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IDENTIFICATION OF C.*1232G>A POLYMORPHISM IN THE *MSTN* GENE IN MEAT SHEEP BREEDS IN POLAND

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ABSTRACT

The aim of the study was to identify a potential occurrance of c.*1232G>A polymorphism in the 3′–UTR region of the myostatin gene (*MSTN*) in sheep of meat breeds: Pomeranian sheep, Suffolk and Berrichon du Cher. The populations of Suffolk and Berrichon du Cher breeds turned out to be monomorphic, whereas in the native Pomeranian sheep, the occurrence of polymorphism in the region of the *MSTN* gene was demonstrated. The Pomeranian sheep were characterized by a higher frequency of the mutated A allele (0.41), the frequency of genotypes AA and GA was 0.18 and 0.46 respectively. The effect of polymorphism c.*1232G>A on the body weight of ewes on the day of license has not been observed.

Key words: *MSTN* gene, polymorphism, body weight, sheep

INTRODUCTION

Myostatin is encoded by the *MSTN* gene [Boman and Våge 2009], growth differentiation factor 8 (GDF-8), which belongs to the family of transforming growth factors-β. After completion of the maturation process, myostatin acts as a negative regulator of skeletal muscle growth in transverse striated muscle [Stefaniuk et al. 2014]. The decrease in myostatin level as well as the inhibition of its activity significantly increases body weight and accelerates growth of muscle tissue [Dominique and Gérard 2006]. It can also cause a decrease in adipose tissue content and increase density of bone tissue [Lin et al. 2002].

The first research on *MSTN* gene in livestock was published as early as 1997, just after the studies describing this phenomenon in mice [Grobet et al. 1997, McPherron et al. 1997]. Polymorphisms were originally described in two breeds of beef cattle: Piemontese and Belgian Blue. The research showed homozygosity of the Belgian Blue cattle population regarding deletion of 11 base pairs in the coding region nt821 (del 11). In Piemotese cattle, a polymorphism in the myostatin gene was also detected, but in this breed it led to the G-A transition, which caused the replacement of tyrosine with cysteine [Kambadur et al. 1997]. Mutations were also identified in other cattle breeds such as Asturiana [Grobet et al. 1997], Maine-Anjou and Charolaise [Grobet et al. 1998], Aubrac, Limousine and Pirenaica [Dunner et al. 2003] as well as in Aberdeen Angus and its crosses [Gill et al. 2009]. Polymorphisms in the myostatin gene has also been described in domestic pigeons, goats, pigs, chickens, rabbits, horses [Baron et al. 2002, Stinckens et al. 2008, Hill et al. 2010, Zhang et al. 2012, Dybus et al. 2013, El-Sabrout and Aggag 2017].

The gene responsible for coding of GDF-8 in sheep is located on the second chromosome. The c.*1232G>A polymorhism originally detected in Texel sheep is characterized by the guanine to adenine transition in the 3′–UTR region. The effect of this mutation is inhibition of myostatin gene translation, i.e. blocking the polypeptide protein synthesis on the mRNA matrix [Clop et al. 2006]. Zhang and co wolkers confirm that miR-27b could promote sheep skeletal muscle satellite cell proliferation by targeting *MSTN* and suppressing its expression [Zhang et al. 2018]. The c.*1232G>A poplymorphism leads to reduction of myostatin circulating in the bloodstream by two thirds in the carriers in comparison to individuals with fully functional copies of the *MSTN* gene [Boman et al. 2010]. Sequencing the open reading frame of the

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MSTN gene in sheep characterised by increased muscle mass allowed to locate the c.960delG (p.K320NfsX39) in the Norwegian WhiteSheep (NWS) breed [Boman et al. 2009] and c.120insA (p.N40MfsX9) in the Norwegian Spaelsau [Boman and Våge 2009].

The aim of the study was to check for the presence of c.*1232G>A polymorphism in the 3′–UTR region of the myostatin gene in meat sheep breeds: Pomeranian sheep, Suffolk and Berrichon du Cher, and if observed, to estimate its influence on the body weight of ewes on the day of getting breeding license.

MATERIAL AND METHODS

The study of polymorphism in the gene encoding the myostatin was conducted on a genomic DNA isolated from blood obtained from unrelated ewes of meat sheep breeds: Pomeranian sheep (n = 83), Suffolk (n = 25) and Berrichon du Cher (n = 25). The body weight of ewes was recorded on the day of obtaining a breeding license (over a year old). The local Krakow Ethics Committee for Experiments with Animals approved all experimental procedures relating to the use of live animals.

Isolation of genomic DNA and genotyping

Blood, from which genomic DNA was subsequently isolated, was collected from the external jugular vein (1 ml) to EDTA tubes. After sampling, it was frozen at –20°C. Genomic DNA isolation was carried out using the commercial reagents kit Sherlock AX (A & A Biotechnology).

For amplification of the *MSTN* gene, primers of the following sequences were used [Clop et al. 2006]:

– F: TTTGGTATATTTTTACAGTAAGGAC

– R: TAAATAGTGTTGCACTTAAGGATTC

As a result of the PCR reaction, a 1003 bp fragment was amplified. The reaction was carried out in a 20 µl reaction mixture, 1×Buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 mM each primer, 0.3 U polymerase (Thermo Scientific) and resulted in obtaining 150–250 ng of genomic DNA. The temperature-time profile of the individual reaction stages was as follows: 3 min – 95°C, 35 cycles: 30' – 95°C, 30' – 59°C, 30' – 72°C; 2 min – 72°C. The digestion of the product was carried out using the HpyCH4V enzyme (New England Biolabs). The visualization of the PCR-RFLP result was performed by electrophoresis in a 2% agarose gel (40 min, 80V, Wide Mini-Sub® Cell GT, BIO-RAD).

Statistical analysis

The influence of *MSTN* polymorphism on the body weight on the day when ewes obtaining a breeding license (over a year old) was examined. The normality of the trait distribution was analyzed by the Shapiro-Wilk test. The Kruskal-Wallis test was used to determine the effect of the genotype on the body weight of ewes. A Hardy-Weinberg equilibrium of *MSTN* genotypic frequencies was also assessed with the χ^2 test. The statistical analyses were conducted using the Origin software (OriginLab, Northampton, MA).

RESULTS

Identification of the transition – polymorphism c.*1232G>A in the 3′–UTR region of the myostatin gene in meat sheep breeds: Pomeranian sheep, Suffolk and Berrichon du Cher showed the presence of three genotypes: GG, GA and AA only in case of the Pomeranian sheep. The Suffolk and Berrichon du Cher sheep were monomorphic, in all ewes only the wild-type GG genotype was observed. The Pomeranian sheep were characterized by a higher frequency of the mutated A allele (0.41), and the frequency of AA and GA genotypes was 0.18 and 0.46 respectively (Table 1).

The analyzed population of the Pomeranian sheep was in the Hardy-Weinberg equilibrium as regards the *MSTN* locus ($\chi^2 = 0.241$).

The polymorphism occurring at the site of the *MSTN* did not have any impact on the body weight of ewes recorded on the day of the license. Mean body weight for genotypes GG, GA and AA was 53.1 kg, 52.05 kg and 52.2 kg, respectively (Table 1).

DISCUSSION

Polymorphisms in the myostatin gene in sheep are associated with increased muscularity and a decrease in body fat. However, the effects caused by the loss of myostatin function are not as drastic as changes occurring in some cattle breeds [Kijas et al. 2007]. The high polymorphism of the *MSTN* gene is proved by 28 polymorphisms that have already been described in sheep [Stefaniuk et al. 2014].

In the present study, the polymorphism c.*1232G>A (g.6223G>A) in the Pomeranian sheep was identified. The study also shown that Suffolk and Berrichon du Cher sheep populations were monomorphic. The obtained results are similar to Kolenda's studies carried out in the population of Pomeranian sheep [Kolenda et al. 2019]. The heterozygous AG carriers were most frequently observed, and the populations were characterized by high variability within the site of the c.*1232G>A mutation. The wild homozygous ewes (GG genotype) in the present study constituted 36% of the analyzed population, which was similar to 38% obtained by Kolenda [2019]. The lowest frequency in the present study was observed for AA homozygote, only 18%. Nevertheless, it was three times

Table 1. Alleles and genotypes frequency at the locus c.*1232G>A in sheep of the studied breeds

Tabela 1. Frekwencja alleli i genotypów w *locus* c.*1232G>A u owiec badanych ras

Breed Rasa	N	Alleles frequency Frekwencja alleli		Genotypes frequency Frekwencja genotypów			χ^2
		G	A	GG	GA	AA	
Pomeranian sheep Owca pomorska	83	0.59	0.41	0.36	0.46	0.18	0.241
Body weight on the license date, kg/SD Masa ciała w dniu licencji, kg/SD				53.1/4.8	52.1/6.1	52.2/5.3	
Suffolk Suffolk	25	1.00		1.00	0.00	0.00	
Berrichon du Cher Berrichon	25	1.00		1.00	0.00	0.00	

The licence date – over a year old

higher in comparison with the analysis by Kolenda et al. [2019], where frequency of AA genotype was only 6%. Both in the present study and in the one carried out by Kolenda et al. [2019], the effect of mutations on the body weight of ewes was not observed. The high frequency of heterozygotes observed in Pomeranian ewes included in both studies may suggest that it will be useful to extend research on polymorphism c.*1232G>A and cover a larger part of population of Pomeranian sheep. It would be interesting to analyse the impact of this popymorphism on body weight of lambs during the rearing period. In the future, this trait may be used for selection works with this breed.

The Pomeranian sheep in the current study were characterized by a higher frequency of the mutated A allele (0.41). The c.*1232G>A polymorphism was also detected in different breeds of sheep kept in Great Britain, with a significant predominance of the transitional allele frequency [Hadjipavlou et al. 2008]. The c.*1232G>A mutation is also present in the British Charolais sheep population. However, a higher frequency of wild-type allele was present in this breed. The study suggested possibility to use the c.*1232G>A mutation in breeding programme for Charolais sheep and selection of individuals carrying two copies of the mutant gene, due to their significant increase of skeletal muscles [Hadjipavlou et al. 2008].

Research on the *MSTN* gene is widely carried out in sheep breeds kept in New Zealand, where c*1232G>A polymorphism was detected in the following breeds: Australian White Suffolk, Dorset Poll, Lincoln [Kijas et al. 2007] and in New Zealand Romney [Hickford et al. 2010]. However, these two studies have not demonstrated any effect of any of the *MSTN* gene variants on traits such as:, birth weight, live weight or growth rate. Another research team found the influence of the *MSTN* gene on the body weight at birth in Romney lambs; additionally the significant relationship between the genotype in the *MSTN* gene and the sex of lambs born was observed, where females constituted majority of heterozygous individuals [Han et al. 2010].

The positive effect on muscularity of one genotype of myostatin was observed in the New Zeland Texel. Individuals characterized by one or two copies of "A" allele were characterised by greater musculature and less fat content in the carcass [Johnson et al. 2009]. Studies on the effect of the c.*1232G>A mutation on adipose tissue in heterozygous crossbred lambs (Scotish Mule ewes by Texel rams) with one copy of an inactive gene, showed a decrease in their fat mass [Masri et al. 2011].

Polymorphisms in the *MSTN* have also been the subject of research in native Russian sheep breeds. In the Dzhalginsky Merinobreed, twenty SNPs were found, of which three SNPs had a negative effect on the performance, causing, among other things, a decrease in body weight and a daily growth rate. Another three SNPs, among them the c.*1232G>A mutation, had no significant effect on the traits [Trukhachev et al. 2015]. Twenty-one SNPs and single mutations consisting of insertions and deletions were identified in the Russian Stavropol Merinosheep. Eight of the observed in the study mutations in the *MSTN* gene have been described for the first time and identified as unique for this sheep breed [Trukhachev et al. 2018].

Research on the polymorphism in the *MSTN* gene in native Polish sheep breeds was carried out so far only by Grochowska et al. [2019] and Kolenda et al. [2019]. Polymorphism c.*1232G>A was identified in Pomeranian and Kamieniecka sheep, while the population of the Colored Merino sheep was monomorphic [Grochowska et al. 2019]. The presence of the A-type allele in the population of Pomeranian and Kamieniecka sheep in the Kolenda's studies as well as in the population of Pomeranian sheep examined in the current study may result from using Texel rams in development of this breeds in order to improve their meat performance. Further studies covering larger population of Pomeranian sheep are required in order to fully explain the relation-

ship between the polymorphism within the *MSTN* gene and the traits of meat performance and consider their possible applications.

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IDENTYFIKACJA POLIMORFIZMU C*1232G>A W GENIE *MSTN* U OWIEC RAS MIĘSNYCH W POLSCE

STRESZCZENIE

Celem badania była identyfikacja mutacji c.*1232G>A w regionie 3′–UTR genu miostatyny (*MSTN*) u owiec ras mięsnych: pomorskiej, suffolk i berrichon du cher. Badane populacje ras suffolk i berrichon du cher okazały się monomorficzne. W rasie pomorskiej wykazano występowanie polimorfizmu w regionie badanego genu. Owce pomorskie charakteryzowały się wysoką częstotliwością zmutowanego allelu A [0,41], frekwencja występowania genotypów GA i AA wynosiła odpowiednio 0.46 and 0.18, co może ułatwić rozprzestrzenianie się mutacji w krótkim czasie w populacji. Wpływ polimorfizmu c.*1232G>A na masę ciała owiec w dniu uzyskania licencji nie został wykazany.

Słowa kluczowe: *MSTN*, gen, polimorfizm, masa ciała, owce

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