

## FATTY ACID BINDING PROTEIN 4 (*FABP4*) AND THYROGLOBULIN (*TG*) POLYMORPHISMS IN RELATION TO MILK PERFORMANCE TRAITS IN THE HOLSTEIN-FRIESIAN CATTLE

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### ABSTRACT

Cows of the Polish Holstein-Friesian black-white variety were tested to identify c.328G>A polymorphism in the *FABP4* gene and g.422C>T polymorphism in the *TG* gene. Three different genotypes (GG, GA and GG) were identified in cows in the *FABP4* gene, with the GG genotype (0.74) and wild type G allele (0.85) occurring most frequently. High frequency of the wild type C allele (0.815) was demonstrated also in the *TG* gene. While the TT homozygotes accounted for only 7% of the total numbers in the herd, the CC made up as much as 70% of the total. The impact of GG and GA genotypes in the *FABP4* gene and of CC and CT in the *TG* gene on yield and composition of milk in the first 3 consecutive lactations of young cows was analyzed. No effect between the tested genotypes and milk performance traits has been demonstrated.

**Key words:** *FABP4* gene, *TG* gene, polymorphism, milk, cattle

### INTRODUCTION

The fatty acid binding proteins (FABP) are a family of small cytoplasmic proteins that bind LCFA (long chain fatty acids) and other hydrophobic ligands. Their main functions are the capture, transport and metabolism of fatty acids. They play a role in  $\beta$ -oxidation of fatty acids by engaging in their transport from the cytoplasmic membrane. Tissue-specific cytoplasmic proteins belonging to this family have been identified, and are known as FABP1 – FABP9. The most common FABP protein isoforms are FABP3, FABP4 and FABP5. The FABP4 protein, also called adipocyte A-FABP, is strongly expressed in mature adipose tissue. The function of this protein in the mammary gland has not yet been well understood [Nafikov 2010]. These proteins were found to be involved in intracellular transport, storage and metabolism of ligands [Prows et al. 1996, Coe and Bernlohr 1998]. Thyroglobulin TG is a glycoprotein produced in the thyroid follicular cells, accounting for 75% of total thyroid

protein and serving as a substrate for synthesis of thyroid hormones.

The *FABP4* and *TG* genes in cattle are located on chromosome 14, where the presence of a number of quantitative traits loci (QTLs) affecting milk production: yield and fat content in milk has been demonstrated [Khatkar et al. 2004, Yardibi et al. 2013]. *FABP4*, otherwise known as A-*FABP4*, has a conservative character, and consists of 4 exons and 3 introns. It is expressed, inter alia, in adipose tissue and in the mammary gland [Chmurzyńska 2006, Kulig et al. 2013]. Links between *FABP4* polymorphism and meat marbling, subcutaneous fat thickness, and carcass weight [Barendse et al. 2009, Michal et al. 2006, Cho et al. 2008, Lee et al. 2010] as well as the profile of fatty acids in intramuscular fat [Hoashi et al. 2008] have been demonstrated.

The *TG* gene is considered to be one of the major genes affecting lipid levels and their metabolism [Yardibi et al. 2013]. *TG* covers a DNA genome area of at least 300 000 bp and contains at least 37 exons; it encodes 8.700 bp

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mRNA [Khatib et al. 2007]. Research focusing on Single Nucleotide Polymorphism (SNP) in the *TG* gene has been carried out on various breeds of cattle and *Bubalus bubalis*. The resulting works [Pannier et al. 2010, Yardibi et al. 2013, Dubey et al. 2014, Dubey et al. 2015] showed a correlation between SNP and meat marbling. The effect of the thyroglobulin gene on fat thickness, to a small extent correlated with meat marbling, was shown in the work of Casas and White [2007]. It was demonstrated that the *TG* gene affects intramuscular fat content in different breeds of cattle [Barendse et al. 2004, Bonilla et al. 2010, Anton et al. 2012, Savasci and Atasoy 2016]. Gan et al. [2008] have demonstrated the effect of SNP in the 3'UTR gene region on meat marbling, while lack of correlation between the gene polymorphism and fat deposition and meat tenderness was noted by other authors [Fortes et al. 2009].

In the studies by Kowalewska-Łuczak et al. [2010] and Anton et al. [2012] carried out on different breeds of cattle, a correlation between SNP in the *TG* gene and milk performance traits has been revealed.

### Purpose of the study

The study aimed to identify the *Hin1III/FABP4* and *PsuI/TG* polymorphisms in a herd of cows of the Holstein-Friesian breed (HF), and to assess the relationship between the detected gene variability and milk yield and composition.

### MATERIAL AND METHODS

HF cows (N = 77) from the Nowe Jankowice farm, born in the years 2008–2016 were studied. Analysis of milk quantity and quality was carried out on the basis of data obtained from the milk performance test using the A4 method: milk yield, content of protein and fat (%), yield of protein and fat (kg) in the first three consecutive lactations were recorded to calculate 305-day yields. Cows were kept tethered and twice a day fed a TMR consisting of: 25 kg of maize silage, 9 kg of lucerne silage, 9 kg of CCM (ensiled corn grain and cobs), 0.5 kg of straw, 0.5 kg of barley middling, 3 kg of soybean middling. The averages for individual milk performance traits in the herd for the three consecutive lactations were as follows (Table 1).

### Isolation of genomic DNA and genotyping

DNA was isolated from whole blood, collected once into tubes containing EDTA. The reagent kit used for purification was the MasterPureTM Genomic Purification Kit (Epicentre Technologies, USA). The PCR reaction for *FABP4* (1142 bp) and *TG* (548 bp) gene fragments was performed at 25 l volume using the Mastercycler Ep Grad

S (Eppendorf, Germany) with the following reagents: 1x buffer and 1U DreamTag polymerase, MgCL2 1.25 mM, dNTP' mix 0.2 mM (ThermoScientific, USA), 150–200 ng genomic DNA and 0.2 uM each primer. Data on PCR reaction and restriction analysis are shown in Table 2. Restriction digestion was checked electrophoretically on agarose gel 1.5–2.0%, ran at 80 V for 45 min.

**Table 1.** Milk performance traits of cows in the first three consecutive 305-day lactations

**Tabela 1.** Parametry użytkowości mlecznej mleka krów w pierwszych trzech kolejnych laktacjach 305-dniowych

Trait – Cecha	1. lactation 1. laktacja	2. lactation 2. laktacja	3. lactation 3. laktacja
Yield, kg – Wydajność, kg	11,070	12,694	12,972
Protein, kg – Białko, kg	375	425	420
Protein, % – Białko, %	3.40	3.40	3.24
Fat, kg – Tłuszcz, kg	401	456	443
Fat, % – Tłuszcz, %	3.66	3.61	3.48

### Statistical analysis

The data were analyzed statistically using the univariate variance analysis approach, taking into account the genotype impact. The significance of differences between means was determined using Duncan's new multiple range test (Statistica® 13.1 PL).

### RESULTS

Amplified fragment analysis of both of the examined genes showed the presence of polymorphism c.328G>A in the *FABP4* gene and g. 422C> T in the *TG* gene (Table 3). Three different genotypes were identified in the *FABP4* gene, with the genotypes GG (0.74) and the wild type G allele (0.85) being the most common. Similarly 2 alleles and 3 genotypes were present also in the *TG* gene, with the TT genotype found only in 7% of the cows tested. In the *FABP4* and *TG* loci the population was at Hardy-Weinberg equilibrium.

The incidence of homozygotes with a mutation in the studied loci was very low, therefore the analysis of correlation between the gene polymorphism and milk production traits in the Nowe Jankowice herd was limited to the GG and GA genotypes for *FABP4* and to CC and CT genotypes for *TG* (Table 4). Analysis of milk yield of cows in consecutive lactations showed an increase in milk yield with age. For cows with different genotypes in the *FABP4* and *TG* loci, in mutation carriers the milk yield was reduced in the 1st lactation. In the subsequent two lactations, an increase in yield in carriers of mutation at the *FABP4* locus was observed, while cows with muta-

**Table 2.** Starters in PCR reaction and restriction enzymes used as RFLP probes

**Tabela 2.** Startery zaprojektowane do reakcji PCR oraz enzymy restrykcyjne użyte w reakcji PCR-RFLP

Gene Gen	Starters – Startery	Ta	Amplicon (bp) Fragment (pz)	Enzyme Enzym	Substitution Substytucja	Fragment length in agarose gel (bp) Wielkość prążków w żelu agorozowym (pz)
<i>FABP4</i>	CCATGTTACCTTTGATATTTAGCTG	59	1142	HinIII	G>A	GG (726, 278 bp/pz)
	ACAACGTATCCAGCAGAAAGTCATG Hoashi et al. [2008]					GA (726, 493, 278 bp/pz) AA (493, 278 bp/pz)
<i>TG</i>	GGGGATGACTACGAGTATGACTG	54	548	PvuI	C>T	CC (366, 178 bp/pz)
	GTGAAAATCTTGTGGAGGCTGTA Khatib et al. [2007]					CT (548, 366, 178 bp/pz) TT (548 bp/pz)

Ta – annealing temperature.

Ta – temperatura przyłączenia starterów.

tion at the *TG* gene locus continued to produce less milk. However, these differences were not statistically significant. No effect of the presence of a mutated allele in the studied loci on milk composition has been determined.

## DISCUSSION

The presence of polymorphism in exon 3 of the *FABP4* gene (c.328 G>A), resulting in a p.V110M missense mutation (valine substituted by methionine) has been demonstrated in HF cows farmed in Nowe Jankowice. This polymorphism has so far been analyzed in cattle primarily with regard to meat performance [Cho et al. 2008, Hoashi et al. 2008], while the relationship with milk performance traits of dairy cattle has been somewhat neglected in research [Nafikov et al. 2013, Zhou et al. 2015].

**Table 3.** Genotype and allele frequency in the studied loci

**Tabela 3.** Frekwencja genotypów i alleli w badanych loci

Gene – Gen	Genotype Genotyp			Allele Allel	
<i>FABP4</i>	GG	GA	AA	G	A
Frequency – Frekwencja	0.74	0.23	0.03	0.85	0.15
<i>TG</i>	CC	CT	TT	C	T
Frequency – Frekwencja	0.70	0.23	0.07	0.815	0.185

The analysis showed relatively low frequency of the mutated (C = 0.815) allele, and showed no association with milk yield and composition in the HF cows. In the study by Kulig et al. [2013], the Polish HF cattle breed showed a higher incidence of carriers of GC genotype (0.3867) and CC homozygotes (0.0995). The aforementioned authors have shown that cows carrying mutations had a significantly lower percentage of fat in milk compared to the wild genotype [Kulig et al. 2013]. In the Jersey breed, where only genotypes CC and CG – with frequencies of 0.89 and 0.11, respectively – were identified, no correlation between polymorphism and milk performance has been found [Kulig et al. 2010]. HF x Jersey hybrids showed considerable variability, as the fol-

lowing polymorphisms: c.328G>A, c.348G>C, c.348 + 34T>C, c.348 + 56T>C, c.348 + 303T>C, and the following 3 haplotypes have been identified: A (GGTTT); B (AGTTT); C (GCCCC). It has been shown that haplotype A is associated with an increase in protein content (%) in milk, while haplotype B – with increased milk yield. Out of the identified six genotypes: AA, AB, AC, BB, BC, CC (with respective frequencies of 0.221; 0.161; 0.328; 0.032; 0.120; 0.138), the cows with AA, AB and AC genotypes produced less milk compared to the BC genotype cows – however, their milk showed increased protein content [Zhou et al. 2015].

In light of the above results, it appears that the effects of *FABP4* polymorphisms are not yet unambiguously clear, but on the basis of available literature it can be posited that this genetic factor may lead to modifications in cow milk fatty acid profile. In the Piedmontese, Valdostana and Jersey cattle breeds, it was shown that animals of the wild genotype AA (g.2989A>G) presented with a higher content of short chain fatty acids (8 C–14 C). Whenever this effect was negative, the share of long chain fatty acids (over 16 C) increased. The genotype resulted also in modification of the sum of saturated fatty acids (SFA) in milk without affecting the content of unsaturated UFAs [Marchitelli et al. 2013]. In American cattle breeds, researchers identified the presence of 4 SNPs: g.3767 T>C, g.3745 T>C, g.3118 G>C, g.3691 G>A as well as three haplotypes: H1 (CCCG), H2 (TTGG) and H3 (TTGA), with frequencies of 0.50; 0.27 and 0.24, respectively. Studies have shown that the presence of a given haplotype is correlated with SFA, UFA and MUFA (monounsaturated fatty acids) content, as well as the SFA/UFA ratio. It was found that haplotype affects also the content of lauric and myristic acid in milk. The presence of the haplotype H3 compared to the haplotype H1 was (statistically significantly) correlated with lower SFA content, higher UFA and MUFA concentration, lower SFA/UFA ratio, as well as lower lauric and myristic acid content [Nafikov et al. 2013]. Variation in the *FABP4* gene may thus be considered as a candi-

**Table 4.** Averages for milk performance traits of cows with different genotypes in the *FABP4* and *TG* loci

**Tabela 4.** Średnie dla parametrów użytkowości mlecznej krów o różnych genotypach w loci *FABP4* i *TG*

Trait – Cecha	Genotype – Genotyp			
	<i>FABP4</i>		<i>TG</i>	
	GG (X ±SE)	GA (X ±SE)	CC (X ±SE)	CT (X ±SE)
1. lactation – 1. laktacja				
Yield, kg – Wydajność, kg	11,176.2 ±366	11,005.6 ±209	11,207.1 ±209	10,727.5 ±428
Protein, kg – Białko, kg	377.4 ±10.11	379.2 ±17.2	381.1 ±6.6	362.72 ±15.1
Protein, % – Białko, %	3.39 ±0.01	3.46 ±0.02	3.4 ±0.03	3.4 ±0.06
Fat, kg – Tłuszcz, kg	398.1 ±2.32	407.4 ±5.19	404.9 ±7.5	404.7 ±18
Fat, % – Tłuszcz, %	3.59 ±2.31	3.7 ±5.11	3.6 ±0.06	3.8 ±0.14
2. lactation – 2. laktacja				
Yield, kg – Wydajność, kg	12,675.2 ±697	13,331.2 ±2	12,881.3 ±241	11,987.7 ±577.6
Protein, kg – Białko, kg	419.6 ±10.11	458.0 ±18.5	429.4 ±7.9	404.0 ±21.2
Protein, % – Białko, %	3.38 ±4.90	3.51 ±9.2	3.4 ±0.03	3.5 ±0.07
Fat, kg – Tłuszcz, kg	454.1 ±20.8	496.4 ±25.2	457.4 ±14.5	450.8 ±25.8
Fat, % – Tłuszcz, %	3.57 ±0.5	3.73 ±0.4	3.6 ±0.1	3.7 ±0.2
3. lactation – 3. laktacja				
Yield, kg – Wydajność, kg	12,923.9 ±100	13,139.0 ±106	12,952.3 ±282	12,473.9 ±407
Protein, kg – Białko, kg	419.4 ±24.9	439.0 ±26.8	423.4 ±11.8	399.1 ±11.1
Protein, % – Białko, %	3.74 ±0.05	3.34 ±0.05	3.2 ±0.03	3.2 ±0.04
Fat, kg – Tłuszcz, kg	446.0 ±27.7	315.0 ±31.7	443.5 ±20.02	432.9 ±22.4
Fat, % – Tłuszcz, %	3.49 ±0.09	2.43 ±0.2	3.5 ±0.1	3.5 ±0.2

date gene for association with the fatty acid profile of cow milk [Marchitelli et al. 2013].

*TG* polymorphism in the 5'-UTR region was also identified in the study and, similarly as was the case for the *FABP4* gene, high incidence of the wild genotype CC was reported (0.70). The TT genotype accounted for only 7% of the herd. It should be noted that the incidence of homozygotic mutation carriers depends on the breed of cattle. For example, the reported frequency was: for the Limousine breed 0.17, in Charolais cows 0.26, in Simmental cows 0.33, for the Hereford breed 0.02 [Pannier et al. 2010], and for the Brahman breed 0.04 [Casas and White 2007]. Cows from the Nowe Jankowice farm had low incidence of the T allele (0.185) – higher frequencies of this allele were found in such breeds as German HF: 0.25 frequency [Thaller et al. 2003] and Jersey – 0.47 [Kowalewska-Łuczak et al. 2010].

The analysis of associations of polymorphisms in the *TG* gene did not show statistically significant correlations, as confirmed by other authors [Khatib et al. 2007]. The link between such polymorphism and milk performance traits has been demonstrated in a number of studies, but often the results were dependent on which lactation was analyzed. Anton et al. [2012] observed the highest milk yield in the 1st lactation in individuals with the TT genotype, and the lowest - in heterozygotes. In the Jersey and Simmental breeds, the highest yields were recorded in subjects with the CC genotype, and the lo-

west – in cows with the TT genotype. Also in the study by Kowalewska-Łuczak et al. [2010] the highest milk yield (though only in the 2nd and 3rd lactations) was noted in Jersey cows with the CC genotype, while the lowest - in individuals with the TT genotype.

In the 2nd and 3rd lactations, Anton et al. [2012] found the highest protein content in milk of HF cows with the CT genotype; in the case of the Simmental and Jersey breeds milk of the TT genotype had the highest (%) protein content. In contrast, Kowalewska-Łuczak et al. [2010] found that in the Jersey breed the TT homozygotes were giving milk with the highest protein content. Analyzing the fat yield (kg) in milk in the 2nd and 3rd lactations, Kowalewska-Łuczak et al. [2010] found that milk of the CC genotype Jersey cows had the highest fat yield. Anton et al. [2012] observed the highest fat content in the milk of TT homozygotes for the Jersey and Simmental cattle breeds.

## SUMMARY

In conclusion, the research conducted - which should be treated as a preliminary study – did not uncover a correlation between polymorphisms in the *FABP4* and *TG* genes and milk performance traits of the Holstein-Friesian cows. However, the research on the topic will continue as part of a study focused on impact of genetic factors on the quality of cow milk.

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## POLIMORFIZM W LOCI *FABP4* I *TG* A SKŁAD MLEKA KRÓW RASY HOLSZTYNO-FRYZYJSKIEJ

### STRESZCZENIE

Krowy rasy polskiej holsztyno-fryzyjskiej poddano analizie identyfikacji polimorfizmu c.328G>A w genach *FABP4* i g.422C>T w *TG*. U krów w genie *FABP4* stwierdzono występowanie 3 różnych genotypów (GG, GA i GG), przy czym najczęściej pojawiał się genotyp GG (0,74) i allel typu dzikiego G (0,85). Również w genie *TG* wykazano dużą frekwencję allelu typu dzikiego C (0,815) natomiast homozygoty TT stanowiły zaledwie 7% a CC aż 70% krów w stadzie. Analizowano wpływ genotypów GG i GA w *FABP4* oraz CC i CT w genie *TG* na ilość i skład mleka w 3 pierwszych kolejnych laktacjach krów. Nie wykazano wpływu badanych genotypów na parametry użytkowości mlecznej krów.

**Słowa kluczowe:** gen *FABP4*, gen *TG*, polimorfizm, mleko, bydło