Polymorphisms of calpastatin gene in sheep

Polimorfizm genu kalpastatyny owiec

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Abstract

Calpastatin plays an essential role in the growth of skeletal muscles and *post mortem* meat tenderness. The objective of this research was to determine polymorphism in the calpastatin gene in a group of 212 sheep (192 ewes and 20 rams) of four breeds: Polish Merino, Berrichon du Cher, Blackheaded Mutton Sheep, and Ile de France. Polymorphism was identified using the PCR-RFLP technique in accordance with methods by Palmer et al. [9]. The amplified product with the length of 622 bp was digested with restriction enzymes *Msp*I and *Nco*I. It was found that the M and N alleles were present in CAST/*Msp*I locus, their frequency being 83.5% and 16.5% respectively. Whereas in CAST/*Nco*I locus the M allele occurred with the frequency of 95.8%, and the N allele with the frequency of 4.2%.

Keywords: polymorphism, calpastatin, sheep, PCR-RFLP

Abstrakt

Kalpastatyna odgrywa ważną rolę we wzroście mięśni szkieletowych i procesie kruszenia mięsa *post mortem*. Celem badań było określenie polimorfizmu w genie kalpastatyny w grupie 212 owiec (192 maciorki i 20 tryków) czterech ras: merynos polski, berrichon du cher, czarnogłówka i ile de france. Polimorfizm został zidentyfikowany metodą PCR-RFLP według metodyki Palmera i wsp. [9]. Zamplifikowany produkt o długości 622 pz poddano trawieniu enzymami restrykcyjnymi *Msp*I i *Nco*I. Badania wykazały występowanie alleli M i N w *locus* CAST/*Msp*I z frekwencją odpowiednio 83,5% i 16,5%. Natomiast w *locus* CAST/*Nco*I allel M wystąpił z częstością 95,8%, a allel N z częstością 4,2%.

Słowa kluczowe: polimorfizm, kalpastatyna, owce, PCR-RFLP

Detailed abstract

Badaniom poddano 212 owiec (20 tryków i 192 maciorki) 4 ras: merynos polski (82 osobniki: 4 tryki i 78 maciorek), berrichon du cher (41osobników: 2 tryki i 39 maciorek), czarnogłówka (59 osobników: 12 tryków i 47 maciorek) oraz ile de france (30 osobników: 2 tryki i 28 maciorek). DNA powielono za pomocą techniki PCR według metodyki Palmera i wsp. [9]. Zamplifikowany produkt o długości 622 pz poddano trawieniu enzymami restrykcyjnymi: *Msp*I i *Nco*I. Rozdział produktów trawienia przeprowadzono w 1,5% żelu agarozowym w buforze 1xTBE przez 50 minut, pod napięciem 120 V w obecności markera wielkości pUC19 DNA/*Msp*I (Fermentas) (Rys. 1 i 2).

Przeprowadzona analiza *locus* CAST/*Msp*I i CAST/*Nco*I wykazała obecność alleli M i N (Tabela 1), oraz genotypów: MM, MN i NN (Tabela 2). W badanej grupie 4 ras owiec z największą częstością wystąpił allel M (83,5% *Msp*I i 95,8% *Nco*I), natomiast frekwencja allela N wyniosła odpowiednio 16,5% i 4,2%. Polimorfizm zidentyfikowany w locus CAST/*Msp*I był znacznie wyższy niż ten wykryty w *locus* CAST/*Nco*I. Allel N wykrywany przez enzym *Nco*I nie wystąpił w ogóle w grupie owiec ras merynos polski, ile de france i berrichon du cher. Wszystkie allele pojawiły się zarówno w grupie maciorek jak i tryków (Tabela 3). Spośród genotypów najliczniejszym był MM/ *Nco*I (92,0%) i MM/*Msp*I (70,7%), a najniższą częstością cechowały się genotypy NN/*Nco*I (0,5%) i NN/*Msp*I (3,8%). Wszystkie genotypy pojawiły się u obu płci, z wyjątkiem NN/*Nco*I, którego nie stwierdzono u maciorek (Tabela 4).

W znanej literaturze nie odnotowano dotychczas informacji na temat frekwencji alleli i genotypów w owczym genie kalpastatyny identyfikowanych przy użyciu restryktazy *Nco*I.

Przeprowadzona analiza locus CAST/*Msp*I wykazała obecność 3 genotypów (MM, MN i NN), których frekwencja jest odzwierciedleniem częstości alleli. Analiza wyników badań własnych oraz literatury tematu wskazuje, że na frekwencję alleli i genotypów CAST/*Msp*I ma wpływ czynnik rasowy. Natomiast badania locus CAST/*Nco*I wykazały małe zróżnicowanie frekwencji genotypów, przy wysokiej częstości allela M (u ras merynos polski, berrichon du cher i ile de france w ogóle nie zidentyfikowano heterozygot i homozygot NN).

Polimorfizm w genie kalpastatyny owiec wykrywano również metodą PCR-SSCP [1, 6]. Badania te wykazały obecność 3 alleli (A, B i C). Stwierdzono, że są one powiązane z takim cechami jak: masa ciała jagniąt przy urodzeniu oraz tempo wzrostu do momentu odsadzenia [1], oraz przyrosty dobowe w poszczególnych okresach życia jagniąt [6]. Dlatego też wnioskuje się, że także polimorfizm owczego genu kalpastatyny zidentyfikowany metodą PCR-RFLP może mieć istotny wpływ na cechy związane z mięsnością jagniąt. Z tego względu konieczne jest

przeprowadzenie oceny przyżyciowej oraz analizy poubojowej jagniąt i stwierdzenie ewentualnych powiązań lub ich braku z polimorficznymi wariantami w owczym genie kalpastatyny.

Introduction

Calpastatin is an endogenous inhibitor of non-lysosomal proteases – calpains, present in cytosol. The calpastatin gene (CAST) in sheep is located in chromosome 5. It is essential for the formation of skeletal muscles during the postnatal period, and protein proteolysis after slaughter, having therefore an effect on traits of particular importance to consumers such as meat tenderness or water binding [8]. It was also proved that there is a relationship between polymorphism in the calpastatin gene in sheep and slaughter traits such as lamb's body weight at birth and its growth rate until weaning [1]. The objective of the research was to determine the calpastatin gene polymorphism in sheep of four breeds: Polish Merino, Berrichon du Cher, Blackheaded Mutton Sheep and Ile de France, maintained in the *kujawsko-pomorskie* province.

Material and Methods

The research included 212 sheep (20 rams and 192 ewes) of 4 breeds: Polish Merino (82 animals: 4 rams and 78 ewes), Berrichon du Cher (41 animals: 2 rams and 39 ewes), Blackheaded Mutton Sheep (59 animals: 12 rams and 47 ewes), and Ile de France (30 animals: 2 rams and 28 ewes). The biological material was constituted by peripheral blood drawn from the jugular vein into test tubes containing the K_2EDTA anticoagulant. Then, DNA was isolated from blood with the use of MasterPure DNA Purification Kit for Blood (Epicentre Biotechnologies) in accordance with the manufacturer's instructions. As the next step, DNA was multiplied by means of the PCR technique in accordance with methods by Palmer et al. [9]. The amplified product with the length of 622 bp was digested with restriction enzymes: *Msp*I and *Nco*I. Digestion with these endonucleases allows differentiation between the M and N alleles. The *Msp*I enzyme recognizing the M allele cuts the product into 336bp, and 286bp fragments, whereas the *Nco*I enzyme while detecting the N allele triggers formation of 374bp, and 248bp products [9]. Separation of the digestion product was conducted in 1.5% agarose gel in a 1xTBE buffer for 50 minutes, with the voltage of 120 V and pUC19 DNA/*Msp*I (Fermentas) marker present.

Results

Figures 1 and 2 show electrophetic separation of PCR products exposed to *Msp*I and *Nco*I restrictases, respectively.

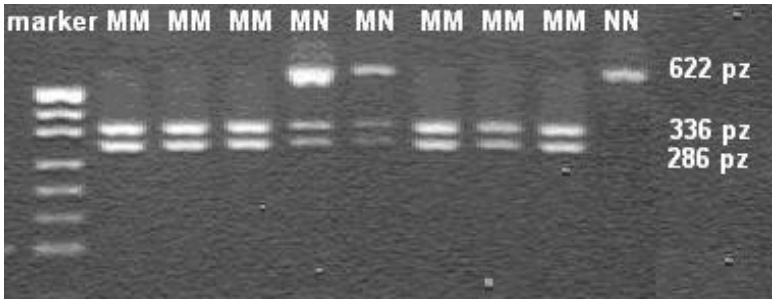

Fig. 1. Electrophetic separation of amplified calpastatin gene fragments exposed to *Msp*I restrictase. Marker – length marker for DNA fragments pUC19 DNA/*Msp*I (Fermentas)
Rys. 1: Rozdział elektroforetyczny amplifikowanych fragmentów genu kalpastatyny poddanych działaniu restryktazy *Msp*I. Marker – marker długości fragmentów DNA pUC19 DNA/*Msp*I (Fermentas)

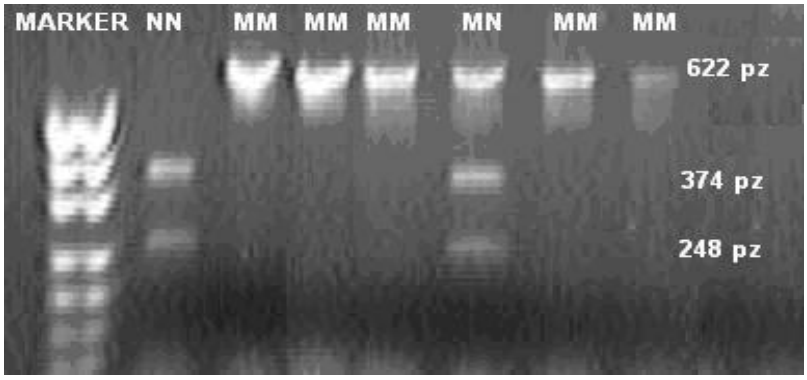

Fig. 2: Electrophetic separation of amplified calpastatin gene fragments exposed to *Nco*I restrictase. Marker – length marker for DNA pUC19 DNA/*Msp*I fragments (Fermentas)
Rys. 2: Rozdział elektroforetyczny amplifikowanych fragmentów genu kalpastatyny poddanych działaniu restryktazy *Nco*I. Marker – marker długości fragmentów DNA pUC19 DNA/*Msp*I (Fermentas)

The analysis of CAST/*Msp*I and CAST/*Nco*I *loci* demonstrated presence of M and N alleles (Table 1), as well as MM, MN, and NN genotypes (Table 2). In the examined group of sheep from 4 breeds the most frequent was the M allele (83.5% *Msp*I and 95.8% *Nco*I), whereas the frequency of the N allele was 16.5% and 4.2% respectively. Polymorphism identified in CAST/*Msp*I locus was considerably higher than that found in CAST/*Nco*I *locus*. The N allele detected by the *Nco*I enzyme did not occur at all in the Polish Merino, Ile de France and Berrichon du Cher sheep. All of the alleles occurred both in the ewes as well as in the rams (Table 3). Amongst the genotypes, the most frequent one was the MM/*Nco*I (92.0%) and the MM/*Msp*I (70.7%). All of the genotypes occurred in both sexes, except for the NN/*Nco*I which was not found in the ewes (Table 4).

Table 1: Frequencies (%) of calpastatin gene alleles depending on the breed of sheep
Tabela 1: Frekwencja (%) alleli genu kalpastatyny w zależności od rasy owiec

Breed/Rasa	Allele/Allel	*Msp*I [%]	*Nco*I [%]
Polish Merino/Merynos polski	M	76.2	100.0
	N	23.8	-
Berrichon du cher	M	92.7	100.0
	N	7.3	-
Blackheaded Mutton	M	81.4	84.7
Sheep/Czarnogłówka	N	18.6	15.3
Ile de France	M	95.0	100.0
	N	5.0	-
Totally/Razem	M	83.5	95.8
	N	16.5	4.2

Table 2: Frequencies (%) of calpastatin gene genotypes depending on the breed of sheep
Tabela 2: Frekwencja (%) genotypów genu kalpastatyny w zależności od rasy owiec

Breed/Rasa	Genotype/ Genotyp	*Msp*I [%]	*Nco*I [%]
Polish Merino/Merynos polski (n=82)	MM	56.1	100.0
	MN	40.2	-
	NN	3.7	-
Berrichon du Cher (n=41)	MM	85.4	100.0
	MN	14.6	-
	NN	-	-
Blackheaded Mutton Sheep/ Czarnogłówka (n=59)	MM	71.2	71.2
	MN	20.3	27.1
	NN	8.5	1.7
Ile de France (n=30)	MM	90.0	100.0
	MN	10.0	-
	NN	-	-
Totally/Razem (n=212)	MM	70.7	92.0
	MN	25.5	7.5
	NN	3.8	0.5

Table 3: Frequencies (%) of calpastatin gene alleles depending on sex
Tabela 3: Frekwencja (%) alleli genu kalpastatyny w zależności od płci

Sex/Płeć	Allele/ Allel	*Msp*I [%]	*Nco*I [%]
Rams/Tryki	M	85.0	90.0
	N	15.0	10.0
Ewes/Maciorki	M	83.3	96.4
	N	16.7	3.6

Table 4: Frequencies (%) of calpastatin gene genotypes depending on sex
Tabela 4: Frekwencja (%) genotypów genu kalpastatyny w zależności od płci

Sex/Płeć	Genotyp	*Msp*I [%]	*Nco*I [%]
Rams/Tryki	MM	75.0	85.0
	MN	20.0	10.0
	NN	5.0	5.0
Ewes/Maciorki	MM	70.3	92.7
	MN	26.0	7.3
	NN	3.7	-

Discussion

The highest differentiation in terms of genotype occurrence in the examined breeds was found in the Blackheaded Mutton Sheep, a meat type breed, in which all possible genotypes occurred. Interestingly, the lowest polymorphism was found in the Ile de France and Berrichon du Cher sheep – another meat type group, where the NN/*Msp*I, MN/*Nco*I, and NN/*Nco*I genotypes were not found at all.

Genotype frequencies similar to those in the Berrichon du Cher sheep were identified in CAST/*Msp*I *locus* in populations of the Tsugai, Valachian, East Fresian, Lacaune breeds as well as in Tsigai and Lacaune crossbreds maintained in Slovakia. The NN genotype was not found either, and the MM and MN genotype frequencies were similar to those discovered in the authors' own research in Berrichon du Cher sheep, and they respectively equalled 87% and 13% [3]. Whereas allele frequencies identified with the *Msp*I enzyme, similar to those found in the Blackheaded Mutton Sheep group, occurred in a Karakul population in Iran. The M allele frequency was 79%, and the N allele 21%, however, the frequency of occurrence for the genotypes differed. In the Karakul population there were significantly fewer NN and MM homozygotes (3% and 61% respectively), meaning that the frequency of MN heterozygotes was higher (36%) as compared to Blackheaded Mutton Sheep examined in the authors' own research [10].

In the examination of the calpastatin gene polymorphism in a population of Arabic sheep from various regions in Iran, occurrence of M and N alleles was identified in CAST/*MspI locus* with frequencies of 85% and 15% respectively, similarly as in the group of animals examined in the authors' own research. The NN homozygotes, however, were present merely in 0.9% of population, and heterozygotes in 28.8% [5]. Furthermore, frequencies of M/*Msp*I and N/*Msp*I alleles similar to those detected in the authors' own research were found in Iran in Kurdi sheep. At the same time, genotype frequency differed, as no NN homozygotes were identified in this case, and the MM and MN genotype frequencies were 76% and 24% respectively [7]. In contrast to the results of the authors' own research, where the M/*Msp*I allele frequency was quite high – from 76.2% in the Polish Merino sheep up to 95.0% in the Ile de France sheep, the research conducted by Elyasi et al. [2] showed frequency of the M allele between 48% and 69% in particular breeds [2].

As has been said earlier, in the examined group of sheep the presence of all genotypes identified with the use of the *Msp*I enzyme was found. The only exception were the sheep of the Berrichon du Cher and Ile de France breeds, in which the NN homozygotes were not present. Whereas in the research conducted by Kaczor [4], 100% of Polish Mountain Sheep were established to be MM homozygotes [4].

In the known literature, no information has so far appeared on allele and genotype frequencies in the calpastatin gene in sheep identified using the *Nco*I restrictase.

The presence of 3 genotypes was found in the analysis of CAST/*Msp*I locus (MM, MN, and NN), the frequency of which reflects the allele frequency. Analysing the results of the authors' own research and the contents of specialist literature one may conclude that the frequency of alleles and CAST/*Msp*I genotypes is influenced by the breed factor. Whereas the examination of CAST/*Nco*I *locus* showed low differentiation of genotype frequencies and high frequency of the M allele (in the Polish Merino, Berrichon du Cher, and Ile de France breeds no NN homozygotes, and heterozygotes were found at all).

Polymorphism in the calpastatin gene in sheep was also detected by means of the PCR-SSCP method [1,6]. The presence of 3 alleles (A, B, and C) was found in the research. It was established that there is a relationship between them and the following traits: lamb body weight at birth, growth rate until weaning[1], and daily gains in specific periods of lamb life [6]. Therefore, it is concluded that also the calpastatin gene polymorphism in sheep, identified by means of the PCR-RFLP method, may have a significant effect on traits related to lamb fleshiness. Hence, it is necessary to conduct a survival assessment and post-slaughter analysis of lambs to establish if any possible links with polymorphic variants in the calpastatin gene are present in sheep.

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