THE EFFECT OF H-FABP GENE POLYMORPHISM ON CARCASS AND MEAT QUALITY IN THE POLISH NATIVE ZŁOTNICKA SPOTTED PIG

WPŁYW POLIMORFIZMU GENU H-FABP NA JAKOŚĆ TUSZY I MIĘSA ŚWIŃ POLSKIEJ RODZIMEJ RASY ZŁOTNICKIEJ PSTREJ

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ABSTRACT

To estimate the relationships between the H-FABP/HinfI genotype and quality of carcass and meat traits, a total of 30 pigs Złotnicka Spotted breed were investigated. Blood samples were taken during slaughter into test tubes containing anticoagulant K_3EDTA and stored at -25ºC until analysed. The H-FABP genotypes were identified with PCR-RFLP method using HinfI restriction endonuclease. Meat physico-chemical traits were determined in the longissimus lumborum (LL) muscle. Two alleles (H and h) and three genotypes (HH, Hh and hh) were identified within studied pig sample. The effect of H-FABP gene polymorphism was showed on body weight at slaughter and cold carcass weight, where both weights of body were higher in hh group (113.33 kg and 84.27 kg) than in Hh (106.06 kg and 76.40 kg) and HH (103.75 kg and 73.40 kg); P≤0.05 and P≤0.01. Differences between the means of backfat thickness for three genotype groups were not significant. However, significant relations were found between the polymorphism at locus H-FABP/HinfI and basic chemical meat composition. The pigs meat with genotype hh were marked by a higher intramuscular fat content (2.64%) compared to HH group (1.99%); P≤0.05. Values of all analysed traits were within ranges indicating good meat quality of the Polish native Złotnicka Spotted pigs.

KEYWORDS: fattened pigs, heart fatty acid-binding protein gene, carcass and meat characteristics

STRESZCZENIE

W celu oszacowania zależności pomiędzy genotypem H-FABP/HinfI a cechami jakości tuszy i mięsa, badaniu poddano łącznie 30 tuczników rasy złotnickiej pstrej. Podczas uboju pobrano od zwierząt krew do probówek zawierających antykoagulant K_3EDTA i przechowywano w temperaturze -25ºC do czasu analizy. Genotypy względem mutacji genu H-FABP, którego produktem są białka wiążące kwasy tłuszczowe identyfikowano metodą PCR-RFLP używając endonukleazy restrykcyjnej HinfI. Cechy fizyko-chemiczne mięsa określono na części lędźwiowej mięśnia najdłuższego grzbietu. Dwa allele (H i h) oraz trzy genotypy (HH, Hh i hh) stwierdzono w analizowanej próbie świń. Wpływ polimorfizmu genu H-FABP wykazano w zakresie masy ciała przy uboju oraz masy tuszy zimnej, obie masy były cięższe w grupie hh (113,33 kg i 84,27 kg) niż wśród osobników Hh (106,06 kg i 76,40 kg) oraz HH (103,75 kg i 73,40 kg); P≤0,05 oraz P≤0,01. Różnice pomiędzy średnią grubością słoniny dla trzech genotypowych grup były nieistotne. Jednakże, istotne różnice stwierdzono pomiędzy polimorfizmem w locus H-FABP/HinfI a podstawowym składem chemicznym. Mięso tuczników o genotypie hh odznaczało się wyższą zawartością tłuszczu śródmięśniowego (2,64%) w porównaniu do mięsa tuczników o genotypie HH (1,99%); P≤0,05. Wartości wszystkich analizowanych cech wskazywały na dobrą jakość mięsa świń polskiej rodzimej rasy złotnickiej pstrej.

SŁOWA KLUCZOWE: tuczniki, gen wiążący kwasy tłuszczowe w sercu, charakterystyka tuszy i mięsa

STRESZCZENIE SZCZEGÓŁOWE

Gen białka wiążącego kwasy tłuszczowe w sercu jest genem kandydującym, który według wielu badaczy kształtuje zawartość tłuszczu śródmięśniowego [9, 6, 5]. Białko H-FABP wiąże i przenosi kwasy tłuszczowe z membran komórkowych do miejsca ich β-oksydacji lub syntezy fosfolipidów. Ponadto, może również regulować koncentrację oraz metabolizm lipidów, a także niektóre inne procesy metabolizmu komórki, szczególnie w mięśniu sercowym i mięśniach szkieletowych [9].

Niniejsza praca stanowi materiał wstępny do określenia wpływu polimorficznych form genu H-FABP na cechy związane z jakością tuszy oraz mięsa świń rodzimej rasy złotnickiej pstrej. Badaniom poddano 30 osobników rasy złotnickiej pstrej (loszki względem wieprzków 1:1). Zwierzęta utrzymywano w jednakowych warunkach i żywiono taką samą paszą. Tucz trwał do masy ciała średnio 104 – 113 kg, i zbliżonego wieku około 200 dni.

Po zakończonym tuczu wszystkie zwierzęta poddano ubojowi, zgodnie z przepisami obowiązującymi w przemyśle mięsnym. Podczas uboju pobrano od zwierząt krew do probówek zawierających antykoagulant K_3EDTA i przechowywano w temperaturze -25ºC do czasu analizy. Identyfikacji polimorfizmu genu H-FABP dokonano metodą PCR-RFLP przy zastosowaniu endonukleazy HinfI według metody Gerbensa i wsp. [7].

Dzień po uboju i wychłodzeniu tusz, prawą półtuszę poddano szczegółowej ocenie. Mierzono grubość słoniny w 5 miejscach (nad łopatką, między ostatnim kręgiem piersiowym i pierwszym lędźwiowym oraz na wysokości kręgów krzyżowych – I, II, III) oraz długość tuszy. Przeprowadzono dysekcję ocenianych półtusz zgodnie z metodyką opracowaną dla polskich Stacji Kontroli Użytkowości Rzeźnej Trzody Chlewnej [16]. Ponadto, określono wielkość przekroju poprzecznego oka polędwicy metodą planimetrowania wykorzystując program komputerowy Lucia. W celu dokonania oceny jakości mięsa z każdej półtuszy pobierano próby z mięśnia longissimus lumborum. W 45 minut po uboju określono stopień zakwaszenia tkanki mięśniowej (pH_1) przy użyciu przenośnego pH-metru wyprodukowanego przez firmę Matthaüs. Końcowe zakwaszenie tkanki mięśniowej (pH_k) określono natomiast w 48 godzin po uboju w wodnych ekstraktach mięsa. Swobodny wyciek soku z mięsa oszacowano na podstawie próby mięsa o masie 150 g, zgodnie z metodą Honikela [10]. Barwę mięsa oceniono przy użyciu fotokolorymetru Minolta CR 310 [11] oraz dodatkowo jasność barwy spektrofotometrem Spekol 11 [17]. Skład chemiczny mięsa (zawartość wody, białka, tłuszczu śródmięśniowego, popiół), oznaczono metodą AOAC [2]. Do opracowania wyników wykorzystano program komputerowy STATISTICA 8.0 PL [20].

W badanej próbie świń rasy złotnickiej pstrej stwierdzono występowanie obydwu allei H i h o zbliżonej częstości wynoszącej odpowiednio: 0,53 i 0,47 oraz wszystkich trzech genotypów względem analizowanej mutacji o częstości: HH 0,27; Hh 0,53 oraz hh 0,20 (tabela 1). Cechy wartości rzeźnej świń przedstawiono w tabeli 2. Stwierdzono istotne różnice pomiędzy badanymi grupami genotypowymi w zakresie masy ciał przed ubojem jak i masy tuszy zimnej. Osobniki o genotypie hh były istotnie cięższe w obydwu przypadkach (odpowiednio: 113,33 kg oraz 84,27 kg) niż tuczniki o genotypie Hh (106,06 kg i 76,40 kg) oraz HH (103,75 kg i 73,40 kg); $P \leq 0,05$ oraz $P \leq 0,01$. W zakresie pomiaru wielkości oka polędwicy nie wykazano różnic statystycznie istotnych, a uzyskane wartości tej cechy były charakterystyczne dla świń rasy złotnickiej pstrej i kształtowały się na poziomie blisko 33 cm^2 (tabela 2). W zakresie oceny jakości mięsa analizie poddano stopień zakwaszenia tkanki mięśniowej (tabela 3). W przypadku pomiaru pH w 45 minut po uboju jak i 48 godzin nie zaobserwowano istotnych różnic pomiędzy grupami genotypowymi. Jasność barwy mięsa oszacowana przy użyciu spektrofotometru mieściła się w granicach od 18,65% (HH) do 19,74% (hh), co było zgodne z wartościami uzyskanymi przy użyciu aparatu Minolta: od 47,32 (HH) L* do 49,02 (hh) L* (tabela 3). Istotne różnice w zakresie składu chemicznego mięsa świń złotnickich pstrych wykazano w przypadku zawartości tłuszczu śródmięśniowego (IMF). Wyższą zawartością i jednocześnie bardziej korzystną IMF cechowało się mięso pochodzące od tuczników o genotypie hh (2,61%) w porównaniu do grupy o genotypie HH (1,99%), $P \leq 0,05$. Wartości wszystkich analizowanych cech charakteryzowały dobrą jakość mięsa świń polskiej rodzimej rasy złotnickiej pstrej.

Stwierdzone niewielkie zależności pomiędzy polimorfizmem genu H-FABP/HinfI a jakością tuszy i mięsa w badanej próbie świń złotnickiej pstrej mogą być rozważane jako interesujące, lecz jednocześnie wymagają dalszej kontynuacji na większej populacji zwierząt.

INTRODUCTION

Intramuscular fat content (IMF) is one of decisive factors influencing the sensory properties of meat, positive correlated with its tenderness, juiciness and taste [4]. Optimal intramuscular fat content level of carcass is considered to oscillate around 2–3 % [4]. Intensive selection carried out in order to reduce pork fat thickness and consequently improve the carcass quality led to a decrease in intramuscular fat content to a value below the allowable level [18]. Besides, the intramuscular fat content depends on various factors, like breed type,

slaughter weight and sex of pigs [3].

Numerous researchers suggest [9, 6, 5] that candidate gene responsible for intramuscular fat content level is the protein gene binding fatty acids in heart – H-FABP, which was mapped in the 6th pig chromosome [7]. The H-FABP protein is one of the short-chain FABP cytoplasm proteins, binding and transporting fatty acids from cell membranes to the place of their β-oxidation or phospholipids synthesis. Furthermore, the gene may also regulate the concentration and lipid metabolism, as well as other processes connected with cell metabolism [9].

Dutch researchers [7] proved, in case of the Duroc breed of pigs, the existence of an important correlation between intramuscular fat content and genotype in the H-FABP gene locus. It has been furthermore proved that the polymorphism identified by the HaeIII enzyme is significantly correlated with pork backfat thickness. Results obtained on the Duroc breed were not however confirmed in the course of the studies carried out by Nechtelberger et al. [13] on Large White and Landrace pig breeds.

Złotnicka Spotted pigs constitute the sole indigenous Polish breed of pigs which was not improved by any other breeds. Meat obtained from Złotnicka Spotted fatteners is distinguished by an overall good quality [15] and the capability of being used in production of traditional dry cured hams [12].

It seem particularly interesting to establish the interdependence between specific forms of H-FABP/HinfI gene and the essential production features of Złotnicka Spotted indigenous breed of pigs.

MATERIAL AND METHODS

The subject of research was 30 purebred Złotnicka Spotted pigs (gilts to barrows 1:1). Animals were managed and feed similarly, according to current standards. Fattened pigs were slaughtered at average 104 to 113 kg live body weight according to the meat industry standards. The age of the pigs was amounted to approximately 200 days. Blood samples were taken from the vena cava anterior or at the slaughter and put into test tubes containing K_3EDTA and stored at -25ºC until analysed. Gene polymorphism of H-FABP was determined with the PCR/RFLP procedure using HinfI restriction enzyme [7]. The studied pig sample was also analysed with respect to influence of RYR1 gene polymorphism. The 16 porkers were heterozygous CT and 14 porkers were homozygous CC at RYR1 gene locus.

One day post-slaughter, the right carcass-sides were dissected and evaluated using the simplified methods applied to Polish Pig Testing Stations [16]. Thickness of the backfat was measured in five points: above the shoulder, between the last thoracic vertebra and the first lumbar vertebra and at the level of sacral vertebra – I, II, III. The loin cross section area was measured behind last rib. The loin eye area was measured by planimetering the photographs with the help of computer Lucia software.

Meat quality traits were measured in the lumbar part of longissimus dorsi muscle (LL). Value of pH_1 was recorded using a pistol pH-meter (Matthaüs company), and ultimate pH_u was measured in minced meat-water slurry 48h after slaughter. Meat colour was determined twofold, first on minced meat samples on Spekol 11 spectrophotometer with a reflectance attachment and using regression equations [17] to estimate brightness and secondly on a second apparatus Chromameter Minolta CR 310 to obtain L, a* and b* values [11]. Drip loss was recorded on approximately 150 g slice of meat as described by Honikel [10]. Basic chemical composition, water, protein and intramuscular fat content (IMF) were assayed according to AOAC [2].

In order to compile the results, the computer program STATISTICA 8.0 PL [20] was used. Differences between groups were defined using one-way analysis of variances and significance by means of the Duncan test.

RESULTS AND DISCUSSION

As a result of the analyses carried out, it has been proved that in examined sample of Złotnicka Spotted pigs, the H and h alleles occurred with similar frequency (0.53 and 0.47 respectively) (Table 1). An assessment of the number of homo- and heterozygous genotypes of H-FABP/HinfI gene was also carried out. Data presented in Table 1 show that heterozygous Hh genotype occurred more frequently (0.53) than homozygous genotypes (0.27 for HH and 0.20 for hh). Urban and Mikolášová [21] analysed frequency within H-FABP/HinfI locus in case animal groups consisting of the three following breeds of pigs: Large White, Landrace and Duroc. Similarly to the study presented herein, the least numerous was the hh homozygous group, both in case of the Large White (0.084), as well Landrace (0.075) breeds. Nechtelberger et al. [13] did not find any occurrence of hh/HinfI genotype animals in the Large White population. However, in the study carried out by Antosik et al. [1] on pigs from Line 890 as well as in study by Sieczkowska et al. [19] among Landrace breed, there have been no animals found with the HH/HinfI genotype and the highest frequency of occurrence applied to animals with the hh/HinfI genotype.

The slaughter value results of carcasses are presented in Table 2. There have considerable differences observed

Table 1. Frequency of genotypes and alleles of the *H-FABP* gene
Tabela 1 Frekwencja genotypów i alleli genu *H-FABP*

	Genotypes *H-FABP*			Alleles *H-FABP*	
	HH	*Hh*	*hh*	*H*	*h*
Number	8	16	6	0.53	0.47
Frequency	0.27	0.53	0.20		

Table 2. Slaughter value of carcasses in relation to the *H-FABP* genotype
Tabela 2 Wartość rzeźna tusz w zależności od genotypu *H-FABP*

Trait		Genotype *H-FABP*		
		HH	*Hh*	*hh*
Body weight at slaughter (kg)	mean	103.75^{a}	106.06^{a}	113.33^{b}
	SD	5.12	6.56	9.54
Cold carcass weight (kg)	mean	73.40^{Aa}	76.40^{a}	84.27^{Bb}
	SD	5.58	6.98	9.27
Carcass length (cm)	mean	83.62	84.41	84.83
	SD	1.68	2.33	3.98
Loin eye area (cm^2)	mean	32.03	32.68	32.87
	SD	5.43	4.35	4.59
Backfat thickness (cm)				
over shoulder	mean	34.50	35.00	39.30
	SD	10.50	5.20	11.80
over last rib	mean	21.50	24.40	24.00
	SD	10.90	4.40	8.60
over sacrum I	mean	25.10	28.00	27.50
	SD	12.40	6.10	9.50
over sacrum II	mean	18.50	23.00	22.00
	SD	8.80	3.60	6.80
over sacrum III	mean	24.10	27.70	31.20
	SD	9.60	5.20	7.80
mean from	mean	24.80	28.50	28.80
5 measurements (mm)	SD	10.00	4.10	8.20

$^{Aa...}$ Within genotype groups means for individual bearing different superscripts differ significantly at: small letters P≤0.05; capitals P≤0.01.

between the body weight of animals prior to slaughter and the weight of cold carcass. The hh fattened pigs were distinguished by higher values of the above features (113.33 kg and 84.27 kg respectively) as compared with Hh group results (106.06 kg and 76.40 kg respectively) and HH group as well (103.75 kg and 73.40 kg); P≤0.05 and P≤0.01. Gerbens et al. [9] in study covering 983 specimens of the Duroc breed did not identify any influence of the H-FABP/HinfI gene polymorphism on animal body weight in the course of fattening up to 110 kg approximately.

The experiment conducted by Sieczkowska et al. [19] on purebred Landrace animals and two-way crossbreeds (Landrace x Yorkshire) did not indicate any influence of the H-FABP gene polymorphism, identified by HinfI restriction enzyme, on the size of the loin eye. The lack of correlations in this scope has also been found in the study presented herein, with all genotype groups bearing traits of similar loin section size (HH 32.03 cm^2; Hh 32.68 cm^2; hh 32.87 cm^2).

Gerbens et al. [8, 9] proved the existence of a significant influence of the analysed polymorphism not only on intramuscular fat content but also on the backfat thickness. According to Gerbens et al. [8], the correlations were significant only in case of Duroc breed of pigs, distinguished by a higher intramuscular fat content. The study presented herein and carried out on Złotnicka Spotted pigs did not confirm the above correlations. Differences in the backfat thickness, taken from the mean value of five measurements, were statistically

Table 3. The physico-chemical traits of meat in relation to the *H-FABP* genotype
Tabela 3 Cechy fizyko-chemiczne mięsa w zależności od genotypu *H-FABP*

Trait			Genotype *H-FABP*		
			HH	*Hh*	*hh*
pH_1		mean	6.27	6.29	6.20
		SD	0.55	0.40	0.43
pH_u		mean	5.48	5.51	5.52
		SD	0.06	0.10	0.09
Drip loss (%)		mean	2.59	2.44	2.57
		SD	0.98	1.62	1.24
Lightness (%)		mean	18.65	19.17	19.74
		SD	3.22	3.43	3.36
Minolta	L*	mean	47.32	48.04	49.02
		SD	1.69	2.81	1.54
	a*	mean	17.23	17.04	16.72
		SD	0.43	0.53	0.51
	b*	mean	2.08	2.39	2.79
		SD	0.68	1.13	0.74
Water content (%)		mean	74.01	73.85	73.51
		SD	0.48	0.66	0.32
Protein content (%)		mean	22.86	22.74	22.75
		SD	0.50	0.48	0.50
Intramuscular fat content (%)		mean	1.99^{a}	2.28^{ab}	2.61^{b}
		SD	0.64	0.58	0.24

[Aa...] Within genotype groups means for individual bearing different superscripts differ significantly at: small letters $P \leq 0.05$.

insignificant. Similarly, Urban et al. [22] did not find any influence of H-FABP/HinfI polymorphism in terms of the backfat thickness, among 97 pigs of Large White and Landrace breed.

The pH_1 and pH_u measured values were not diversified between the analysed genotype groups (Table 3). The pH value measured 45 minutes after slaughter was between 6.20 (hh) to 6.29 (Hh). Pulkrábek et al. [14] lead that the values of pH exceeding 5.8 obtained 45 to 60 minutes after slaughter are considered as normal while those equal to or lower than 5.8 indicate a reduced quality of pig meat. The pH value measured 48 hours after slaughter was between 5.48 (HH) to 5.52 (hh). The lack of correlations between these features and H-FABP gene was observed by Nechtelberger et al. [13], among the most important breeds of pigs found in Austria (Pietrain, Large White and Landrace).

Meat colour measurement was carried out with the aid of Spekol 11 spectrophotometer and a Minolta CR 310 camera (Table 3). The most crucial element of meat colour measurement is its lightness. In an assessment done by means of the above spectrophotometer, the meat lightness ranged approximately from 18.65% (HH) to 19.74% (hh) and according to results obtained by means of Minolta camera: from 47.32 (HH) L* to 49.02 (hh) L*. These values confirm good meat quality with the desirable, dark meat colour. In 2001 year, Nechtelberger et al. [13] carried out an assessment of meat colour measurement depending on the H-FABP/HinfI genotype and with the aid of the Göttinger photometer. Significant differences with regard to this feature between the HH and hh genotypes were observed among Large White breed, however, in case of the remaining two breeds (Landrace and Pietrain) there have been no correlations found.

In the course of study presented herein, a significant influence has been observed of analysed polymorphism on the meat's chemical composition, mainly on the intramuscular fat content (Table 3). The higher and at the same time more advantageous IMF content was the feature of the meat of specimens with hh genotype (2.61%), as compared with the HH genotype group (1.99%), $P \leq 0.05$. Slight correlations ($P<0.06$) between three forms of the H-FABP/HinfI gene and the intramuscular fat content were observed by Urban et al. [22] among Large White and Landrace breeds. The subject of study carried out by Gerbens et al. [9] were, on the other hand, three mutations of the said gene, identified by MspI, HaeIII and HinfI endonuclease. In all the above cases, the authors found significant correlation between genotype of pigs and the IMF level. The study carried out by Nechtelberger [13], did not confirm these correlations. Intramuscular fat content level in three populations of pigs (Large White,

Landrace and Pietrain) remained on a similar level in all analysed genotype groups.

The presented results of the study conducted on the Polish native Złotnicka Spotted pigs with regard to H-FABP/ HinfI genotype showed an essential influence on quality of meat, first of all in relation to the level of intramuscular fat content. The meat of the Złotnicka Spotted breed of pigs was good quality, with an appropriate dark colour and an optimal intramuscular fat content level.

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