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ACETYL-CoA CARBOXYLASE α AND STEAROYL-CoA DESATURASE GENES POLYMORPHISM AND THEIR INFLUENCE ON FATTY ACID PROFILE IN MILK OF POLISH HOLSTEIN-FRIESIAN COWS*

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Abstract

The aim of the study was to analyse the association of *ACACA* and *SCD1* polymorphism with milk composition, fatty acid profile in milk fat and milking performance of Polish Holstein-Friesian cows. The animals were divided according to criteria: lactation – 1st, 2nd, 3rd, 4th; *ACACA* polymorphism – CC, CG, GG; *SCD1* polymorphism – AA, VA, VV. The presence of A293V polymorphism of *SCD1* gene in the population of Polish Holstein-Friesian cattle has been confirmed. In the analysed fragment of *ACACA* gene presence of a novel SNP has been revealed. The SNP AJ312201.1g.1488C>G consists of a substitution G>C in 1488 position. This *ACACA* polymorphism influenced C13:0, C14:1, C16:1 and CLA, while the analysed *SCD1* polymorphism influenced C14:1. Interestingly, C16:0, C18:0 and C14:1 were influenced by fat content; while C16:1 was influenced by lactation stage; and CLA was influenced by both lactation stage and fat content. Although the novel SNP on *ACACA* gene and A293V on *SCD1* showed only slight influence on fatty acid profile in this study, these genes are still potential candidate genes for fat content and composition in milk, but require further research.

Key words: fatty acids, *ACACA*, *SCD1*, milk, cattle

Fatty acids play an important role in many biological reactions in the body, e.g. in synthesis of glycerophospholipids and sphingolipids, important compounds of cell membranes (Hames and Hooper, 2012). As milk is a significant component of human diet, in recent years more and more attention is paid to milk fat of cows, in particu-

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lar to the participation of unsaturated fatty acids. Long chain fatty acids (more than 16 carbon atoms) enter milk from blood. *De novo* synthesis of fatty acids in milk-producing cells include short and medium chain fatty acids (from 4 to 16 carbon atoms). Conversion of saturated to monounsaturated fatty acids (C10:0–C18:0) may also occur in the mammary gland (Conte et al., 2010). Fatty acid synthesis involves a great number of enzymes, including: Acetyl-CoA Carboxylase α, Fatty Acid Synthase, Stearoyl-CoA Desaturase and Diglyceride Acyltransferase 1 (Shingfield et al., 2010; Kęsek et al., 2014).

Acetyl-CoA Carboxylase α catalyzes the first step of fatty acids synthesis in the cytosol, i.e. the carboxylation of Acetyl-CoA to Malonyl-CoA. This enzyme is encoded by *ACACA* gene which is located on chromosome 19 q13–14 in cattle (Mao et al., 2001). Its transcription is controlled by four promoters PI, PIA, PII, and PIII (Barber et al., 2005). Promoters PI (human) and PIA (rodents and ruminants) are responsible for gene expression in nervous system and white adipose tissue. Promoter PII (mammals) is transcribed in all tissues and is considered as a housekeeping gene (Mao and Seyfert, 2002). Transcript of the promoter PIII is detected in humans and ruminants, and it is responsible for gene expression in the mammary gland during lactation (Mao et al., 2002). Zhang et al. (2009) analysed the impact of *ACACA* gene polymorphism on fatty acid composition in beef, while Moioli et al. (2013) and Signorelli et al. (2009 a) in milk of sheep and goat, respectively. The only study on dairy cattle was conducted by Matsumoto et al. (2012).

Stearoyl-CoA Desaturase is encoded by *SCD* gene located on the long arm of chromosome 26 in cattle, and shows *SCD1* isoform, which is expressed in adipose and milk producing tissues (Lengi and Corl, 2007; Schennink et al., 2008). The *SCD* gene is approx. 17 kbp in length and is composed of 6 exons, plus 5 introns; it encodes a protein of 359 amino acids. Northern Blot analysis showed that a single transcript of approx. 5 kbp is present in the mammary gland and adipose tissue, which was characterised by an unusually long 3`UTR region of 3.8 kbp (Bernard et al., 2001). Stearoyl-CoA desaturase is responsible for the addition of a double bond between the 9th and the 10th carbon atom in the carbon chain (Pereira et al., 2003). A well-known *SCD1* gene polymorphism is the substitution A293V placed on exon 5, consisting of replacement of T with C, and resulting in exchange of valine for alanine. Its association with milk fat and fatty acid profile in milk of cows has been described by many authors (Moioli et al., 2007; Schennink et al., 2008; Kgwatalala et al., 2009; Signorelli et al., 2009 b; Conte et al., 2010; Bouwman et al., 2011; Duchemin et al., 2013).

Milk composition and fatty acid profile depend also on many non-genetic factors, such as feeding, milk performance or lactation stage and energy status (Stoop et al., 2009; Kęsek et al., 2014). The aim of the study was to analyse the association of lactation and polymorphisms of *ACACA* and *SCD1* (A293V) genes with milk composition, fatty acids profile in milk fat and milking performance of Polish Holstein-Friesian cows.

Material and methods

Animals

The material consisted of blood and milk samples of Polish Holstein-Friesian cows (n=100) in the first phase of lactation (20–100 DIM). Cows were fed Total Mixed Ration with corn silage as primary forage component. All blood and milk samples were collected the same day. All experimental procedures were licensed by the 2nd Local Ethics Committee at Wrocław University of Environmental and Life Sciences, Poland.

Milk analyses

Protein, fat, lactose, solids percentages were determined in all milk samples with use of Infrared Milk Analyser 150 (Bentley Instruments, Chaska, MN, USA). The fatty acid profile was determined using 7890 gas chromatograph with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). Milk fat was extracted by the Folch method (Folch et al., 1957). Esterification was performed using KOH in methanol-hexane. For separation of the obtained esters of fatty acids a HP–88 capillary column (100 m × 0.25 mm × 250 μm) was used. The oven temperature from initial isotherm of 100°C to 140°C increased at a rate of 4°C/min.; and then to 240°C at a rate of 2°C/min. The FAME mixture (1 μl) was automatically injected in split mode 20:1 with injector temperature of 250°C and detector temperature of 270°C. Helium was the carrier. Identification of particular fatty acids esters was performed on the basis of standard mixture of methyl-esters of fatty acids Supelco™ 37 (Sigma Aldrich, St Louis, MO, USA) plus CLA *cis*-9*, trans*-11 and *trans*-10*, cis*-12 (Larodan, Malmö, Sweden), with use of Agilent ChemStation software (Agilent Technologies).

ACACA and *SCD1* genes polymorphism

The polymorphism in the non-coding region for the *ACACA* gene will be indicated with the nucleotide name, while the SNP in the coding region of the *SCD1* gene will be indicated using the name of the coded amino acid. DNA was isolated from blood with use of GenElute™ Blood Genomic DNA Kit (Sigma Aldrich) according to manufacturer's instructions. The analysis of polymorphism (SNP) in *ACACA* gene was based on publication by Matsumoto et al. (2012). A 344 nucleotide fragment, nearing the fragment analysed by Matsumoto et al. (2012), was chosen from GenBank AJ312201.1, which corresponds to: *Bos taurus* partial acc1 gene for acetyl-CoA-carboxylase alpha, exon 5A (mammary gland specific isoform) and is composed of 3690 nucleotides. Gene amplification by PCR of random DNA samples was performed (n=9). PCR reactions were carried out using HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany), buffer (10X PCR Buffer, and Q-Solution), dNTPs mix (Qiagen), primers, deionized water, and the DNA template. Thermal program for the reaction in the thermocycler was empirically refined (Veriti® Thermal Cycler, Applied Biosystems, Foster City, CA, USA). In order to verify presence and quality of the amplified gene, an electrophoresis on 2% agarose gel for 60 min. at 65 V was carried out. After the reaction, the PCR products were purified using USB ExoSAP-IT PCR Product Clean-Up (Affymetrix, Wycombe Lane, Wooburn Green,

UK). Sequencing reactions were performed with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) by modified methods. Then the sequencing reaction products were purified using BigDye xTerminator Purification Kit (Applied Biosystems). Reading of sequences was performed in 3130xl Genetic Analyser (Applied Biosystems). After detection of SNP on *ACACA* gene, a restriction enzyme was selected in NEBcutter V2.0 tool (Vincze et al., 2003). The remaining samples (n=38) were analysed by the restriction fragment length polymorphism method (PCR-RFLP) with HpyF10VI (MwoI) restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA). Primers were designed using Primer3Plus (Untergasser et al., 2007): forward 5'-ctgccggataaactgctacgtca-3', reverse 5'-gaggcactctcgggaatcaagct-3'.

Single Nucleotide Polymorphism A293V on *SCD1* gene was chosen for analysis. Primers (Heck, 2009) and restriction enzyme (Matsuhashi et al., 2011) for this SNP were chosen from the literature. Its presence in the population was determined by PCR-RFLP with NcoI restriction enzyme (Thermo Fisher Scientific). The primers for *SCD1* gene were: forward 5'-tcatttaacccctcattacctca-3', reverse 5'-tgtaaaatactag-gctttctgg-3'.

For the amplification of the examined fragments by PCR-RFLP, Maxima™ Hot Start Green PCR Master Mix (2X) (Thermo Fisher Scientific) was used according to the manufacturer's instructions. The PCR reactions were performed on Thermal Cycler Bio-Rad C1000 (Bio-Rad Laboratories, Hercules, CA, USA) according to the following procedures:

– for *ACACA*: initial denaturation (94°C for 4 min.), 35 PCR cycles (94°C for 30 s; 62°C for 30 s; 72°C for 30 s), final elongation (72°C for 10 min.),
– for *SCD1*: initial denaturation (94°C for 5 min.), 36 PCR cycles (94°C for 30 s; 55°C for 45 s; 68°C for 90 s), final elongation (68°C for 10 min.).

Then the restriction enzyme digestion of the PCR products was performed. The reaction mixture was incubated at 37°C for 4 hours. Restriction enzyme digestion products were separated by electrophoresis on 1.5% agarose gel for 30 min. at 100 V.

Statistical analysis

The animals were divided according to the following criteria: lactation (L) – 1st, 2nd, 3rd, 4th; *ACACA* polymorphism – CC, CG, GG; *SCD1* polymorphism – AA, VA, VV; fat class (F) – 1 (<4.25%), 2 (>4.25%). The statistical analysis was performed by mixed-effects models' ANOVA using the following model:

$$Y = \mu + A + S + L + F + Si + e$$

where:

Y is phenotypic value of each trait,
μ is the overall mean,
A is the effect of *ACACA* allele (CC – 0; CG – 1; GG – 2),
S is the effect of *SCD1* allele (AA – 0; VA – 1; VV – 2),
L is the effect of lactation,
F is the fat class,
Si is the random effect of sire,
e is the random error.

As post-hoc, the Tukey's test was used. Statistical analysis was performed in R (R Core Team, 2016). Statistically significant differences were accepted at $P \leq 0.05$. Consistence of polymorphisms and alleles frequencies with Hardy-Weinberg's equilibrium was checked using χ^2 test. The association of lactation, polymorphisms and alleles of both SNPs with milk composition, fatty acid profile in milk fat and milk yield of cows in 305-day lactation was analysed.

Results

In the analysed fragment of *ACACA* gene presence of novel SNP has been revealed. The SNP AJ312201.1g.1488C>G (g.1488C>G) consists in a substitution C>G in 1488 position of sequence no. AJ312201.1 (Figure 1). Verification in online tool ORF Finder (www.ncbi.nlm.nih.gov/gorf/gorf) showed that this SNP is located outside any Open Reading Frame. The presence of A293V polymorphism of *SCD1* gene in the analysed population has been confirmed. Three polymorphisms for each SNP were found (g.1488C>G – CC, CG, GG and A293V – AA, VA, VV). Most of animals were homozygous: CC in *ACACA* gene and AA in *SCD1* gene. Frequencies of alleles and polymorphisms are presented in Table 1. No significant deviation from Hardy-Weinberg's equilibrium was found for both SNPs. Such low frequencies of GG in *ACACA* gene and VV in *SCD1* gene may result from human selection of animals targeting milk production which negatively affected the occurrence of alleles G and V.

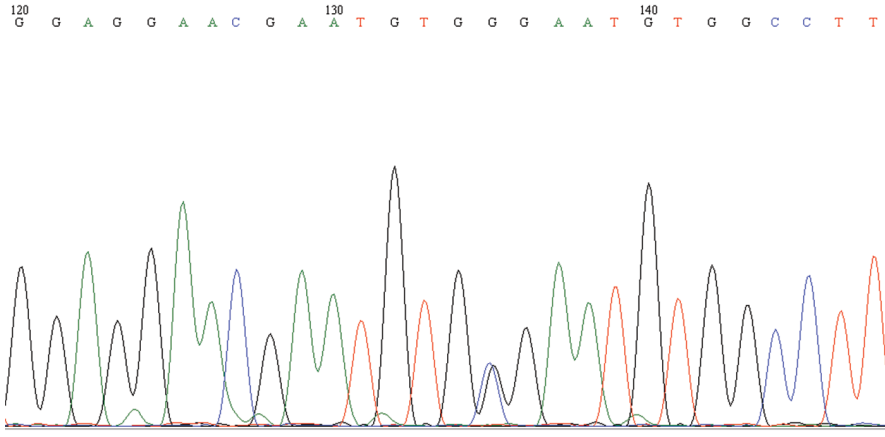

Figure 1. Sequence of AJ312201.1g.1488C>G polymorphism of *ACACA* gene

ANOVA did not show any differences in milk composition among polymorphisms of both SNPs, but showed a few differences in fatty acid profile. Cows with AA (1.08%) on *SCD1* gene had a higher ($P \leq 0.05$) content of C14:1 fatty acid than

cows with VA (0.88%). Whereas *ACACA* gene showed an influence on two fatty acids. ANOVA showed statistically significant influence of *ACACA* on C16:1 (P≤0.01) and C13:0 (P≤0.05) content, although Tukey's post-hoc test did not show any indication of specific differences between pairs. While CLA content was significantly higher (P≤0.01) in milk from cows with CC (0.56%) compared to GG (0.26%).

Table 1. Frequencies of alleles and polymorphisms of SNP J312201.1g.1488C>G of *ACACA* gene and SNP A293V of *SCD1* gene with χ^2 test and P-value

	ACACA AJ312201.1g.1488C>G			*SCD1* A293V		
Alleles frequencies	C		0.79	A		0.77
	G		0.21	V		0.23
Expected Hardy-Weinberg frequencies	n=63	CC	0.63	n=59	AA	0.576
	n=32	CG	0.32	n=33	VA	0.358
	n=4	GG	0.04	n=7	VV	0.056
x^2 test		0.0006			0.623	
P-value		0.979			0.43	

P-value with 1 degree of freedom.

Concerning the remaining analysed factors, the fat class influenced C16:0 content, which was higher (P≤0.05) in milk from cows with <4.25% of milk fat (30.83%) compared to cows with >4.25% (29.58%). The same relation (P≤0.05) was observed for C14:1, cows with <4.25% had more (1.02%) of this acid in milk than cows with >4.25% (0.92%). Also CLA content was significantly higher (P≤0.05) in milk from cows with <4.25% (0.48%) compared to those with higher fat percentage (0.41%). Contrary to C18:0, the content of which was significantly (P≤0.01) lower in milk with lower fat percentage (10.34%) than in milk with higher fat percentage (11.37%). Lactation influenced highly significantly C16:1 content, higher lactation – higher content (in 1st – 1.99%; in 4th – 3.77%). Contrary to CLA, the content of which was the highest (P≤0.01) in 1st lactation (0.67%) compared to the three others, and significantly (P≤0.05) higher between the 3rd (0.40%) and 4th (0.28%) lactations. Also, C20:1 content was related to lactation number. ANOVA showed significantly higher (P≤0.05) content that was found in milk from primiparous cows compared to the others, although Tukey's post-hoc test did not show any indication of specific differences between pairs. Summarizing, C16:0, C18:0 and C14:1 were influenced by fat content; while C16:1 was influenced by lactation stage; and CLA was influenced by both lactation stage and fat content. Moreover, *SCD1* polymorphism influenced C14:1, while *ACACA* polymorphism influenced C13:0, C14:1, C16:1 and CLA. Milk composition and percentages of fatty acids in milk depending on fat class and lactation are presented in Table 2, while milk composition and percentages of fatty acids in milk depending on polymorphisms are presented in Table 3. Milk performance depending on polymorphisms and fat class is presented in Table 4.

Table 2. Percentage (mean±SE) of fatty acids, depending on lactation and fat class

	Lactation				ANOVA	Fat class		ANOVA
	1st	2nd	3rd	4th	P-value	<4.25%	>4.25%	P-value
1	2	3	4	5	6	7	8	**9**
Milk fat	4.39±0.33	4.34±0.28	4.27±0.36	3.76±0.53	0.74	-	-	-
Proteins	2.94±0.16	3.11±0.14	3.20±0.18	3.20±0.26	0.70	3.15±0.12	3.08±0.12	0.40
Lactose	4.97±0.1	4.83±0.08	5.03±0.11	5.01±0.16	0.09	4.95±0.08	4.97±0.08	0.67
C4:0	1.03±0.15	0.84±0.13	0.87±0.16	0.93±0.24	0.84	0.85±0.11	0.99±0.11	0.09
C6:0	1.00±0.11	1.05±0.09	1.05±0.12	1.08±0.18	0.98	0.99±0.08	1.10±0.08	0.09
C8:0	0.74±0.09	0.87±0.07	0.87±0.1	0.87±0.14	0.75	0.82±0.07	0.86±0.06	0.52
C10:0	1.84±0.29	2.39±0.25	2.45±0.32	2.59±0.47	0.49	2.34±0.22	2.29±0.21	0.78
C12:0	2.29±0.36	2.91±0.3	2.99±0.39	3.19±0.58	0.53	2.94±0.27	2.75±0.26	0.34
C13:0	0.09±0.02	0.11±0.02	0.13±0.03	0.20±0.04	0.10	0.14±0.02	0.12±0.02	0.28
C14:0	8.74±0.84	9.43±0.7	9.47±0.91	9.87±1.35	0.90	9.77±0.63	8.99±0.61	0.10
C15:0	1.02±0.11	0.95±0.09	0.96±0.12	1.17±0.18	0.71	1.07±0.08	0.98±0.08	0.13
C16:0	31.63±1.09	28.99±0.91	29.37±1.18	30.85±1.75	0.33	30.83±0.8 a	29.58±0.79 a	**≤0.05**
C17:0	0.58±0.04	0.62±0.04	0.60±0.05	0.70±0.07	0.50	0.61±0.03	0.64±0.03	0.18
C18:0	10.65±0.68	11.46±0.57	10.93±0.74	10.43±1.1	0.57	10.34±0.51 A	11.39±0.5 A	**≤0.01**
C20:0	0.13±0.02	0.12±0.02	0.12±0.02	0.09±0.03	0.61	0.11±0.01	0.12±0.01	0.37
SFA	59.73±2.23	59.79±1.86	59.86±2.41	61.99±3.59	0.94	60.84±1.66	59.84±1.63	0.42
C14:1	0.99±0.08	1.00±0.07	0.94±0.09	0.95±0.13	0.83	1.02±0.06 a	0.92±0.06 a	**≤0.05**
C16:1	1.99±0.23 ABC	3.36±0.19 A	3.26±0.25 B	3.77±0.37 C	≤0.01	3.14±0.17	3.05±0.17	0.44
C17:1	0.35±0.06	0.43±0.05	0.37±0.07	0.46±0.1	0.52	0.40±0.05	0.41±0.04	0.71
C18:1 ω-9 c	26.47±2.07	23.60±1.73	23.37±2.23	22.43±3.33	0.71	23.40±1.54	24.53±1.51	0.32
C18:1 ω-8 c / C18:1 ω-11 c	1.14±0.12	1.18±0.1	1.20±0.13	1.23±0.19	0.97	1.15±0.09	1.22±0.09	0.28
C18:1 ω-9 t	0.44±0.06	0.57±0.05	0.55±0.05	0.45±0.1	0.46	0.52±0.05	0.49±0.05	0.37
C18:1 ω-7 t	2.28±0.32	1.99±0.26	2.31±0.34	1.64±0.51	0.43	2.08±0.24	2.03±0.23	0.80
other 18:1	0.19±0.12	0.44±0.1	0.37±0.13	0.24±0.19	0.40	0.34±0.09	0.27±0.09	0.30

Table 2 – contd.

1	2	3	4	5	6	7	8	9
MUFA	33.84±2.26	32.55±1.89	32.37±2.44	31.16±3.64	0.94	32.05±1.68	32.92±1.65	0.49
C18:2 ω-6 c	2.09±0.17	2.53±0.15	2.57±0.19	2.74±0.28	0.20	2.51±0.13	2.46±0.13	0.60
CLA	0.67±0.06 ABC	0.43±0.05 A	0.39±0.06 Ba	0.28±0.09 Ca	**≤0.01**	**≤0.05**	0.48±0.04 a	0.41±0.04 a	**≤0.05**
C18:3 ω-3	0.31±0.02	0.34±0.02	0.34±0.02	0.32±0.04	0.70	0.32±0.02	0.33±0.02	0.55
C20:1	0.25±0.03	0.16±0.02	0.17±0.03	0.08±0.04	**≤0.05**	0.16±0.02	0.17±0.02	0.26
C20:4 ω-6	0.14±0.03	0.13±0.03	0.15±0.03	0.15±0.05	0.88	0.15±0.02	0.13±0.02	0.51
PUFA	3.45±0.21	3.59±0.17	3.62±0.23	3.57±0.34	0.95	3.61±0.16	3.50±0.15	0.35

a, b, c, d – values in rows with different letters differ significantly (P≤0.05); A, B, C, D – as above for P≤0.01. In rows with P-value bolded but without letters indicating differences, the ANOVA showed significant differences, but Tukey's post-hoc test did not show any indication of specific differences between pairs. In the fatty acid name c stands for *cis*; t stands for *trans.*

Table 3. Percentage (mean±SE) of fatty acids, depending on AJ312201.1g.1488C>G polymorphism of *ACACA* gene, A293V polymorphism of *SCD1* gene

	ACACA			ANOVA	*SCD1*			ANOVA
	CC	CG	GG	P-value	AA	VA	VV	P-value
Milk fat	4.28±0.15	4.30±0.23	3.98±0.52	0.81	4.06±0.21	4.33±0.22	4.17±0.41	0.48
Proteins	2.97±0.08	2.95±0.11	3.42±0.26	0.18	3.12±0.1	3.22±0.11	3.00±0.2	0.33
Lactose	4.93±0.05	4.96±0.07	4.99±0.16	0.92	5.02±0.06	4.89±0.07	4.97±0.13	0.16
C4:0	0.94±0.07	1.05±0.11	0.77±0.24	0.30	0.93±0.1	0.86±0.1	0.96±0.19	0.71
C6:0	1.04±0.05	1.12±0.08	0.97±0.17	0.42	0.98±0.07	1.01±0.07	1.13±0.14	0.51
C8:0	0.81±0.04	0.87±0.06	0.84±0.14	0.68	0.76±0.06	0.82±0.06	0.93±0.11	0.27
C10:0	2.09±0.14	2.25±0.21	2.60±0.46	0.51	1.99±0.19	2.29±0.19	2.67±0.37	0.15
C12:0	2.57±0.17	2.73±0.25	3.23±0.57	0.51	2.45±0.23	2.77±0.24	3.32±0.45	0.13
C13:0	0.08±0.01	0.11±0.02	0.20±0.04	**≤0.05**	0.11±0.02	0.11±0.02	0.17±0.03	0.11
C14:0	8.83±0.39	9.41±0.59	9.89±1.32	0.55	8.77±0.54	9.09±0.55	10.28±1.05	0.32
C15:0	0.90±0.05	0.95±0.08	1.23±0.18	0.20	1.02±0.07	0.97±0.07	1.09±0.14	0.59
C16:0	30.23±0.5	29.79±0.76	30.59±1.71	0.76	30.40±0.7	30.62±0.72	29.61±1.36	0.67
C17:0	0.60±0.02	0.58±0.03	0.69±0.07	0.25	0.64±0.03	0.64±0.03	0.59±0.05	0.49
C18:0	10.72±0.31	11.01±0.48	10.86±1.07	0.82	11.00±0.43	11.22±0.45	10.37±0.85	0.47
C20:0	0.12±0.01	0.12±0.01	0.11±0.03	0.92	0.13±0.01	0.12±0.01	0.10±0.02	0.46
SFA	58.93±1.03	60.02±1.56	62.07±3.5	0.62	59.20±1.42	60.56±1.47	61.26±2.78	0.63
C14:1	0.96±0.04	1.04±0.06	0.90±0.13	0.22	1.08±0.05 a	0.89±0.05 a	0.94±0.1	**≤0.05**
C16:1	2.68±0.11	2.73±0.16	3.88±0.36	**≤0.01**	3.02±0.15	3.11±0.15	3.16±0.29	0.81
C17:1	0.42±0.03	0.40±0.04	0.39±0.1	0.91	0.45±0.04	0.42±0.04	0.34±0.08	0.37
C18:1 ω-9 c	26.64±0.96	24.94±1.45	20.32±3.25	0.15	25.41±1.32	24.18±1.37	22.31±2.58	0.44
C18:1 ω-8 c / C18:1 ω-11 c	1.22±0.05	1.20±0.08	1.13±0.18	0.89	1.20±0.07	1.18±0.08	1.18±0.15	0.96
C18:1 ω-9 t	0.42±0.03	0.44±0.05	0.64±0.1	0.11	0.48±0.04	0.53±0.04	0.49±0.08	0.45
C18:1 ω-7 t	2.00±0.15	2.31±0.22	1.85±0.5	0.26	1.81±0.2	2.12±0.21	2.24±0.39	0.33
other 18:1	0.20±0.05	0.25±0.08	0.47±0.18	0.36	0.36±0.07	0.23±0.08	0.33±0.15	0.22

Table 3 – contd.

MUFA	34.55±1.04	33.32±1.58	29.58±3.55	0.38	33.81±1.44	32.64±1.49	30.99±2.82	0.56
C18:2 ω-6 c	2.51±0.08	2.42±0.12	2.52±0.27	0.72	2.59±0.11	2.49±0.11	2.37±0.22	0.55
CLA	0.56±0.03	0.51±0.04	0.26±0.09	**≤0.01**	0.48±0.04	0.44±0.04	0.41±0.07	0.46
C18:3 ω-3	0.32±0.01	0.31±0.02	0.35±0.04	0.47	0.33±0.01	0.32±0.01	0.33±0.03	0.99
C20:1	0.19±0.01	0.20±0.02	0.10±0.04	0.09	0.18±0.02	0.14±0.02	0.18±0.03	0.16
C20:4 ω-6	0.15±0.01	0.15±0.02	0.11±0.05	0.73	0.16±0.02	0.13±0.02	0.12±0.05	0.39
PUFA	3.73±0.1	3.59±0.15	3.35±0.33	0.44	3.73±0.13	3.53±0.14	3.41±0.26	0.30

a, b, c, d – values in rows with different letters differ significantly (P≤0.05); A, B, C, D – as above for P≤0.01. In rows with P-value bolded but without letters indicating differences, the ANOVA showed significant differences, but Tukey's post-hoc test did not show any indication of specific differences between pairs. In the fatty acid name c stands for *cis*; t stands for *trans*.

Table 4. Milk performance (means±SE) depending on AJ312201.1g.1488C>G polymorphism of *ACACA* gene, A293V polymorphism of *SCD1* gene and fat class

		Mean [L]	SE [L]	ANOVA
				P-value
ACACA	CC	9188.63	354.35	0.62
	CG	8643.23	591.82	
	GG	8389.77	1194.54	
SCD1	AA	9380.66	520.94	0.28
	VA	8405.38	523.33	
	VV	8435.58	1034.77	
Fat class	<4,25%	9041.73	584.60	0.24
	>4,25%	8439.35	593.90	

Discussion

Presented results differ from results of Artegoitia et al. (2013), who found that SFA group in milk fat was higher, and MUFA group was lower in multiparous cows compared with primiparous cows. Moreover, authors demonstrated an interaction between age (as consecutive lactations) and lactation week *vs* SFA and MUFA proportions (increase of SFA and decrease of MUFA content between 2nd and 8th week of lactation in primiparous cows). However, authors did not observe any influence of age on PUFA content in milk fat. Nałęcz-Tarwacka et al. (2009) showed slight, but statistically significant differences in CLA level in milk from different lactations, which is consistent with the presented study.

Matsumoto et al. (2012) detected 3 SNPs on promoter III of *ACACA* gene in dairy cattle. Two of them were in linkage disequilibrium and showed significant effect on fatty acid composition in milk of Holstein-Friesian cows. CCT/CCT type (two SNPs on promoter III and one on promoter IA) were associated with higher content of C14:0, compared with CCT/GTC type (for homozygote GTC/GTC the differences were not significant). Also, significant differences were observed between CCT/CCT and GTC/GTC for C16:0. Moreover, cows with GTC genotype had higher level of C18:0. Research conducted on sheep also demonstrated significant association of SNP g.1330T>C in *ACACA* gene (located in PIII region) with milk fat content (Moioli et al., 2013). Similar conclusions were presented by Zhang et al. (2009) who found that eight novel SNPs (g.2064T>A, g.2155C>T, g.2203G>T, g.2268T>C, g.2274G>A, g.2340A>G, g.2350T>C, g.2370A>G) in PI region of *ACACA* gene were significantly related with fatty acid composition of beef fat. These findings, as well as results from the presented study show that there might be a relation between the *ACACA* gene and milk composition. However, any confirmation or rejection of these associations requires further research.

Frequencies of alleles and polymorphisms of A293V of the *SCD1* gene observed in the presented study are comparable with frequencies obtained by other authors (Schennink et al., 2008; Kaneda et al., 2011; Matsuhashi et al., 2011). Duchemin et al. (2013) observed that in winter and summer allele V was negatively linked

with C18:0, from C10:1 to C14:1 *cis*-9 and C18:1 *trans*-11, C18:3 *cis*-9,12,15, plus with unsaturation indices of several fatty acids (from C10 to C14). In contrast, for C8:0 to C14:0, C16:1 *cis*-9, CLA and unsaturation indices of C16 and CLA, a positive relation with this allele in the same seasons was demonstrated. Authors also showed a strong influence of A293V on C18:1 *trans*-11 content. Bouwman et al. (2011) analysed Dutch population of Holstein-Friesian cattle, and found that allele A of this polymorphism is linked with higher level of C10:1, C12:1 and C14:1, as well as with lower level of C10:0, C14:0 and C16:1. Similar effect was found for C12:0 and C12:1, but for the saturated fatty acid this influence was not significant. Authors noticed that such an influence of polymorphism of this gene on medium-chain UFA group and their SFA analogues is in accordance with the function of the encoded enzyme. Comparable results were obtained by Schennink et al. (2008), who analysed the influence of this SNP on fatty acids unsaturation indices in milk of Holstein-Friesian cows. They showed that allele V was related with higher content of C10:0, C12:0, C14:0, C16:1 and CLA, as well as with lower level of C10:1, C12:1, C14:1, C18:0 and C18:1 *trans*-11. Furthermore, this polymorphism was significantly affecting unsaturation indices of different fatty acids. For C10, C12 and C14 these indices were lower in presence of allele V, in contrast to indices for C16, C18 and CLA. Authors suggest that the activity of the encoded enzyme may be changed by this SNP, as this polymorphism causes a substitution of valine to alanine in 293 position, which is located in 3rd histidine-rich region of the enzyme. Such regions show strong catalytic activity (Shanklin et al., 1994). Conte et al. (2010) hold that probably *SCD1* gene is not the only genetic factor controlling fatty acid unsaturation. Authors confirmed that VV genotype is associated with higher level of C14:1 *cis*-9 and saturation index for C14. Similar relation was observed in Canadian Jersey cattle (Kgwatalala et al., 2009). Allele A was positively linked with unsaturation of C10, C12 and C14, but did not show any effect on unsaturation of C16 and C18. Authors suggest that A293V SNP of *SCD1* gene may be used as genetic marker in selection of cattle aiming at amelioration of unsaturation of several fatty acids (C10, C12 or C14). Contrary to other authors, in the presented study no relations were observed between A293V SNP on *SCD1* gene and fatty acid profile (except for C14:1).

Nowadays, more attention is paid to composition of milk fat and its role in human health. In the presented study, gene and lactation number effect on fatty acids profile was analysed. The finding of a novel SNP in the *ACACA* gene (AJ312201.1g.1488C>G) and its relation with milk yield in 305-day lactation indicate that there are as yet unknown associations between this gene and milk production. It is therefore necessary to carry out further studies on dairy cows to better understand this impact.

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