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FABP3 POLYMORPHISM IN RELATION TO GROWTH TRAITS IN SIMMENTAL AND SALERS COWS

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ABSTRACT

The aim of this study was to estimate the associations between the FABP3 genotypes and the growth traits (body weight at 210 days of age, average daily body weight gain to 210 days of age, withers height, sacrum height, chest girth) as well as longevity in 98 Simmental and 86 Salers cows. The analysis of the first calving was also carried out. Statistically significant associations between the FABP3A/G polymorphism and the body weight at 210 days of age, daily body weight gain to 210 days of age and longevity in Simmental cows were found; higher values of these traits were observed in cows with the G allele. No significant associations were found between the above polymorphism and the all analyzed traits in Salers cows. The study results indicated that the selection for the animals with the G allele might contribute to an increased some weight traits and longevity in Simmental cows. Additionally, the largest number of individuals with this allele were characterized by easy calving. It is necessary to continue research on the larger groups of animals.

Key words: genetic polymorphism, beef cattle, body weight

INTRODUCTION

Genetic research is carried out to create modern breeding selection programs. Nowadays, the main purpose of breeding programs is to improve the accuracy of the selection process in farm animals. Economically important traits in beef cattle which are subject to continuous improvement include fertility and growth. Growth traits in beef cattle from birth to adulthood are of particular significance to breeders and farmers due to their association witch meat production [Snelling et al. 2010, Buzanskas et al. 2014]. Birth weight determines animal viability and is used in selection to improve calving ease, weaning weight is an excellent indicator of beef production systems, and yearling weight is associated with the mature weight [Snelling et al. 2010, Jahuey-Martinez et al. 2016]. Studies on QTL for body weight and growth traits have identified hundreds of genes or markers that affect economically important traits in beef cattle [Bolormaa et al. 2011].

Fatty acid binding protein 3 (FABP3), also known as heart-type FABP, has an important role in intracellular fatty acid transport and metabolism also improving insulin sensitivity [Shearer et al. 2005, Li et al. 2016]. Fatty acids play important functions in the body, and are primary sources of energy stored in triacylglycerol. They are produced in the liver and muscles and take part in the formation of complex lipids, hormones and signaling compounds [Chmurzyńska 2006]. The protein is one of nine cytosolic FABPs (FABP1-FABP9), which consist of 126–134 amino acids, have the molecular weight of 14 to 15 kDa, and are engaged in the preservation of energy supply to many organs such as heart, skeletal muscles, liver, and adipocyte tissues. FABP3 binds non-esterified saturated and unsaturated long-chain fatty acids and other lipids for transportation or storage inside a cell [Shimano et al. 1996, Qu et al. 2017]. FABP3 is distributed in smaller quantities in the brain, kidneys, adrenal glands, lungs, testis as well as lymphocytes [Yardibi et al. 2013, Zhang



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et al. 2013]. It is found in tissues with a higher demand for fatty acids, such as the lactating mammary gland, and cardiac and skeletal muscles [Tsukahara et al. 2014]. *FABP3* gene contains 4 exons and its coding gene has been mapped to a region on bovine chromosome 2 [Calvo et al. 2004].

The aim of this study was to estimate allele and genotype frequencies of the SNP in *FABP3* gene in Simmental and Sales cows and to establish possible associations between the genotypes and the growth traits.

MATERIAL AND METHODS

The study was conducted on herds of Salers (86 individuals) and Simental (98 individuals) cows. The animals were kept in similar environmental conditions. From spring to autumn they raised on pasture and in the winter they were fed with haylage and hay with mineral and vitamin supplements. Water was available *ad libitum*.

Blood samples taken from the external jugular vein were collected into anticoagulant tubes containing K₃EDTA. Genomic DNA was extracted using MasterPureTM DNA Purification Kit for Blood (Symbios) according to the methodology recommended by the manufacturer. The isolated DNA was suspended in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA) and stored at 4°C.

The studied AG transition in the FABP3 gene was described by Wu et al. [2005]. A 438 base pair long gene fragments were amplified with the following primer pairs: 5'-GTG AGT TGA GGA AGG GGC TGT G-3' and 5'-TAG GTC TCC ACC TCT TGT CCT TCA G-3' [Wu et al. 2005]. The PCR was performed in a 15 µL volume mixture containing approximately 70 ng DNA, 15 pmol of each primer, 0.5 mM dNTP, 4 mM MgCl₂ and 0.3 U of Taq polymerase (A&A Biotechnology). The amplification included the following steps, initial denaturation for 3 minutes at 95°C, followed by 30 cycles: 45 s of denaturation at 94°C, 50 s of annealing at 55°C, 1 minute of elongation at 72°C ending with a final elongation for 8 minutes at 72°C. The amplicons were digested using the AciI restriction enzyme, then the obtained fragments were separated on 2% agarose gels with ethidium bromide (0.5 $\mu g \times ml^{-1}$) in the presence of the pUC19/MspI DNA standard. Electrophoretic results were analyzed using the software for the photodocumentation of electrophoretic separation, and image storage (Vilber Lourmat®).

Statistical analysis

In the first stage, the *FABP3* genotype and allele frequencies in the studied herds of cows were estimated. In the next stage, the analysis of association between genotypes and the average value of the following traits was performed: body weight at 210 days of age (kg), average daily

body weight gain (g) to 210 days of age, withers height (cm), sacrum height (cm), chest girth (cm) and longevity (days).

In addition, the analysis of the delivery process (first calving) was carried out within each genotype group, in accordance with the following types: 1 – easy pull with no assistance, 2 – easy pull with some assistance; 3 – hard pull with assistance/mechanical means/veterinary intervention, 4 – caesarean section, 5 – embryotomy, 6 – abortion [PZHiPBM 2016].

The analyzed traits data came from the breeding documentation of the herds, carried out in order the recommendations of the International Committee for Animal Recording (ICAR).

The statistical analysis was carried out using a oneway analysis of variance (ANOVA). The significance of differences between mean values of traits was calculated with the Duncan multiple range test. The calculations were made using the Statistica 12.5 PL Software.

RESULTS

The frequencies of the analyzed *FABP3A/G* alleles and genotypes are presented in the Table 1. Within the both analyzed cows' breeds the *GG* genotype was the most frequent.

Table 1. Genotype and allele frequencies of the FABP3A/G

Tabela 1. Częstość występowania genotypów oraz alleli *FABP3A/G*

Breed Rasa	Genotype/Allele Genotyp/Allel	Frequency Częstość występowania	
	AA	0.17	
	AG	0.37	
Simmental	GG	0.46	
	A	0.36	
	G	0.64	
	AA	0.22	
Salers	AG	0.36	
	GG	0.42	
	A	0.40	
	G	0.60	

Mean values and standard deviations (SD) for the analyzed traits of Simmental and Salers cows with different *FABP3A/G* genotypes are given in the Table 2.

The study results show statistically significant differences between the mean values of body weight at 210 days of age and daily body weight gain to 210 days of age in Simmental cows with different genotypes. The AG genotype cows were characterized by a significantly (P \leq 0.05) higher value of body weight at 210 days than the AA genotype cows; the difference amounted to 3

Table 2. Means and standard deviations of growth traits in cows with different FABP3A/G genotypes

Tabela 2. Średnie wartości i odchylenia standardowe dla cech wzrostu krów z różnymi genotypami FABP3A/G

Genotype Genotyp	N	BW210, kg	ADG210, g	Sacrum height, cm Wysokość w krzyżu, cm	Wither height, cm Wysokość w kłębie, cm	Chest girth, cm Obwód klatki piersiowej, cm
				Simmental		
AA	17	268 ± 15^a	1123 ± 76^{a}	144.2 ± 2.6	140.0 ± 3.6	205.1 ± 2.6
AG	36	281 ± 16^a	1178 ± 78^a	144.3 ± 3.4	141.2 ± 2.8	203.9 ± 4.4
GG	45	278 ± 24	$1166\pm\!108$	144.9 ± 3.8	141.3 ± 3.9	204.3 ± 4.7
Total Ogółem	98	278 ±20	1163 ±94	144.6 ±3.5	141.1 ±3.5	204.3 ±4.3
				Salers		
AA	19	251 ± 17	$1037 \pm \! 79$	147.0 ± 3.1	142.2 ± 3.9	203.5 ± 4.4
AG	31	251 ±24	$1036\pm\!114$	145.7 ± 4.4	141.6 ± 4.5	202.7 ± 3.5
GG	36	251 ±30	1035 ± 141	144.9 ± 4.2	141.2 ± 4.4	203.1 ±4.3
Total Ogółem	86	251 ±25	1036 ±119	145.6 ±4.1	141.6 ±4.3	203.1 ±4.0

BW365, kg – body weight at 210 days of age, kg; ADG210, g – average daily body weight gain to 210 days of age, g; N – number of individuals; the means in columns marked with the same superscript letter differ significantly ($P \le 0.05$).

BW210, kg – masa ciała w 210. dniu życia, kg; ADG210, g – średni dobowy przyrost masy ciała do 210. dnia życia, g; N – liczba osobników; średnie wartości w kolumnach oznaczone tą samą literą różnią się między sobą istotnie ($P \le 0.05$).

kg. Significant differences ($P \le 0.05$) in the daily body weight gain to 210 days of age were also found between cows with the AA and AG genotypes. The difference was 45 g, with a higher value, similar to the previously analyzed trait, in cows with the heterozygous genotype. When analyzing the wither height and the sacrum height, it was found that cows with the GG genotype were characterized by the highest values of these traits, and the smallest values were observed in cows with the AA genotype. The analysis of the chest girth showed that the highest value of this trait was characteristic for cows with the AA genotype, while the smallest for heterozygous cows. In case of the wither height, the sacrum height and the chest girth, the differences between individuals with different genotypes have not been confirmed statistically.

In the case of Salers cows no significant associations were found between the FABP3A/G polymorphism and the all above analyzed traits. However, homozygous individuals with the AA genotype were characterized by the highest value of each analyzed trait. On the other hand, cows with homozygous GG genotype were characterized by the smallest value of each trait except for chest girth, where the smallest value was found in cows with the AG genotype.

The longevity of cows with different genotypes was also analyzed (Table 3). The highest value of this trait was noted for the GG genotype individuals, both in a herd of Simmental and Salers. In terms of Simmental cows, statistically significant differences (P \leq 0.05) were found between cows with the AA and AG as well as AA and GG genotypes. The differences amounted to 669 and 566 days, respectively. In the case of Salers the differences between the respective FABP3A/G genotypes were statistically no significant.

Analysis of the first calving are presented in the Table 4. In the vast majority of cows, the delivery was classified as easy, without human assistance. The largest number of individuals with the GG and AG genotypes were characterized by such a calving in Simmental and Salers herds, respectively. In Simmental cows, dystocia belonging to categories 3 and 5, was observed in individuals with the AG and GG genotypes and amounted to 8 and 4% of all births, respectively. There was no delivery with caesarean section. In Salers cows, hard calvings belonging to categories 3 and 4 were observed in individuals with the GG genotype and amounted to 3% of all births. There was no case of embryotomy.

DISCUSSION

The Salers and Simmental breeds are resistant to difficult environmental conditions and not demanding in terms of nutrition. They easily adapt to different environmental conditions. Moreover, they do not require housing in winter. High resistance, calf viability, good fertility, easy calvings and good fattening results [Choroszy and Choroszy 2011] predispose them to breeding under local conditions, especially in Western Pomerania [Pilarczyk and Wójcik 2007, Czerniawska-Piątkowska et al. 2012].

Both Salers and Simmental cows analyzed in the present study were maintained under similar conditions. Most often, the calving was easy, generally without human help. However, a slightly higher percentage of this type of births was observed in Salers cows, which was consistent with the study conducted by Pilarczyk and Wójcik [2008]. It is worth mentioning that Salers cows are characterized by the wide, well-shaped pelvic area, and thus less stress for both mother and calf du-

ring delivery [Dobicki et al. 2007, Gołębiewski 2017]. Additionally, the birth weight of Simmentals is larger

Table 3. Means and standard deviations of longevity in cows with different *FABP3A/G* genotypes

Tabela 3. Średnie wartości i odchylenia standardowe dla długości użytkowania krów z różnymi genotypami *FABP3A/G*

Breed – Rasa	Genotype Genotyp	Longevity, days Długość użytkowania, dni	
	AA	1275 ±662 ^{ab}	
Simmental	AG	1944 ± 1128^a	
Similiental	GG	$1948 \pm \! 1068^b$	
	Total – Ogółem	1841 ± 1059	
	AA	1249 ±950	
Salers	AG	1534 ± 987	
Saicis	GG	1589 ± 786	
	Total – Ogółem	$1490 \pm \! 892$	

The means in columns marked with the same superscript letter differ significantly ($P \le 0.05$).

Średnie wartości w kolumnach oznaczone tą samą literą różnią się między sobą istotnie ($P \le 0.05$).

Table 4. Analysis of the first calving in cows with different *FABP3A/G* genotypes

Tabela 4. Analiza pierwszego wycielenia u krów z różnymi genotypami *FABP3A/G*

Calving type	Genotype – Genotyp			Total			
Typ wycielenia	AA	AG	GG	Ogółem			
Simmental							
1	11 (64.7%)	28 (77.8%)	39 (86.7%)	78 (79.7%)			
2	6 (35.3%)	5 (13.9%)	4 (8.9%)	15 (15.3%)			
3	_	2 (5.5%)	_	2 (2.0%)			
4	_	-	1 (2.2%)	1 (1.0%)			
5	_	1 (2.8%)	1 (2.2%)	2 (2.0%)			
6	_	-	_	-			
Total – Ogółem	17 (100%)	36 (100%)	45 (100%)	98 (100%)			
Salers							
1	15 (78.9%)	27 (87.1%)	27 (75.0%)	69 (80.2%)			
2	4 (21.1%)	4 (12.9%)	7 (19.4%)	15 (17.4%)			
3	_	-	1 (2.8%)	1 (1.2%)			
4	-	-	1 (2.8%)	1 (1.2%)			
5	-	-	_	-			
6	-	-	_	-			
Total – Ogółem	19 (100%)	31 (100%)	36 (100%)	86 (100%)			

than that of Salers [Pilarczyk and Wójcik 2007]. A reduction in dystocia, according to Pilarczyk and Wójcik [2007], could be caused by changes that have taken place in recent years, namely the effectiveness of selection and the maintenance system. The advantage of both breeds is their strong mothering instinct and caring for calves, especially in Simmental, as well as the high milk

yield of cows, due to which daily body weight gains of calves until weaning are large [Pilarczyk and Wójcik 2007, Dobicki et al. 2007, Czerniawska-Piątkowska et al. 2012].

Both body weight at 210 days of age, daily body weight gain at 210 days of age and the measurements characterizing the caliber of the animal in this study were consistent with those given by PZHiPBM for the breed standard [PZHiPBM 2016].

Genomewide association studies enable the identification and improvement of many economically important traits in beef cattle such as growth traits including body weight, body length, height at withers, hip width and average daily body weight gain. Genetic improvement of growth, carcass and meat quality traits is an important tool to evaluate the genetic merit of livestock. Recent reports found, that key traits such as growth and meat quality are dependent on many genes and affected by a variety of environmental factors and nutrition [Liu et al. 2015, Ma et al. 2015, Santiago et al. 2017]. In the studies by Jahuey-Martinez et al. [2016] five genes were identified as potential candidate for growth traits such as birth, weaning and yearling body weights in Charolais cattle [Jahuey-Martinez et al. 2016]. Snelling et al. [2010] stated highly significant associations between analyzed SNP genotypes and growth traits of Simmental, Angus, Charolais, Hereford, Limousine; it was consistent with the detected QTL for growth traits. The SNPs localized in QTL for growth suggest that there are highly significant associations between genotype and traits growth. Zhang et al. [2016] identified 18 candidate genes which may affect the growth and carcass traits in Simmental cattle [Zhang et al. 2016].

The results of the present study show that the analyzed polymorphism significantly affects the body weight at 210 days of age and daily body weight gain to 210 days of age in Simmental cows. No studies on the analysis of the *FABP3* gene polymorphism in relation to the growth trait could be found, and therefore the results need to be verified in other studies on cattle herds. The *G* allele was more frequent in the studied herds, which is consistent with the results obtained in the study conducted on Holstein-Friesian [Kulig et al. 2013] and Wagyu x Limousin cattle [Wu et al. 2005] Contrary, in Jersey [Kulig et al. 2010], Sahiwal and Karan Fries cattle [Yadov et al. 2018] the A allele occurred most frequently.

It was suggested that *FABP3* gene might be applied to selection program with regard to meat quality traits. Significant association between *FABP3* polymorphism and intermuscular fat [Li et al. 2016] and rib eye area [Blecha et al. 2015] in beef cattle was found. The *FABP3* polymorphism was also analyzed in dairy cattle and in the other livestock species. It has been associated with protein and fat content in milk of Jersey cows [Kulig

et al. 2010] as well as with breeding value for fat yield in Holstein-Friesian cows [Kulig et al. 2013]. Yadov et al. [2018] conducted an analysis of the *FABP3* polymorphism in relation to performance traits of Sahiwal and Karan Fries dairy cattle. The results showed a statistically significant effect of genotypes on fatty acid composition and estimated breeding value for fat content in milk. The polymorphism in *FABP3* gene was also analyzed in riverine buffaloes [Dubey et al. 2015], but no significant association with milk traits were found. Calvo et al. [2004] Similarly, no association was found between estimated breeding values for milk trait such as milk yield, fat and protein contents of dairy sheep. However, in within-family analysis, a significant effect on fat content in one family was shown.

CONCLUSION

The study results indicated that there are statistically significant associations between the FABP3A/G polymorphism and the body weight at 210 days of age, daily body weight gain to 210 days of age and longevity in Simmental cows, with the G as a desirably allele. It is worth mentioning that the largest number of individuals with G was also characterized by easy calving. The results might be useful in selection toward increasing the growth traits and longevity of Simmental cattle. The study should be treated as a preliminary, so is necessary to continue research on the larger groups of animals.

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POLIMORFIZM FABP3 W ODNIESIENIU DO CECH WZROSTU KRÓW RAS SIMENTAL I SALERS

STRESZCZENIE

Celem badania było oszacowanie zależności między genotypami *FABP3* a cechami wzrostu (masa ciała w 210. dniu życia, średni dobowy przyrost masy ciała do 210. dnia życia, wysokość w kłębie, wysokość w krzyżu, obwód klatki piersiowej), a także długości użytkowania w stadach 98 krów rasy simental i 86 krów rasy salers. Przeprowadzono również analizę pierwszego wycielenia. Stwierdzono istotne statystycznie zależności między polimorfizmem *FABP3A/G* a masą ciała w 210. dniu życia, średnim dobowym przyrostem masy ciała do 210. dnia życia i długością użytkowania u krów rasy simental, przy czym większe wartości tych cech obserwowano u krów z allelem *G*. Nie stwierdzono istotnych zależności między powyższym polimorfizmem a analizowanymi cechami u krów rasy salers. Wyniki wskazują, że selekcja uwzględniająca zwierzęta z allelem *G* może przyczynić się do zwiększenia wybranych cech wzrostu i długowieczności u krów rasy simental. Ponadto, największą liczbę osobników z tym allelem charakteryzowały łatwe wycielenia. Konieczne jest jednak kontynuowanie badań na większych grupach zwierząt.

Słowa kluczowe: polimorfizm genetyczny, bydło mięsne, masa ciała