 desaturation. Bulls had a higher ($p \leq 0.05$) proportion of total and some individual saturated FA (SFA) (C10:0, C12:0, C15:0, C18:0 and C20:0) and a higher ($p \leq 0.05$) proportion of total and all individual polyunsaturated FA (PUFA), except the C20:2n-6.

Table 3. Effect of sex and DGAT1 gene polymorphism on intramuscular fat content (*m. longissimus dorsi*) and fatty acid composition (g/100g FA) in Simmental beef cattle

Variable	Sex		p	DGAT1		p
	Bulls (n=13)	Heifers (n=13)		AA (n=15)	KA (n=8)	
IMF (g/kg)	12.02 ±3.09	27.00 ±3.37	*	19.77 ±2.75	19.24 ±3.80	ns
C10:0	0.10 ±0.004	0.07 ±0.004	*	0.09 ±0.003	0.08 ±0.005	ns
C12:0	0.09 ±0.003	0.07 ±0.003	*	0.08 ±0.003	0.07 ±0.004	ns
C14:0	2.55 ±0.12	2.50 ±0.13	ns	2.58 ±0.10	2.47 ±0.14	ns
C14:1	0.44 ±0.03	0.54 ±0.04	*	0.44 ±0.03	0.54 ±0.04	t
C15:0	0.50 ±0.02	0.41 ±0.02	*	0.49 ±0.02	0.42 ±0.03	t
C16:0	23.83 ±0.40	23.37 ±0.44	ns	23.84 ±0.36	23.36 ±0.49	ns
C16:1	2.72 ±0.17	3.29 ±0.19	*	2.92 ±0.15	3.08 ±0.21	ns
C17:0	1.52 ±0.06	1.47 ±0.07	ns	1.57 ±0.06	1.41 ±0.08	ns
C18:0	15.43 ±0.35	14.10 ±0.39	*	15.00 ±0.32	14.52 ±0.43	ns
C18:1	35.21 ±0.99	44.39 ±1.07	*	39.00 ±0.88	40.60 ±1.22	ns
C18:2n-6	10.54 ±0.70	5.34 ±0.73	*	8.04 ±0.60	7.84 ±0.82	ns

cont. Table 3.

C18:3n-3	0.35 ±0.02	0.20 ±0.02	*	0.28 ±0.02	0.27 ±0.02	ns
C20:0	0.11 ±0.003	0.08 ±0.003	*	0.10 ±0.003	0.09 ±0.003	t
C20:1	0.29 ±0.03	0.41 ±0.03	*	0.34 ±0.03	0.36 ±0.03	ns
C20:2n-6	0.15 ±0.02	0.12 ±0.02	ns	0.14 ±0.02	0.14 ±0.02	ns
C20:3n-6	0.61 ±0.04	0.46 ±0.04	*	0.53 ±0.04	0.54 ±0.05	ns
C20:4n-6	3.34 ±0.23	1.77 ±0.25	*	2.71 ±0.21	2.41 ±0.29	ns
C20:5n-3	0.18 ±0.01	0.10 ±0.02	*	0.15 ±0.01	0.14 ±0.02	ns
C22:4n-6	0.46 ±0.03	0.29 ±0.03	*	0.39 ±0.02	0.36 ±0.03	ns
Σ SFA	44.12 ±0.50	42.12 ±0.55	*	43.75 ±0.45	42.44 ±0.62	ns
Σ MUFA	38.65 ±1.06	48.63 ±1.15	*	42.69 ±0.94	44.58 ±1.30	ns
Σ PUFA	16.23 ±1.01	8.68 ±1.10	*	12.72 ±0.89	12.20 ±1.23	ns
C 14 index	14.58 ±0.77	17.82 ±0.84	*	14.66 ±0.68	17.73 ±0.94	*
C16 index	10.18 ±0.50	12.27 ±0.55	*	10.86 ±0.45	11.59 ±0.61	ns
C18 index	69.42 ±0.79	75.91 ±0.86	*	71.94 ±0.70	73.38 ±0.97	ns

ns = $p>0.05$; t = $p\leq0.10$; * = $p\leq0.05$.

Neither IMF content nor FA profile of MLD was significantly affected by DGAT1 genotype (Table 3). However, the index for C14 desaturation was higher ($p\leq0.05$) for DGAT1 KA animals, which also showed a tendency ($p\leq0.10$) for higher C14:1 proportion in IMF compared to DGAT1 AA animals. In addition, the proportion of C15:0 and C20:0 tended ($p\leq0.10$) to be higher in DGAT1 AA animals.

DISCUSSION

Genotype and allele frequencies of DGAT1 gene found in the present study are in accordance with indications described in literature, that the K allele is the minor allele in most European *Bos taurus* breeds (Kaupe *et al*., 2004; Ripoli *et al*., 2006). However, the overall allelic frequency of 17% K allele observed presently was higher than those reported recently for Simmental populations in other countries, ranging from less than 1% in Italy (Scotti *et al*., 2010) to 6% in Ireland (Pannier *et al*., 2010). Simmental breed is known for its dual (milk and meat) production purpose and its breeding type in Croatia has changed over the years in accordance with the alteration of breeding goals (Štoković *et al*., 2009). It is possible that the somewhat higher frequency of DGAT1 K allele observed in present study is a result of frequent importation of breeding animals from Germany (around 34 000 heads in 2000-2010 period, Croatian Agriculture Agency, personal communication) as in German Fleckvieh the lysine-encoding allele has being associated with the high breeding values for milk fat content (Winter *et al.*, 2002).

It is well-known that sex of farm animals is a factor with a large influence on carcass fatness. In beef cattle, the order of leanness in young slaughter animals is normally bulls >steers > heifers (De Smet *et al*., 2004). Present results confirmed this pattern well; the amount of cover fat, as well as dissected fat tissue in the carcass and most of its parts was significantly higher in heifers than in bulls. For both sexes the highest fat deposition existed on the ribs, lower under-shoulders, brisket and belly part, all of which fall into the third category of beef. Regarding to association of DGAT1 gene and fat deposition traits, the K allelic frequency of DGAT1 was too low to produce sufficient numbers of KK genotype for a complete association study. For the remaining KA and AA genotypes, association was only observed with the carcass shares of beef retail categories, with about 1% higher share of the fattest third category beef in lysine carrying KA heterozygous. However, this single finding can hardly be sufficient to fully support the previous observations on association of DGAT1 K allele with several carcass fatness traits in beef cattle (Gill *et al*., 2009; Curi *et al*., 2011).

Regarding to amount and type of fat in the muscle, heifers had more IMF with a higher proportion of MUFA, whilst bulls had more saturated and polyunsaturated IMF. These differences in intramuscular FA composition between bulls and heifers can be attributed to the difference in deposition of fat in the muscle. In muscle lipids, the triaciylglycerol/phosholipid ratio increases with fat accretion with an associated increase in C18:1 and decrease in C18:2n-6 (Wood *et al*., 2008). Besides, the activity of delta-9-desaturase, which converts SFA into MUFA, increases significantly with an increase in adipose tissue mass (Smith *et al*., 2006), leading to a general increase in fat unsaturation. Thus, the greater muscle fattening in heifers was associated with significantly higher indices of FA desaturation and MUFA proportion, and lower SFA proportion, as compared with bulls. In the present study there was no effect of DGAT1 locus on IMF content or FA composition. The lack of consistent association between bovine DGAT1 polymorphism and IMF deposition has already been reported (Fortes *et al*., 2009; Pannier *et al*., 2010). This is opposes to the work done by Thaller *et al*. (2003) and recently by Anton *et al*. (2011), in which the animals with the K allele at DGAT1 were found to have increased marbling. However, in these studies on Holstain and Angus populations the significant effect on IMF content was observed for DGAT1 KK genotype which was not found in the Simmental population examined in the present study or elsewhere (Scotti *et al*., 2010; Pannier *et al*., 2010). Potentially interesting result of the present study is a higher index of C14:0 desaturation in DGAT1 KA animals, which might imply a higher muscle fattening. However, such an implication was not supported by other results.

In conclusion, the bovine DGAT1 gene polymorphism studied in Croatian population of Simmental bulls and beef heifers confirmed the low variability at the DGAT1 locus with a low frequency of lysine allele already described for this breed in the literature. As expected, heifers had a higher degree of carcass and muscle fattening with more unsaturated IMF than bulls; however, no interaction between the sex and DGAT1 was observed. Despite a few differences between genotypes at DGAT1, i.e., the slightly higher share of the fattest beef category in the carcass and higher intramuscular C14:0 desaturation index in KA heterozygous, in general there was no significant association of DGAT1 polymorphism with fat deposition traits. Nevertheless, this study was conducted on a small sample population and without the lysine carrying homozygous for testing. Hence, the effect of DGAT1 polymorphisms on fat deposition traits in Simmental beef cattle should be further investigated.

ACKNOWLEDGEMENT:
This research was supported through the EUREKA programme (Project E! 3983-RFA) and Bjelovar-Bilogora County. The authors are grateful to Ivan Jurić for his collaboration and to the »Association of baby-beef producers« Gudovac and »MI Ivanec« (meat industry) for technical assistance.

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EFEKAT POLA I POLIMORFIZMA DGAT1 GENA NA KARAKTERISTIKE DEPONOVANJA MASTI KOD SIMENTALSKIH GOVEDA

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SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja genske mutacije K232A gena za diacilglicerol O-aciltransferazu 1 (DGAT1) kod simentalskih goveda i njeni efekti na karakteristike deponovanja masti. U ispitivanja je bilo uključeno 26 jednogodišnjih bikova i junica iz intenzivnog tova. Udeo masti u trupovima je procenjivan metodom potpune disekcije i masne naslage u mišićima su utvrđivane korišćenjem Soxhlet ekstrakcije sa hidrolizom. Koncentracija masnih kiselina u mišićima je utvrđivana tečnom gasnom hromatografijom korišćenjem trans-esterifikacionog metoda *in situ.* DNK je ekstrahovana iz mišića i primenjena je PCR-RFLP metoda (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) za analizu 411 bp fragmenta na DGAT1 genu. DGAT1 K alel je bio manje zastupljen kod junica nego kod bikova, sa ukupnom frekvencijom od 17% K alela. Samo su KA i AA genotipovi dobijeni bez odstupanja od Hardy-Weinberg-ovog ekvilibrijuma. Kod junica je utvrđen veći stepen taloženja sala na trupu i u mišićima sa više nezasićenih masnoća u intramuskularnom salu nego kod bikova. Nije dokazana korelacija između polova i DGAT1 gena. Generalno, nije bilo značajne razlike između DGAT1 AA i KA kod ispitivanih životinja, osim blago povećanog udela masti kod najdebljih bikova i višeg intramuskularnog indeksa desaturacije C14:0 kod heterozigota KA.