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Polymorphism of insulin-like growth factor *IGF-1* gene in selected Polish sheep breeds

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Abstract: Polymorphism of insulin like growth factor IGF-1 gene in selected Polish sheep breeds. Research was carried out in 2009-2013 on 1,751 sheep bred in Poland (1366 $\cite{1}$; 385 $\cite{1}$) – 4 meatwool breeds: Polish Merino, Old type Polish Merino, Corriedale and Żelaźnieńska sheep and 3 meat breeds (Berrichone du cher, Suffolk, Charolaise) from 34 flocks selected randomly across the country. All animals were subjected to identification factor insulin-IGF-1 gene, in the assessment of C and T alleles. Summing up, it should be noted that in four meat-wool breeds and two meat breeds (Berrichone du cher, Suffolk) there were no polymorphism of alleles and genotypes of insulin-like growth factor (IGF-1) gene, limiting its scope to determine C allele and CC genotype. Only one Charolaise ewe (breed imported from France) had T allele and C:T genotype. That result indicates the need for further research about sheep imported and adapted in Polish production conditions and assess the adaptation process.

Key words: sheep, *IGF-1*, distribution of alleles and genotypes

INTRODUCTION

Protein *IGF-1* is one of the key components of the pathway of growth hormone (Franco et al. 2005). *IGF-1* is produced in liver and is responsible for cell growth and body treatments. It is believed that the effect of growth hormone occurs in

the tissue in cooperation with local somatomedin e.g. IGF-1 and stimulates the secretion of hypothalamic somatostatin inhibits secretion of growth hormone (Krzymowski et al. 1998). Insulin growth factor gene is listed among the conditions which were useful to the identification of breeds, as demonstrated in the Mediterranean countries (Pariset et al. 2006). Niżnikowski et al. (2013, 2014) presented a different view in relation to the research carried out on domestic sheep breeds compared to the ancestor of the sheep - European mouflon (Ovis aries musimon). Taking into account the fact reported in the cited work conditions influence the incidence of insulin-like gene IGF-1, it was decided to examine its frequency of occurrence in meat-wool breeds: Polish Merino, Old type Polish Merino, Corriedale and Żelaźnieńska sheep and meat breeds: Berrichone du cher, Suffolk, Charolaise (PZO 2014).

MATERIAL AND METHODS

The studies were conducted in 2009–2013 on meat-wool sheep breeds: Polish

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Merino (three flocks from Wielkopolskie Voivodeship, eight flocks from Kujawsko-Pomorskie Voivodeship); Old type Polish Merino (one flock from Wielkopolskie Voivodeship, five flocks from Łódzkie Voivodeship, eight flocks from Kujawsko-pomorskie Voivodeship); Żelaźnieńska Sheep (two flocks from Podlaskie Voivodeship, one flock from Łódzkie Voivodeship) and meat breeds: Berrichone du cher (one flock from Wielkopolskie Voivodeship), Suffolk (two flocks from Wielkopolskie Voivodeship) and Charolaise (two flocks

from Wielkopolskie Voivodeship). Ewes and rams were from herd replacement stuff and were randomly selected for sampling (Table 1). For the isolation of genomic DNA blood samples were obtained of animals from *vein jugula-ris* into tubes containing anticoagulant EDTA.

DNA was isolated from blood leukocytes. In order to obtain high quality DNA suitable for multiple use, blood was purified from the heme compounds, which were erythrocyte lysis products. DNA was isolated by chromatography

TABLE 1. Experimental material used in the study in years 2009-2013

D 1	Sex		N. 1. C. 1	
Breed	9	3	Number of ewes and rams	
Polish Merino	297	68	2010 - 40	
Old type Polish Merino	459	82	2009 − 296 ♀, 10 ♂ 2010 − 59 ♀, 30 ♂ 2011 − 31 ♀, 10 ♂ 2012 − 48 ♀, 26 ♂ 2013 − 25 ♀, 6 ♂	
Corriedale	134	11	2011 − 26 ♀, 5 ♂ 2013 − 108 ♀, 6 ♂	
Żelaźnieńska Sheep	284	155	$2010 - 44 \ \bigcirc, 63 \ \circlearrowleft$ $2011 - 39 \ \bigcirc, 40 \ \circlearrowleft$ $2012 - 46 \ \bigcirc, 27 \ \circlearrowleft$ $2013 - 155 \ \bigcirc, 25 \ \circlearrowleft$	
Berrichone du cher	134	39	$2010 - 14 \bigcirc , 6 \bigcirc $ $2011 - 41 \bigcirc , 16 \bigcirc $ $2012 - 24 \bigcirc , 5 \bigcirc $ $2013 - 55 \bigcirc , 12 \bigcirc $	
Suffolk	38	20	$ \begin{array}{c} 2010 - 8 & \bigcirc \\ 2011 - 16 & \bigcirc, 8 & \bigcirc \\ 2013 - 14 & \bigcirc, 12 & \bigcirc \end{array} $	
Charolaise	20	10	2010 − 3 ♀ 2011 − 10 ♀, 5 ♂ 2013 − 7 ♀, 5 ♂	
Total within sex	1 366	385	- ×	
Total	1 7	51		

on mini-columns of silicate (A&A Biotechnology, Poland, www.aabiot.com), and subsequently served as a template DNA for amplification of polymorphic gene allele fragment. Sample genotyping was performed with KASPar® system (www.kbioscience.co.uk), which uses a single nucleotide polymorphism (SNP) based on primers listed in Table 2.

and Germany, obtained similar results, because Charolaise (meat breed) originated from France and its herd were established in Poland through imports. It worth noted the fact that Charolaise breed was brought to Poland relatively later than Suffolk and Berrichone du cher (PZO 2014). Other tested sheep breeds showed no polymorphism of *IGF-1*

TABLE 2. The primers and SNP of IGF-1 gene (Pariset et al. 2006)

Locus	Name	Starters 3' do 5' (forward/reverse)	SNP	Localization
IGF-1	insuline-like factor	CACACACCTTGTTGCACTCC/ /GCTGAGTTGGTTGGATGCTCT	AY737509: 211 C>T	Exon 3

A high reliability of SNP method compared to the sequencing method was proved by Green et al. (2006). Based on genotyped DNA samples of ewes and rams, distributions of alleles and genotypes were showed separately for each breed of studied sheep. Alleles and genotypes frequencies were compared depending on sheep breed using χ^2 test and SPSS v.21 software, with assessed range of alleles and genotypes frequency between breeds, sexes and the differences between the sexes in terms of individual alleles and genotypes.

RESULTS AND DISSCUSION

Studies showed no statistical significance effect of breed and sex within breed on distribution of factor *IGF-1* gene alleles and genotypes. Among 1,751 collected samples only one Charolaise ewe had T allele and C:T genotype. In comparison with the results of Pariset et al. (2006) which mainly describing sheep from region of Mediterranean Sea, Black Sea

gene alleles and genotypes. That was confirmed by the results of Niżnikowski et al. (2013, 2014) in studies conducted on meat-wool sheep breeds and north--east short-tailed sheep breeds. It is expected that the distribution of the conditions occurring in sheep breeds in Poland was typical (except Charolaise breed) for this region of the World and different from the observed trends in other European countries (Pariset et al. 2006). Perhaps it has to do with another course of life processes of growth and development, which wrote Krzymowski et al. (1998). In this situation, can be useful series of studies on sheep imported into Poland about polymorphism of alleles and genotypes of insulin-like growth factor gene changed due to processes of adaptation - which requires further work in this area. Generally, it should be noted that four meat-wool breeds and two meat breeds showed no polymorphism alleles and genotypes of IGF-1, limiting its scope to determine C allele and genotype CC. In imported breed from France only one ewe had T allele and C:T genotype. That result indicates the need for further research about sheep imported and adapted in Polish production conditions and assess the adaptation process, especially on Charolaise breed.

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Streszczenie: Polimorfizm genu insulinopodobnego czynnika wzrostu IGF-1 u wybranych polskich ras owiec. Badania przeprowadzono na 1751 owcach ras hodowanych w Polsce $(1366 \ \ \ \ i \ 385 \ \ \ \ \) - 4 \ ras \ wełnisto-mięsnych:$ merynos polski, merynos polski starego typu, corriedale i owca żelaźnieńska oraz 3 ras mięsnych (berrichone du cher, suffolk i charolaise) w latach 2009-2013, pochodzących z 34 stad wybranych losowo na obszarze całego kraju. Wszystkie zwierzęta poddane były identyfikacji genu czynnika insulinopodobnego IGF-1, w zakresie oceny występowania alleli C i T. Podsumowując, stwierdzić należy, iż u badanych czterech ras wełnistomięsnych oraz dwu ras mięsnych (berrichone du cher i suffolk) nie wykazano polimorfizmu występowania alleli i genotypów genu insulinopodobnego czynnika wzrostu IGF-1, ograniczając jego zakres do ustalenia jedynie do allelu C i genotypu CC. U importowanej z Francji rasy charolaise stwierdzono odstępstwo od tej reguły tylko u jednej maciorki mającej allel T i genotyp C:T. Wynik ten wskazuje na potrzeby prowadzenia dalszych badań z tego zakresu u owiec pochodzących z importu i adaptowanych w polskich warunkach środowiska produkcyjnego, na podstawie którego można będzie ocenić zakres procesów adaptacyjnych.

Słowa kluczowe: owce, *IGF-1*, rozkład alleli i genotypów

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