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Effect of *DGAT1* gene mutation in sows of dam-line on the composition of the produced milk and piglet rearing during 21-day lactation

Magdalena Szyndler-Nędza[1]* and Katarzyna Piórkowska[2]

[1]Department of Animal Genetics and Breeding, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland.
[2]Laboratory of Genomics, National Research Institute of Animal Production, Krakowska 1, 32-083 Balice, Poland.

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Diacylglycerol acyltransferase 1 gene (*DGAT1*) involved in the synthesis and transport of triglycerides is located on chromosome 4 in pigs, in the region with about 200 QTLs responsible among other things for: fat thickness, daily gains, fat content and composition of fatty acids. It is thus probable that the gene polymorphism (as in cows) may affect the fat content in colostrum and milk of sows. The objective of the experiment was to assess the effect of *DGAT1* polymorphism on the milk composition of sows and as a result on piglet rearing during the suckling period. The experiment was performed on 207 sows of breeds used in breeding program as a dam-line: polish large white (PLW) and polish landrace (PL). Colostrum and milk of sows were collected at 1, 7, 14 and 21 days of lactation to assay solids, total protein, fat and lactose. Data on piglet rearing performance were collected at 1, 7, 14 and 21 days of lactation. The tests performed showed that A/G rs45434075 *DGAT1* mutation occurring in PLW and PL sows did not significantly affect the quality of their colostrum and milk expressed as solids content, and at the same time on piglet rearing performance. However, it was observed that PLW sows of the *DGAT1*^{GG} genotype was characterised by a higher fat content in colostrum, whereas PL sows of the same genotype had an increased protein content and a reduced lactose content in milk.

Key words: *DGAT1*, Polymorphism, milk, rearing of piglets, maternal breed.

INTRODUCTION

The effect of gene mutations on the chemical composition of porcine milk has not been widely studied so far. There are few publications on this subject, and they suggest certain correlations between genotype of sows and milk composition. In their studies on transgenic sows, Monaco et al. (2005) revealed that increased mammary gland expression of the *IGF1* gene (lactation regulator) did not cause an increase in the amount of the

*Corresponding author. E-mail: magdalena.szyndler@izoo.krakow.pl. Tel: +48 666081142.

Abbreviations: PLW, Polish large white; **PL,** polish landrace.

produced milk or a change in its composition. Babicz (2008) analysed the effect of polymorphism of selected genes on the level of chemical components of colostrum and he observed that polymorphisms of *RYR1/HinfI, GH/MspI, PRL/HpyCH4III* genes significantly affected protein content of milk in Puławska sows. Szyndler-Nędza et al. (2013) investigated the effect of *MC4R*(G1426A) and *LEPR/HpaII* polymorphisms on the composition of colostrum from PLW and PL sows which demonstrated that analysed polymorphisms had a similar effect in both breeds. The $MC4R^{A}$ and $LEPR^{B}$ alleles reduce the content of fat, protein and solids in the colostrum of sows. Another interesting gene in this respect might be diacylglycerol acyltransferase 1 (*DGAT1*), whose expression in mammals occurs mainly in the small intestine. This gene participates in intestinal synthesis of triglycerides and their transport to the lymphatic system (Nagalski and Bryła, 2007). Studies conducted on cattle revealed that *DGAT1* polymorphism (K232A-replacement of lysine by alanine) had a significant influence on milk yield and composition (Grisart at al., 2002; Thaller et al., 2003; Kuehn et al., 2007; Streit et al., 2011). Cows with lysine variant in position 232 aa sequence of the *DGAT1* protein were characterised with a higher content mainly of milk fat, but also of protein. The effect of this mutation was confirmed in three subsequent lactations of cows (Thaller et al., 2003). Moreover, studies on Charolaise, Limousin and Retina cattle (Aviles et al., 2013) showed that K232A polymorphism of the *DGAT1* gene affected thickness of cattle fat cover.

Previously, literature has not reported publication concerning the QTL associated with the porcine milk composition and piglet rearing during lactation. Nonneman and Rohrer (2002) and Szczerbal et al. (2007) mapped *DGAT1* gene in pigs and demonstrated that it was located on chromosome 4, in the region with about 200 QTLs responsible for: fat thickness, weight gains, fat content, composition of fatty acids, etc. Cui et al. (2011) analysed *DGAT1* and *DGAT2* gene expression in the longest muscle (*longissimus muscle dorsi*), liver and fat of three different pig breeds. Its correlation with backfat thickness, showed that the highest expression of *DGAT1* gene occurred in the liver and it was positively correlated with this trait ($P \leq 0.05$). Therefore, *DGAT1* polymorphism in pigs (similarly as in cows) may affect the fat content in colostrum and milk of sows.

The objective of the present study was to assess the effect of *DGAT1* polymorphism g. 32748 A>G (NW 003534570.2) identified in intron 2 on the milk composition of sows and its influence on the piglet rearing during the suckling period.

MATERIALS AND METHODS

The studies were performed on 207 sows, including 107 PL sows (58 sows in the second lactation and 49 sows in the third lactation) and 100 PLW sows (53 in the second lactation and 47 in the third lactation). All sows were kept at the Experimental Station of the National Research Institute of Animal Production Ltd. in Żerniki Wielkie, Poland. Sows used for reproduction were maintained under the same feeding and housing conditions. The feeding system is compliant with the farm standards and it was adopted according to various reproductive stages of sows (sows in early gestation, in late gestation, in lactation). In the experiment, only sows in similar condition on the day of mating were used. Their condition was determined based on body weight and last rib (P2) backfat thickness, measured with an ultrasonic device (Piglog 105). Blood was collected from all sows to determine the polymorphisms of *DGAT1* gene. Samples of colostrum and milk were collected from sows during the second and third lactation. After 1 h following delivery the colostrum was milked and collected, and in turn milk 2 h following the morning feeding on 7, 14 and 21 day of lactation. Samples of colostrum and milk were collected from the first, third and sixth teat in the total amount of 50 ml (one sample of milk). The samples were then labelled and cooled to 4°C. Cooled samples of fresh milk were supplied to the Laboratory of Milk Assessment and Analysis of the Wrocław University of Environmental and Life Sciences in order to determine basal composition of the milk. Solids, total protein, fat and lactose were determined in colostrum and milk.

The determinations were performed with the instrument Milko-Scan 133 B by Foss Electric, with the use of infrared analysis according to the enclosed application by Foss. Reproductive performance of the experimental sows was determined based on 207 L, which were analysed for the number and body weight of piglets at birth and at 7, 14 and 21 days of age.

DNA was isolated from leukocytes with the use of the Genomic Wizard Purification Kit (Promega, Madison, WI, USA). Mutation in *DGAT1* gene was determined with PCR-RFLP methods according to Nonneman and Rohrer (2002), with modification of primer pair, which were designed in Primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Forward-GCATCCTGAATTGGTGTGTG and Revers - GGCCATTCAGAACAG primers amplified a 257 bp PCR product, which was then digested with *Ava*II restriction enzyme, which recognizes *A/G* rs45434075 (g. 32748 A>G (NW 003534570.2)) substitution. The data were statistically analysed using the procedures of Statisticaver.10 (2011, StatSoft Inc.). The differences between groups were estimated by ANOVA analysis according to the following statistical model:

$$Y = a_i + b_j + c_k + d_l + e_{ijkl}$$

Where, ai = breed (i = 1,2), bj = lactation (j=1,2), ck= lactation period (k=1-4), dl = genotype (l= 1-3). Differences between the means of individual traits were tested at 0.05 and 0.01 P-value using Duncan's multiple range test.

RESULTS AND DISCUSSION

In comparison with Polish Landrace sows (Table 1), Polish Large White sows had a slightly higher body weight on the day of mating and statistically significantly thinner backfat ($P \leq 0.05$). Differences observed in backfat thickness between PLW and PL breeds are compliant with findings presented by Eckert at al. (2013) for sows of these breeds in the national population. Also, Knecht et al. (2014) performing measurements with an ultrasonographic device Aloka SSD-500, showed that PL sows had significantly higher backfat thickness at P2 ($P \leq$

Table 1. Means and standard deviations of body weight and fat thickness on day of mating in PLW and PL sows.

Parameter	PLW		PL		P- value of
	Mean	SD	Mean	SD	PLW/PL
Mean body weight on day of mating (kg)	192.99	27.62	186.34	23.98	0.051
Mean backfat thickness on day of mating P2 (mm)	13.25	3.53	14.57	3.67	0.005

Table 2. Genotype and allele frequency of *DGAT1* gene.

Breed	n	*DGAT1*			Allele frequency		HWE
		AA	*AG*	*GG*	*A*	*G*	P-value
PLW	100	47.00	45.00	8.00	69.5	30.5	0.54
PL	107	25.23	55.14	19.63	52.80	47.20	0.25

0.05) in comparison with PLW sows. Sows of both breeds were characterised with a the lowest genotype frequency of *DGAT1*^{GG} gene (Table 2). In PLW breed, the most frequent were individuals with *DGAT1*^{AA} genotype (47%). The least frequent were homozygous *DGAT1*^{GG} individuals (8%). In PL breed, the most frequent were heterozygous *DGAT1*^{AG} individuals (55.14%). Similarly to PLW, the least frequent were individuals with *DGAT1*^{GG} genotype (19.63%). Both analyzed populations were in HW-equilibrium, it suggests that polymorphism *A/G* rs45434075 of *DGAT1* gene is not associated with pig traits, which were considered during selection in order to improve their reproductive performance. Differences between breeds in the frequency of alleles for *A/G DGAT1* polymorphism were also reported by Mercadé et al. (2005). They showed that *A* allele was not present in the Iberianpigs as well as in the Landrace population, in contrast to the Meishan breed where *A* allele was observed with 35% frequency.

The analysis of the effect of rs45434075 *DGAT1* gene polymorphism on the backfat thickness and body weight of sows on day of mating prior to second and third litter showed no statistically significant differences among the analysed genotypes of *DGAT1* gene (Tables 3 and 4). Sows of both breeds, representing each of the polymorphic forms of *DGAT1* gene, had similar values for these traits; therefore it was not necessary to include these factors in the statistical model. Different results were obtained for cattle. Studies conducted on Charolaise, Limousin and Retina breeds showed that Lys232 variant of *DGAT1* protein increased fat cover thickness of cows, in contrast to Ala232 variant. The authors also showed that homozygous CC (Lys, Lys, KK) cows, with only alanine variant in *DGAT1* protein were the least common in the analysed populations (Aviles et al., 2013). The basic objective of the present study was to assess the effect of *DGAT1* gene polymorphism on basic composition of colostrum and milk and on possible differences in piglet rearing in maternal breeds used in Polish breeding. Results of the analysis are presented in Table 3 for PLW sows and in Table 4 for PL sows.

It can be concluded from the results obtained, that the A/G rs45434075 DGAT1 gene polymorphism in both breeds had an effect on changes in basic components of colostrum and milk. However, this effect was different in each breed. A statistically significant correlation was observed, between *DGAT1* genotype and fat content in colostrum of PLW sows and between the content of protein and lactose in milk of PL sows. PLW sows with *DGAT1*^{GG} genotype had significantly more fat in colostrum (by 1.86%, P≤0.05) than sows with *DGAT1*^{AA} genotype. The obtained results without significances may have been caused due to the small statistical groups representing *DGAT1*^{GG} (8 sows). PL sows with *DGAT1*^{GG} genotype were reported to have significantly higher protein content (by 1.01%, P≤0.05) and significantly lower lactose content in milk (by 0.63% P≤0.05) than individuals with *DGAT1*^{AA} genotype. Differences in the effect of the analysed polymorphism on milk composition between breeds were also observed in cattle. Suchocki et al. (2010) showed that *DGAT1* polymorphism had a more significant influence on the majority of milk components in Jersey cows than in Holstein-Friesians cows. They also observed epistatic effect between *LEPR* and *DGAT1* gene polymorphisms on milk composition of Holstain-Friesians cows. On the other hand, Marchitelli at al. (2013) showed that in Jersey cows, *DGAT1* polymorphism affected only the fat content in milk.

Piglet rearing performance during lactation is affected by numerous factors for example higher milk yield and higher content of basic milk components (Skrzypczak et al., 2012b; Schmidely et al., 2002; Barłowska et al., 2007; Buczyński et al., 2008; Babicz et al., 2011). Studies on the effect of milk protein polymorphism in sows (Skrzypczak et al., 2012b) on piglet rearing performance showed that sows of *CSN1S1*^{AA} and *CSN2*^{BB} genotypes

Table 3. Means and standard deviations for sows condition on the day of mating, chemical composition of colostrum and milk from 21-day lactation, and rearing performance of piglets from sows of PLW breed with different *DGAT1* genotypes.

Parameter			*DGAT1* PLW		
			AA **47**	***AG*** **45**	***GG*** **8**
Condition	Body weight at mating (kg)		195.00±27.91	192.78±26.31	197.00±38.08
	Backfat thickness (P2) (mm)		13.36±4.04	13.16±3.44	13.38±1.99
Composition of colostrum and milk from 21-day lactation	Fat (%)	Colostrum	3.47a±1.63	3.60±2.97	5.33a±2.09
		Milk	7.08±1.13	6.97±1.06	7.13±1.08
	Protein (%)	Colostrum	14.72±3.65	12.76±2.44	13.42±2.31
		Milk	5.03±1.31	4.99±1.32	4.61±0.09
	Lactose (%)	Colostrum	2.33±0.76	2.75±0.82	2.65±0.40
		Milk	5.49±0.67	5.46±0.57	5.79±0.31
	Solids (%)	Colostrum	21.45±3.21	19.92±2.84	21.70±3.59
		Milk	17.97±1.73	17.84±1.33	17.82±0.87
Rearing of piglets during 21-day lactation	Number of piglets at days of age (head)	1	11.67±1.17	11.93±0.75	11.50±1.07
		7	11.04±1.33	11.07±1.01	10.88±1.13
		14	10.39±1.82	10.67±1.13	10.63±1.30
		21	10.06±1.91	10.31±1.12	10.38±1.19
	Weight of piglets at days of age (kg)	1	1.48±0.17	1.43±0.12	1.55±0.18
		7	2.60±0.32	2.66±0.32	2.75±0.33
		14	3.94±0.45	4.11±0.50	4.16±0.39
		21	5.66±0.65	5.72±0.51	5.88±0.48
	Weight gain of piglet until day (kg)	7	1.12±0.28	1.24±0.31	1.20±0.31
		14	2.45±0.43	2.68±0.48	2.61±0.33
		21	4.17±0.62	4.29±0.47	4.33±0.47

Means in rows with the same small letters differ significantly at P≤0.05.

Table 4. Means and standard deviations for sows condition on the day of mating, chemical composition of colostrum and milk from 21-day lactation, and rearing performance of piglets from sows of PL breed with different *DGAT1*genotypes.

Parameter			*DGAT1* PL		
			AA **27**	***AG*** **59**	***GG*** **21**
Condition	Body weight at mating (kg)		189.15±28.03	185.3±23.32	186±20.97
	Backfat thickness (P2) (mm)		15.5±4.65	14.42±3.23	14.29±2.43
Composition of colostrum and milk from 21-day lactation	Fat (%)	Colostrum	5.50±1.71	5.32±2.46	4.10±1.72
		Milk	7.23±0.68	7.28±1.03	7.09±1.65
	Protein (%)	Colostrum	16.05±3.09	14.74±2.67	14.57±3.18
		Milk	4.51a±0.37	4.70±0.84	5.52a±2.50
	Lactose (%)	Colostrum	1.80±0.81	2.38±2.78	2.13±0.93
		Milk	5.74a±0.26	5.52b±0.55	5.11ab±1.01
	Solids (%)	Colostrum	23.79±2.66	22.58±3.11	21.37±3.13
		Milk	17.81±0.86	17.82±1.39	18.07±1.62

Table 4. Contd.

Rearing of piglets during 21-day lactation	Number of piglets at days of age (head)	1	11.00±1.90	11.51±1.17	11.76±0.54
		7	10.56±1.85	10.93±1.26	11.23±0.83
		14	10.33±1.84	10.61±1.29	10.90±0.83
		21	10.04±1.74	10.29±1.38	10.52±1.66
	Weight of piglets at days of age (kg)	1	1.51±0.11	1.48±0.15	1.49±0.13
		7	2.86±0.37	2.78±0.35	2.75±0.33
		14	4.18±0.48	4.18±0.46	4.26±0.62
		21	5.82±0.65	5.66±0.57	5.83±0.62
	Weight gain of piglet until day (kg)	7	1.35±0.32	1.30±0.34	1.26±0.34
		14	2.67±0.42	2.70±0.46	2.77±0.62
		21	4.31±0.59	4.18±0.57	4.34±0.61

Means in rows with the same small letters differ significantly at $P \leq 0.05$.

of casein genes produced more milk, and as a result achieved higher piglet rearing performance, that is, higher body weight and fast body weight gain. Similar studies conducted in goats (Schmidely et al., 2002; Barłowska et al., 2007) showed that some variants of both casein genes affected higher protein and fat content in milk, which was translated into better rearing performance of offspring. In studies on sows of maternal breeds, Babicz et al. (2011) showed that a higher protein and solids content in milk might be one of the factors affecting increased piglet growth and body weight during 21-day lactation. Studies on sows of Złotnicka White breed (Buczyński et al., 2008) concerning the effect of milk composition on piglet rearing performance showed that daily gains in 21th-day of lactation were higher in piglets of sows with a higher fat content in milk (over 7 %). Another factor influencing piglet body weight gain during lactation is milk pH and somatic cells content (Skrzypczak et al., 2012a). The authors of this paper showed negative correlation coefficients among these parameters in sows' milk and piglet body weight in 21th-day of lactation.

Differences observed in the present studies in individual milk parameters of both breeds were not big enough to use the *DGAT1* polymorphism as a genetic marker in selection of solid milk content in investigated sows of dam-line. Therefore, it may be concluded that *A/G* rs45434075 of *DGAT1* mutation, does not change basal milk composition in sows of both breeds and does not also affect piglet rearing performance. The analysis of changes in piglet body weight and weight gain during 21 days of lactation in relation to *DGAT1* genotype of both breeds (Tables 3 and 4) show that there were no statistically significant differences in the analysed traits between polymorphic forms of this gene. The investigated polymorphism g.32748A>G, NW003534570.2 in the second intron was identified in 2002 by Nonneman and Rohrer and added to GenBank in 2004. Recently, SNPdb was added as new interesting missense mutations located in 1, 5 and 7 exon, which could be tested in further study. It can be observed that the last mutation changing aa in protein domain is associated with membrane-bound O-acylo transferase (Ensemble Browser).

Summarising the findings of the performed tests, it can be observed that mutation *A/G* of *DGAT1* gene participating in intestinal synthesis and transport of triglycerides to the lymphatic system - occurring in PLW and PL sows did not significantly affect the quality of their colostrum and milk expressed as solids content, and at the same time on piglet rearing performance. It was only observed that PLW sows of the *DGAT1*^{GG} genotype were characterised by a higher fat content in colostrum, whereas, PL sows of this genotype had an increased protein content and a reduced lactose content in milk. It must be emphasised that the least common in both breeds were individuals with *DGAT1*^{GG} genotype.

Conflict of interests

The authors did not declare any conflict of interest.

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