# The effects of FASN and DGAT1 gene polymorphism on colostrum and milk fatty acid composition of Charolais cows and growth in Charolais calves

Maja Maurić Maljković<sup>1\*</sup>, Tomislav Mašek<sup>2</sup>, Marina Vranić<sup>3</sup>, and Kristina Starčević<sup>4</sup>

<sup>1</sup>Department of Animal Breeding and Livestock Production, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

<sup>2</sup>Department of Animal Nutrition and Dietetics, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

<sup>3</sup>Department of Field Crops, Forage and Grassland, Faculty of Agriculture, University of Zagreb, Croatia <sup>4</sup>Department of Chemistry and Biochemistry, Faculty of Veterinary Medicine, University of Zagreb, Croatia

MAURIĆ MALJKOVIĆ, M., T. MAŠEK, M. VRANIĆ, K. STARČEVIĆ: The effects of *FASN* and *DGAT1* gene polymorphism on colostrum and milk fatty acid composition of Charolais cows and growth in Charolais calves. Vet. arhiv 92, 389-397, 2022.

### **ABSTRACT**

Fatty acid composition of milk has become an important trait in dairy production, but there has not been a large amount of research on milk fat composition in beef cows. The aim of this study was to evaluate the effects of polymorphisms in the fatty acid synthase (*FASN*) and acyl-CoA diacylglycerol acyltransferase (*DGAT1*) gene on milk and colostrum fatty acid composition, and their direct (calf) and indirect (cow/mother) relationships with calf birth weight and weight gain over 14 days. Eight Charolais cows and eight calves were genotyped using the PCR-RFLP method. The cows' milk and colostrum fatty acid profiles and their calves' weight at birth and 14 day weight gain were analysed. In the colostrum, cows with *DGAT1* genotype KA had a significantly higher content of C4:0 and lower C10:0, and with *FASN* diplotype TW/AR a tendency for higher C14:0 and C16:0 colostrum FA content. In the milk, Charolais cows with diplotype TW/AR tended to have higher C14:1, C16:0 and C16:1, but lower C18:0 content. At the same time, no associations were found between *DGAT1* genotypes and the fatty acid composition of milk. There were also no significant associations found between the studied SNPs and the calf's birth weight and weight gain over 14 days, although calves with *DGAT1* KA genotype tended to have higher birth weight.

Key words: milk; colostrum; fatty acid; DGAT1; FASN; Charolais

#### Introduction

In cow-calf production systems, the development and weights of calves are determining factors for productivity and the main source of farm income that greatly depend on both the genetic potential of the calf (direct effect) and the effect of the dam (maternal effect) (PACHECO CONTRERAS et al., 2015; CORTÉS-LACRUZ et al., 2017). The first mammary secretion produced directly before giving birth and over the following days, that then rapidly turns into milk, is defined as colostrum (PUPPEL et al., 2019). Colostrum and milk are the primary food consumed by calves from birth

Maja Maurić Maljković, DVM, PhD, Assist. Prof., Department of Animal Breeding and Livestock Production, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia; Phone: +385 1 2390 235; E-mail: mmauric@vef.unizg.hr

<sup>\*</sup>Corresponding author:

to weaning. Therefore, its production is considered a key component of maternal ability and, in beef cattle, milk production is a major factor influencing pre-weaning growth. Consequently, milk yield and quality have been proposed as significant factors influencing the development of calves during the preweaning period (PACHECO CONTRERAS et al., 2015; CORTÉS-LACRUZ et al., 2017).

During the last few decades, improving milk fat content has been one of the focuses of the dairy industry. However, lately more and more emphasis has been given to its fatty acid composition, as this could have a major impact on human health (GUTIÉRREZ-GIL et al., 2010; BARTOŇ et al., 2016; KALE et al., 2021). Therefore, candidate genes involved in fat synthesis and metabolic pathways are being investigated for potential improvement in milk production and composition traits (MATSUMOTO et al., 2012). Many studies with diverse dairy cattle breeds have indicated FASN and DGAT1 as promising candidate genes for milk fatty acid composition (LI et al., 2016). Fatty acid synthase (FASN), a multifunctional enzyme complex that catalyses the de novo biosynthesis of long-chain fatty acids, has been mentioned as a promising candidate gene for milk and beef fat composition (ABE et al., 2009; SCHENNINK et al., 2009; MATSUMOTO et al., 2012; LI et al., 2016). Two nonsynonymous SNPs with a potential link to lactation traits were found on exon 34 - the A/G substitution at position 5848 that was predicted to cause amino acid substitution from threonine to alanine (T1950A), and the T/C at position 5863 that results in tryptophan to arginine substitution (W1955R; MATSUMOTO et al., 2012). The DGAT1 gene encodes the acyl-CoA diacylglycerol acyltransferase 1 enzyme that catalyses the final step in triglyceride synthesis (PANNIER et al., 2010). A nonconservative dinucleotide polymorphism (AA>GC) causing lysine to alanine substitution (K232A) is associated with variations in milk production, and milk and intramuscular fat content (KUHN et al., 2004; ANTON et al., 2011).

Even though fatty acid composition has become an important trait in dairy production, there has not been a great deal of emphasis on milk fat composition in beef cows. Previous studies have examined the influence of milk yield and composition on preweaning growth and weaning weights (WANG et al., 2009, PACHECO CONTRERAS et al., 2015, SAPKOTA et al., 2020), as well as the fatty acid composition of beef and the influence of different SNPs on it (GUTIÉRREZ-GIL et al., 2010; BARTOŇ et al., 2016; PAPALEO MAZZUCCO et al., 2016). However, the milk fatty acid composition in Charolais cattle, and the influence of different SNPs on it and its relationship with preweaning growth have not yet been assessed. Given the importance of milk quality, the present study aimed to estimate the frequency of the polymorphic alleles of FASN and DGAT1 genes in Charolais cattle. The aim was to evaluate the relationship of these polymorphisms with colostrum and milk fat composition, and their direct (calf) and indirect (cow/mother) relationships with calf birth weight and their weight gain over 14 days.

#### Materials and methods

Animals. A total of eight, clinically healthy Charolais cows and eight calves, kept in a grass-based cow-calf production system, were included in this trial. The animals were kept on pasture most of the year, but during the winter and early spring period (when calvings took place – late March/early April), they were housed in a barn. While housed, the cows were fed a basal TMR diet, consisting of haylage and corn concentrate, and had constant access to drinking water. The calves were kept together with their mothers and allowed to nurse ad libitum. Milk samples were obtained on the 1st (6 h after calving; colostrum) and 14th days of lactation. Calves were weighed at birth and at 14 days of age.

*Genotyping*. Following protocol instructions, DNA was extracted from blood samples using a GenElute<sup>TM</sup> Blood Genomic DNA Kit (Sigma-Aldrich, Germany). The amplification primers and PCR reaction protocol for *FASN* T1950A and W1955R used were according to MATSUMOTO et al. (2012), and for *DGAT1* K232A according to KUHN et al. (2004). The *FASN* PCR products (5 μL) were digested with *Hha* I (T1950A) and *Nci* I (W1955R) restriction enzymes, and *DGAT1* K232A with *Cfr* I restriction enzyme, following

the supplier's manual. The resulting fragments for *DGAT1* (allele K one uncut fragment of 411 bp, allele A two fragments of 208 and 203 bp) and *FASN* (T1950A: allele A one uncut fragment of 336 bp, allele G two fragments of 262 and 74 bp; W1955R: allele T one uncut fragment of 336 bp, allele C two fragments of 247 and 89 bp) were separated on a 3% agarose gel (3 hours/140V). As the genotypes of T1950A corresponded to those of W1955R, as earlier reported by ABE et al. (2009) and MATSUMOTO et al. (2012), they are later analysed as diplotypes (TW/TW, TW/AR, AR/AR).

Analysis of fatty acid composition in milk. Milk fat extraction and fatty acid analysis procedure were done according to MAŠEK et al. (2014). The fatty acid composition was calculated as individual fatty acid percentages relative to the total fatty acids.

Statistical analysis. Calculation of allele and genotype frequencies, polymorphism deviation from Hardy-Weinberg equilibrium, and population genetic indices (observed heterozygosity -  $H_{\rm o}$ , expected heterozygosity -  $H_{\rm E}$  and fixation index -  $F_{\rm IS}$ ) were performed by POPGENE32 software, version 1.32 (YEH et al., 2000). Polymorphism information content (PIC) was calculated according to BOTSTEIN et al. (1980). Statistical analyses were performed using Statistica v.13

(TIBCO Software Inc, 2017). Data were assessed for significance by the Mann-Whitney U-test. Significance was determined at P<0.05.

#### Results

The allelic frequencies, genotypic frequencies, genetic indices and Hardy-Weinberg equilibrium of the FASN and DGAT1 genes are presented in Table 1. The most frequent diplotype of the FASN gene was AR/AR (0.69), with haplotype AR (0.78) more frequent. In DGAT1, the genotype KA prevailed (0.56), whereas the genotype KK was not found. Consequently, allele K was less frequent (0.28). From the two examined genes, a heterozygosity deficiency was found in the FASN gene, while a heterozygosity excess was found in the DGAT1 gene. The distribution of diplotypes / genotypes was in the Hardy-Weinberg equilibrium for the DGAT1 gene (P>0.05), but not for the FASN gene (P=0.045). This could be attributed to the fact that breeding is not random but done with planned bull substitutions. According to the classification of PIC (PIC value < 0.25 - low polymorphism; 0.25 < PIC value < 0.50 - intermediate polymorphism; and PIC value > 0.50 – high polymorphism), the studied loci possessed intermediate genetic diversity.

Table 1. Allele/haplotype and genotype/diplotype frequencies, genetic indices and Hardy-Weinberg equilibrium of *FASN* and *DGAT1* genes.

Gene	Haplotype/ alelle	Haplotype/ allele frequency	Diplotype/ genotype	Diplotype/ genotype frequency	H <sub>o</sub>	$H_{E}$	F <sub>IS</sub>	PIC	H-W (P)
	TW	0.22	TW/TW	0.13		0.35	0.45	0.28	0.045
FASN	AR	0.78	TW/AR	0.19	0.19 0.35				
			AR/AR	0.69					
	K	0.28	KK	0					
DGAT1	A	0.72	KA	0.56	0.56	0.42	-0.39	0.32	0.14
			AA	0.44					

Total FA composition and associations between the studied *FASN* and *DGAT1* polymorphisms and FA composition of colostrum and milk are presented in Tables 2 and 3. In the colostrum, cows with *DGAT1* genotype KA had a significantly higher content of C4:0 and lower C10:0 (P<0.05). For the *FASN* gene, cows with diplotype TW/AR tended to

have higher C14:0 and C16:0 colostrum FA content (P=0.07). In milk, Charolais cows with diplotype TW/AR tended to have higher C14:1, C16:0 and C16:1, while lower C18:0 content (P=0.07). There were no associations found between DGATI genotypes and the FA composition of milk (P>0.05).

Table 2. The effect of *FASN* T1950A and W1955R diplotype and *DGAT1* K232A on colostrum FA composition in Charolais cows. Values are expressed as mean  $\pm$  standard deviation ( $\overline{X} \pm SD$ ).

	Total	FAS	SN	DGAT1		
FA	Total	TW/AR	AR/AR	KA	AA	
	$\overline{X} \pm SD$					
C4:0	$4.12 \pm 1.14$	$3.7 \pm 1.77$	$4.26 \pm 1.05$	5.29 ± 0.53**	$3.42 \pm 0.7**$	
C6:0	$1.18 \pm 0.22$	$1.02 \pm 0.02$	$1.23 \pm 0.23$	$1.12 \pm 0.12$	$1.21 \pm 0.27$	
C8:0	$0.67 \pm 0.15$	$0.59 \pm 0.004$	$0.7 \pm 0.16$	$0.59 \pm 0.04$	$0.72 \pm 0.17$	
C10:0	$1.42 \pm 0.28$	$1.28 \pm 0.2$	$1.47 \pm 0.3$	$1.12 \pm 0.02**$	$1.6 \pm 0.16**$	
C12:0	$2.46 \pm 0.24$	$2.59 \pm 0.03$	$2.41 \pm 0.27$	$2.28 \pm 0.31$	$2.56 \pm 0.14$	
C14:0	$12.57 \pm 1.27$	$13.76 \pm 0.48*$	$12.17 \pm 1.21*$	$12.3 \pm 1.94$	$12.73 \pm 0.92$	
C14:1	$0.91 \pm 0.73$	$0.89 \pm 0.03$	$0.91 \pm 0.87$	$0.56 \pm 0.27$	$1.11 \pm 0.87$	
C15:0	$1.35 \pm 0.24$	$1.29 \pm 0.38$	$1.37 \pm 0.22$	$1.4 \pm 0.36$	$1.32 \pm 0.18$	
C16:0	$37.12 \pm 3.62$	$40.96 \pm 0.01*$	$35.83 \pm 3.24*$	$36.38 \pm 4.05$	$37.57 \pm 3.76$	
C16:1	$3.34 \pm 1.99$	$3.06 \pm 0.46$	$3.44 \pm 2.33$	$2.63 \pm 0.71$	$3.77 \pm 2.46$	
C17:0	$0.92 \pm 0.19$	$0.87 \pm 0.02$	$0.94 \pm 0.23$	$1.05 \pm 0.23$	$0.85 \pm 0.14$	
C18:0	$9.9 \pm 2.13$	$8.32 \pm 1$	$10.43 \pm 2.2$	$10.89 \pm 2.95$	$9.31 \pm 1.56$	
C18:1n9	$19.01 \pm 1.56$	$17.56 \pm 1.61$	$19.5 \pm 1.32$	$18.39 \pm 2.56$	$19.39 \pm 0.7$	
C18:1 t11	$1.88 \pm 0.29$	$1.57 \pm 0.25$	$1.99 \pm 0.24$	$1.95 \pm 0.48$	$1.84 \pm 0.17$	
C18:2n6 cis	$2.16 \pm 1.56$	$1.55 \pm 0.11$	$2.37 \pm 1.8$	$3 \pm 2.55$	$1.66 \pm 0.42$	
C18:3n3	$0.98 \pm 0.16$	$0.98 \pm 0.14$	$0.99 \pm 0.18$	$1.05 \pm 0.17$	$0.94 \pm 0.16$	
SCFA	$7.39 \pm 1.17$	$6.59 \pm 1.55$	$7.66 \pm 1.05$	$8.12 \pm 0.69$	$6.95 \pm 1.23$	
SFA	$71.71 \pm 3.51$	$74.39 \pm 1.69$	$70.81 \pm 3.58$	$72.42 \pm 2.84$	$71.28 \pm 4.11$	
MUFA	$25.15 \pm 3.47$	$23.08 \pm 1.44$	$25.84 \pm 3.76$	$23.53 \pm 1.97$	$26.12 \pm 3.99$	
PUFA	$3.15 \pm 1.67$	$2.53 \pm 0.26$	$3.35 \pm 1.93$	$4.05 \pm 2.69$	$2.6 \pm 0.54$	

FA - % from total FA content; SCFA - short chain fatty acid; SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsturated fatty acid

Table 3. The effect of *FASN* T1950A and W1955R diplotype and *DGAT1* K232A on milk FA composition in Charolais cows. Values are expressed as mean  $\pm$  standard deviation ( $\overline{X} \pm SD$ )

	Total	FA	ISN	DGAT1		
FA	Total	TW/AR	AR/AR	KA	AA	
	$\overline{X} \pm SD$					
C4:0	$1.84 \pm 0.28$	$1.88 \pm 0.08$	$1.82 \pm 0.33$	$1.79 \pm 0.38$	$1.86 \pm 0.25$	
C6:0	$1.94 \pm 0.16$	$1.94 \pm 0.14$	$1.94 \pm 0.18$	$1.96 \pm 0.23$	$1.93 \pm 0.14$	
C8:0	$1.34 \pm 0.15$	$1.31 \pm 0.13$	$1.35 \pm 0.17$	$1.4 \pm 0.2$	$1.31 \pm 0.12$	
C10:0	$2.89 \pm 0.35$	$2.91 \pm 0.34$	$2.88 \pm 0.38$	$3.07 \pm 0.35$	$2.77 \pm 0.32$	
C12:0	$3.25 \pm 0.52$	$3.53 \pm 0.41$	$3.16 \pm 0.55$	$3.18 \pm 0.87$	$3.29 \pm 0.3$	
C14:0	$11.47 \pm 0.58$	$11.86 \pm 0.48$	$11.34 \pm 0.59$	$11.66 \pm 0.17$	$11.36 \pm 0.74$	
C14:1	$0.62 \pm 0.15$	$0.79 \pm 0.06$ *	$0.56 \pm 0.12*$	$0.66 \pm 0.16$	$0.59 \pm 0.16$	
C15:0	$1.85 \pm 0.31$	$1.95 \pm 0.65$	$1.81 \pm 0.21$	$1.61 \pm 0.19$	$1.99 \pm 0.29$	
C16:0	$36.65 \pm 2.77$	$39.59 \pm 0.18*$	$35.67 \pm 2.47*$	$36.83 \pm 2.77$	$36.54 \pm 3.09$	
C16:1	$1.92 \pm 0.36$	$2.36 \pm 0.35*$	$1.77 \pm 0.23*$	$1.96 \pm 0.57$	$1.89 \pm 0.25$	
C17:0	$0.84 \pm 0.11$	$0.79 \pm 0.13$	$0.86 \pm 0.11$	$0.79 \pm 0.11$	$0.87 \pm 0.12$	
C18:0	$13.14 \pm 2.21$	$10.45 \pm 0.06$ *	$14.04 \pm 1.73*$	$12.81 \pm 2.32$	$13.35 \pm 2.4$	
C18:1n9	$16.63 \pm 2.25$	$15.73 \pm 1.22$	$16.93 \pm 2.52$	$16.06 \pm 1.59$	$16.97 \pm 2.68$	

<sup>\*</sup>differing significantly at P<0.1 (within the same gene and row)

<sup>\*\*</sup>differing significantly at P<0.05 (within the same gene and row)

### M. Maurić Maljković et al.: The effects of *FASN* and *DGAT1* gene polymorphism on milk fatty acid composition and growth in Charolais calves

Table 3. The effect of *FASN* T1950A and W1955R diplotype and *DGAT1* K232A on milk FA composition in Charolais cows. Values are expressed as mean  $\pm$  standard deviation ( $\overline{X} \pm SD$ ) (continued)

	Total	FA	ISN	DGAT1		
FA	Total	TW/AR	AR/AR	KA	AA	
	$\overline{X} \pm SD$					
C18:1 t11	$1.41 \pm 0.27$	$1.4 \pm 0.09$	$1.42 \pm 0.31$	$1.37 \pm 0.15$	$1.43 \pm 0.33$	
C18:2n6 cis	$3.37 \pm 1.06$	$2.66 \pm 0.82$	$3.61 \pm 1.08$	$4 \pm 1.09$	$3 \pm 0.94$	
C18:3n3	$0.85 \pm 0.09$	$0.86 \pm 0.05$	$0.85 \pm 0.11$	$0.84 \pm 0.1$	$0.85 \pm 0.1$	
SCFA	$8.01 \pm 0.75$	$8.04 \pm 0.69$	$8 \pm 0.83$	$8.23 \pm 0.89$	$7.88 \pm 0.73$	
SFA	$75.21 \pm 1.91$	$76.22 \pm 2.49$	$74.87 \pm 1.82$	$75.11 \pm 0.76$	$75.27 \pm 2.46$	
MUFA	$20.58 \pm 2.39$	$20.27 \pm 1.72$	$20.68 \pm 2.72$	$20.05 \pm 1.93$	$20.89 \pm 2.8$	
PUFA	$4.22 \pm 1.08$	$3.51 \pm 0.77$	$4.46 \pm 1.11$	$4.84 \pm 1.19$	$3.85 \pm 0.93$	

FA - % from total FA content; SCFA - short chain fatty acid; SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsturated fatty acid

The effects of the cow's and calf's FASNT1950A and W1955R diplotype and DGAT1 K232A on the birth weight and weight gain over 14 days of calves are presented in Tables 4 and 5. The only effect

found was the effect of the calf's *DGAT1* gene on its birth weight – the calves with the KA genotype tended to have higher birth weight (P=0.07).

Table 4. The effects of the cow's/mother's FASN T1950A and W1955R diplotype and DGAT1 K232A on the calf's birth weight and weight gain over 14 days. Values are expressed as mean  $\pm$  standard deviation.

		FA	ISN	DGAT1		
	Total	$TW/AR$ $\overline{X}\pm SD$	$\begin{array}{c} AR/AR \\ \overline{X} \pm SD \end{array}$	$KA \over \overline{X} \pm SD$	$AA$ $\overline{X} \pm SD$	
Birth weight (kg)	$54.5 \pm 3.63$	$54 \pm 4.24$	54.67 ±3.83	$55 \pm 1.73$	$54.2 \pm 4.6$	
Weight gain in 14 days (kg)	$15.86 \pm 3.16$	$14.75 \pm 0.35$	$16.3 \pm 3.75$	$14.67 \pm 3.75$	$16.75 \pm 2.84$	

Table 5. The effects of the calf's FASN T1950A and W1955R diplotype and DGAT1 K232A on birth weight and weight gain over 14 days. Values are expressed as mean  $\pm$  standard deviation.

		FASN			DGAT1	
	Total	TW/TW \bar{X} \pm SD	TW/AR#	$\begin{array}{c} AR/AR \\ \overline{X} \pm SD \end{array}$	$KA$ $\overline{X} \pm SD$	$AA$ $\overline{X} \pm SD$
Birth weight (kg)	$54.5 \pm 3.63$	$54 \pm 4.24$	55	$54.6 \pm 4.28$	56 ± 2.68*	50 ± 1.41*
Weight gain in 14 days (kg)	$15.86 \pm 3.16$	$14.75 \pm 0.35$	15.5	$16.5 \pm 4.3$	$15 \pm 2.69$	18 ± 4.24

#excluded from further analysis

<sup>\*</sup> differing significantly at P<0.1

#### Discussion

While information exists about the influence of DNA polymorphism on intramuscular FA composition (ABE et al., 2009; GUTIÉRREZ-GIL et al., 2010; PAPALEO MAZZUCCO et al., 2016), there is almost no data on its influence on milk and, especially, colostrum FA composition in beef cattle. Therefore, in our study we explored genotypic frequencies for the *FASN* and *DGAT1* gene and their influence on the fatty acid composition of milk and colostrum in purebred Charolais cattle, as well as their direct (calf) and indirect (cow/mother) relationships with calf birth weight and their weight gain over 14 days.

In our research, the TW haplotype was less frequent in the FASN gene. Similar results for the TW haplotype were reported by ABE et al. (2009) in Holstein (0.171), Angus (0.015), and Hereford (0.071) breeds, while the same authors found a frequency of 0.667 in Japanese Black. The same allele was also found in a frequency of 0.37 (CIECIERSKA et al., 2013) in the Holstein breed and 0.225 in Fleckvieh bulls (BARTOŇ et al., 2016). Regarding the DGAT1 gene, allele K being the less frequent was in agreement with some earlier findings for Charolais (0.18), as well as for Simmental (0.06), Limousin (0.12), Friesian (0.13), Angus (0.18), Hereford (0), Belgian Blue (0.03), Blond d'Aquitaine (0.17), Salers (0) (PANNIER et al. 2010) and Fleckvieh cattle (0.058) (BARTOŇ et al., 2016), while others found a frequency of 0.82 in Charolais (AVILÉS et al., 2013), 0.84 and 0.64 in Limousin (AVILÉS et al., 2013; SEDYKH et al., 2021), and 0.64 in Hereford (SEDYKH et al., 2021). This wide range of frequencies indicates the considerable genetic variability of these SNPs in different breeds and populations (CIECIERSKA et al., 2013).

The fatty acid composition of colostrum and milk was not influenced very much by the researched SNPs of *FASN* and *DGAT1* genes. This may be partially due to the low number of animals involved in the study. For the *FASN* gene, only tendencies (P=0.07) were found between diplotypes and milk FA composition (Table 3). In dairy cattle, SCHENNINK et al. (2009) reported that the T1950A (A>G) GG genotype decreased

C14:0 and increased C18:1n9 in Dutch Holstein-Friesian cows. In contrast to this, LI et al. (2016) reported that in Chinese Holstein cattle the T1950A (A>G) GA genotype had higher C10:0, C12:0 and C14:0 than the GG genotype. Other researchers found that the TW/AR diplotype increased C18:1n9 while decreased C14:0 and C14:1 in Simmental cattle (MAURIC et al., 2019). From all this, it may be concluded that the association of this FASN SNP with milk production traits varies depending on breed and population. Although there were no associations found between DGAT1 genotypes and FA composition of milk (P>0.05) in this study, other authors found different results in dairy cattle. SCHENNINK et al. (2007) found that allele K of SNP K232A led to an increase in C16:0 and a decrease in C14:0 and unsaturated C18 in Dutch Holstein Friesian cows. These results were in agreement with those found by BOVENHUIS et al. (2016) in Danish Jersey, and Danish and Dutch Holstein, and by JUHLIN et al. (2012) in Swedish Holstein and Swedish Red.

Interest in the composition of cow colostrum is, for the most part, orientated towards the macro components, with more in-depth research focusing on the protein content, particularly immunoglobulins. One of the least considered colostrum components is fat, and even in studies on colostrum fat, the main attention has been focused on its total content and not its composition (CONTARINI et al., 2014; O'CALLAGHAN et al., 2020). To our knowledge, there are no studies focused on the influence of FASN and DGAT1 genes on the FA composition of colostrum, whether in dairy or in beef cattle. The results of this study show that cows with DGAT1 genotype KA had a significantly higher content of C4:0 and lower C10:0 (P<0.05), and those with FASN diplotype TW/AR tended to have higher C14:0 and C16:0 colostrum FA content (P=0.07). Considering that the most critical time for calves, during which most problems occur from disorders of the digestive system due to poor colostrum quality or poor husbandry, is the first two weeks (PUPPEL et al., 2019), more importance should be ascribed to colostrum fat quality and composition. Colostrum fat provides energy for heat production to maintain body temperature (thermogenesis), and FA oxidation helps continue active gluconeogenesis to maintain glucose homeostasis (HAMMON et al., 2012). Moreover, some FAs are beneficial for their nutritional properties and specific health effects (HILL et al., 2011).

In beef cattle, the uterine environment during pregnancy, colostrum and milk are considered to be the essential maternal effects influencing preweaning calf growth (PACHECO CONTRERAS et al., 2015; CORTÉS-LACRUZ et al., 2017). As both investigated genes are involved in fatty acid synthesis, their genetic variations could influence not only milk quality but also fatty acid composition and intramuscular fat level (SCHENNINK et al., 2007; GUTIÉRREZ-GIL et al., 2010; ANTON et al., 2011, BARTON et al., 2016; PAPALEO MAZZUCCO et al., 2016). Based on the knowledge of genetic control regulating fatty acid metabolic pathways, the present study investigated whether there are also direct (calf) and indirect (cow/mother) relationships of FASN T1950A and W1955R and DGAT1 K232A with calf birth weight and their weight gain in the most critical period, the first 14 days (Tables 4 and 5). Among the examined polymorphisms and effects, only the effect of the calf's DGAT1 gene on its birth weight was found, where the calves with the KA genotype had a tendency for higher birth weight (P=0.07).

#### **Conclusions**

In this study, not many associations were found between the investigated SNPs of *FASN* and *DGAT1* genes and the studied traits. It must be noted that this study was conducted on a small number of animals due to the specificity of the cow-calf production system, in which it might be challenging to obtain all the necessary data. Nevertheless, these results indicate potential associations between the genes analysed and the FA composition of colostrum and milk, as well as the calf's birth weight and weight gain over 14 days. Thus, further investigation is warranted in a larger population, as the development and weight of calves are determining factors for productivity, and the main source of farm income.

#### References

ABE, T., J. SABURI, H. HASEBE, T. NAKAGAWA, S. MISUMI, T. NADE, H. NAKAJIMA, N. SHOJI, M. KOBAYASHI, E. KOBAYASHI (2009): Novel Mutations of the *FASN* Gene and Their Effect on Fatty Acid Composition in Japanese Black Beef. Biochem. Genet. 47, 397-411.

DOI: 10.1007/s10528-009-9235-5

- ANTON, I., K. KOVACS, G. HOLLO, V. FARKAS, L. LEHEL, Z. HAJDA, A. ZSOLNAI (2011): Effect of leptin, DGAT1 and TG gene polymorphisms on the intramuscular fat of Angus cattle in Hungary. Livest. Sci. 135, 300-303. DOI: 10.1016/j.livsci.2010.07.012
- AVILÉS, C., O. POLVILLO, F. PEÑA, M. JUÁREZ, A. L. MARTÍNEZ, A. MOLINA (2013): Associations between *DGAT1*, *FABP4*, *LEP*, *RORC*, and *SCD1* gene polymorphisms and fat deposition in Spanish commercial beef. J. Anim. Sci. 91, 4571-4577.

  DOI: 10.2527/jas.2013-6402
- BARTOŇ, L., D. BUREŠ, T. KOTT, D. ŘEHÁK (2016): Associations of polymorphisms in bovine DGAT1, FABP4, FASN, and PPARGC1A genes with intramuscular fat content and the fatty acid composition ofmuscle and subcutaneous fat in Fleckvieh bulls. Meat Sci. 114, 18-23. DOI: 10.1016/j.meatsci.2015.12.004
- BOTSTEIN, D., R. L. WHITE, M. SKOLNICK, R. W. DAVIS (1980): Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32, 314–331.
- BOVENHUIS, H., M. H. P. W. VISKER, N. A. POULSEN, J. SEHESTED, H. J. F. VAN VALENBERG, J. A. M. VAN ARENDONK, L. B. LARSEN, A. J. BUITENHUIS (2016): Effects of the diacylglycerol o-acyltransferase 1 (DGAT1) K232A polymorphism on fatty acid, protein, and mineral composition of dairy cattle milk. J. Dairy Sci. 99, 3113-3123.

DOI: 10.3168/jds.2015-10462

- CIECIERSKA, D., A. FROST, W. GRZESIAK, W. S. PROSKURA, A. DYBUS, A. OLSZEWSKI (2013): The influence of fatty acid synthase polymorphism on milk production traits in Polish Holstein-Friesian cattle. J. Anim. Plant. Sci. 23, 376-379.
- CONTARINI, G., M. POVOLO, V. PELIZZOLA, L. MONTI, A. BRUNI, L. PASSOLUNGO, F. ABENI L. DEGANO (2014): Bovine colostrum: Changes in lipid constituents in the first 5 days after parturition. J. Dairy Sci. 97, 5065-5072.

DOI: 10.3168/jds.2013-7517

CORTÉS-LACRUZ, X., I. CASASÚS, R. REVILLA, A. SANZ, M. BLANCO, D. VILLALBA (2017): The milk yield of dams and its relation to direct and maternal genetic

components of weaning weight in beef cattle. Livest. Sci. 202, 143-149.

DOI: 10.1016/j.livsci.2017.05.025

GUTIÉRREZ-GIL, B., P. WIENER, R. I. RICHARDSON, J. D. WOOD, J. L. WILLIAMS (2010): Identification of QTL with effects on fatty acid composition of meat in a Charolais? Holstein cross population. Meat Sci. 85, 721-729

DOI: 10.1016/j.meatsci.2010.03.031

HAMMON, H. M., J. STEINHOFF-WAGNER, U. SCHÖNHUSEN, C. C. METGES, J. W. BLUM (2012): Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. Domest. Anim. Endocrinol. 43, 171-185.

DOI: 10.1016/j.domaniend.2012.02.005

HILL, T. M., M. J. VANDEHAAR, L. M. SORDILLO, D. R. CATHERMAN, H. G. BATEMAN II, R. L. SCHLOTTERBECK (2011): Fatty acid intake alters growth and immunity in milk-fed calves. J. Dairy Sci. 94, 3936-3948.

DOI: 10.3168/jds.2010-3935

JUHLIN, J., W. F. FIKSE, J. PICKOVA, A. LUNDÉN (2012): Association of *DGAT1* genotype, fatty acid composition, and concentration of copper in milk with spontaneous oxidized flavor. J. Dairy Sci. 95, 4610-4617.

DOI: 10.3168/jds.2011-4915

KALE, D. S., J. SINGH, Y. B. SATHE, D. V. PATIL (2021): FASN Gene And Its Role In Bovine Milk Production. Int. J. Biotech Trends Technol. 11, 20-25.

DOI: 10.14445/22490183/IJBTT-V11I1P604

KUHN, C., G. THALLER, A. WINTER, O. R. P. BININDA-EMONDS, B. KAUPE, G. ERHARDT, J. BENNEWITZ, M. SCHWERIN, R. FRIES (2004): Evidence for multiple alleles at the DGAT1 locus better explains a quantitative trait locus with major effect on milk fat content in cattle. Genetics 167, 1873-1881.

DOI: 10.1534/genetics.103.022749

LI, C., D. SUN, S. ZHANG, M. A. ALIM, Q. ZHANG, Y. LI, L. LIU (2016): Genetic effects of *FASN*, *PPARGC1A*, *ABCG2* and *IGF1* revealing the association with milk fatty acids in a Chinese Holstein cattle population based on a post genomewide association study. BMC Genetics 17, 110.

DOI: 10.1186/s12863-016-0418-x.

MAURIC, M., T. MASEK, D. BENDELJA LJOLJIC, J. GRBAVAC, K. STARCEVIC (2019): Effects of different variants of the *FASN* gene on production performance and milk fatty acid composition in Holstein × Simmental dairy cows. Vet. Med. 64, 101-108.

DOI: 10.17221/73/2018-VETMED

MAŠEK, T., L. KRSTULOVIC, D. BROZIC, M. VRANIC, M. MAURIC, M. BAJIC, K. STARCEVIC (2014): Cow colostrum and early milk enriched with eicosapentaenoic and docosahexaenoic fatty acid. Eur. Food Res. and Technol. 238, 635–640.

DOI: 10.1007/s00217-013-2144-1

MATSUMOTO, H., S. INADA, E. KOBAYASHI, T. ABE, H. HASEBE, S. SASAZAKI, K. OYAMA, H. MANNEN (2012): Identification of SNPs in the FASN gene and their effect on fatty acid milk composition in Holstein cattle. Livest. Sci. 144, 281-284.

DOI: 10.1016/j.livsci.2011.12.003

O'CALLAGHAN, T. F., M. O'DONOVAN, J. P. MURPHY, K. SUGRUE, D. MANNION, W. P. MCCARTHY, M. TIMLIN, K. N. KILCAWLEY, R. M. HICKEY, J. T. TOBIN (2020): Evolution of the bovine milk fatty acid profile e From colostrum to milk five days post parturition. Int. Dairy J. 104, 104655.

DOI: 10.1016/j.idairyj.2020.104655

PACHECO CONTRERAS, V. I., G. M. P. BRACAMONTE, L. A. L. BUSTAMANTE, V. R. M. MEDINA, A. M. SIFUENTES RINCÓN (2015): Milk composition and its relationship with weaning weight in Charolais cattle. R. Bras. Zootec. 44, 207-212.

DOI: 10.1590/S1806-92902015000600002

PANNIER, L., A. M. MULLEN, R. M. HAMILL, P. C. STAPLETON, T. SWEENEY (2010): Association analysis of single nucleotide polymorphisms in *DGAT1*, *TG* and *FABP4* genes and intramuscular fat in crossbred *Bos taurus* cattle. Meat Sci. 85, 515-518.

DOI: 10.1016/j.meatsci.2010.02.025

PAPALEO MAZZUCCO, J., D. E. GOSZCZYNSKI, M. V. RIPOLI, L. M. MELUCCI, A. M. PARDO, E. COLATTO, A. ROGBERG-MUÑOZ, C. A. MEZZADRA, G. J. DEPETRIS, G. GIOVAMBATTISTA, E. L. VILLARREAL (2016): Growth, carcass and meat quality traits in beef from Angus, Hereford and cross-breed grazing steers, and their association with SNPs in genes related to fat deposition metabolism. Meat Sci. 114, 121-129.

DOI: 10.1016/j.meatsci.2015.12.018

PUPPEL, K., M. GOŁEBIEWSKI, G. GRODKOWSKI, J. SLÓSARZ, M. KUNOWSKA-SLÓSARZ, P. SOLARCZYK, M. ŁUKASIEWICZ, M. BALCERAK, T. PRZYSUCHA (2019): Composition and Factors Affecting Quality of Bovine Colostrum: A Review. Animals 9, 1070. DOI: 10.3390/ani9121070

SAPKOTA, D., A. K. KELLY, P. CROSSON, R. R. WHITE, M. MCGEE (2020): Quantification of cow milk yield and pre-weaning calf growth response in temperate pasturebased beef suckler systems: A meta-analysis. Livest. Sci. 241, 104222.

DOI: 10.1016/j.livsci.2020.104222

## M. Maurić Maljković et al.: The effects of *FASN* and *DGAT1* gene polymorphism on milk fatty acid composition and growth in Charolais calves

SEDYKH, T.A., L.A. KALASHNIKOVA, R. S. GIZATULLIN (2021): Carcass quality traits of beef cattle with different *DGAT1* genotypes. IOP Conf. Ser.: Earth Environ. Sci. 624, 012148.

DOI: 10.1088/1755-1315/624/1/012148

SCHENNINK, A., W. M. STOOP, M. H. P. W. VISKER, J. M. L. HECK, H. BOVENHUIS, J. J. VAN DER POEL, H. J. F. VAN VALENBERG, J. A. M. VAN ARENDONK (2007): *DGAT1* underlies large genetic variation in milk-fat composition of dairy cows. Anim. Genet. 38, 467-473. DOI: 10.1111/j.1365-2052.2007.01635.x

SCHENNINK, A., H. BOVENHUIS, K. M. LEON-KLOOSTERZIEL, J. A. M. VAN ARENDONK, M. H. P. W. VISKER (2009): Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Anim. Genet. 40, 909-916. DOI: 10.1111/j.1365-2052.2009.01940.x

WANG, X. Z., M. A. BROWN, F. Q. GAO, J. P. WU, D. L. LALMAN, W. J. LIU (2009): Relationships of Milk Production of Beef Cows to Postweaning Gain of the Calves. Prof. Anim. Sci. 25, 266-272.

DOI: 10.15232/S1080-7446(15)30717-8

YEH, F. C., R. YANG, T. J. BOYLE, Z. YE, J. M. XIYAN (2000): POPGENE 32, Microsoft Window-based Freeware for Population Genetic Analysis, Version 1.32. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Received: 6 October 2021 Accepted: 26 November 2021

MAURIĆ MALJKOVIĆ, M., T. MAŠEK, M. VRANIĆ, K. STARČEVIĆ: Utjecaj polimorfizama gena *FASN* i *DGAT1* na sastav masnih kiselina u kolostrumu i mlijeka krava Charolais pasmine te prirast njihove teladi. Vet. arhiv 92, 389-397, 2022.

#### SAŽETAK

Za razliku od mliječne industrije u kojoj je masnokislinski sastav mlijeka postao važno obilježje, u mesnih krava nema mnogo istraživanja o njegovom sastavu. Cilj ovog istraživanja bio je procijeniti učinke polimorfizama sintaze masnih kiselina (FASN) i acylCoA diacilglicerol aciltransferaze (DGAT1) na sastav masnih kiselina mlijeka i kolostruma te njihov izravnan (tele) i neizravan (krava/majka) utjecaj na tjelesnu masa teladi pri porođaju i prirast u prvih 14 dana. Metodom PCR-RFLP genotipizirano je ukupno 16 goveda Charolais pasmine (8 krava i 8 teladi). Analiziran je masnokiselinski sastav mlijeka i kolostruma te porođajna masa teladi i prirast u prvih 14 dana. Krave *DGAT1* genotipa KA imale su znakovito veći udio C4:0 i niži C10:0, a krave *FASN* diplotipa TW/AR imale su tendenciju većeg udjela C14:0 i C16:0 u kolostrumu. U mlijeku, krave s diplotipom TW/AR imale su tendenciju za većim udjelom C14:1, C16:0 i C16:1, te nižim udjelom C18:0. Između genotipova *DGAT1* i sastava masnih kiselina mlijeka nije pronađena povezanost. S obzirom na porođajnu masu i prirast u prvih 14 dana, jedina pronađena povezanost bila je u teladi s genotipom *DGAT1* KA koja su imala tendenciju veće porođajne mase.

Ključne riječi: mlijeko; kolostrum; masne kiseline; DGATI; FASN; Charolais