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THE EFFECT OF POLYMORPHISM WITHIN EXON 12 OF *IGF1R* GENE ON MEAT PRODUCTION TRAITS IN LIMOUSIN AND HEREFORD CATTLE

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ABSTRACT

The aim of the research was to genetically describe the cattle herd containing Hereford cattle (n = 141) and Limousin cattle (n = 161) based on the polymorphism in the 12th exon of the gene coding for insulin-like growth factor I receptor (*bIGF1R/e12/Msp*I) and an attempt to estimate the potential relation between genetic variants and selected meat production traits features. The identification of the genotypes of particular individuals was carried out with PCR-RFLP. The most common genotype in the analysed herd was *GG*. There were no individuals with the *AA* genotype found. The statistical analysis revealed that the *GG* genotype was correlated with higher values of the analysed features compared to heterozygotes. The differences were statistically significant in most cases.

Key words: beef cattle, IGF1R gene, polymorphism, daily gaining

INTRODUCTION

Insulin-like growth fator 1 (IGF-I) is a main factor mediating in growth hormone function. The influence of IGF-I on an organism's growth and metabolism is based upon IGR-IR [Obrępalska-Stęplowska et al. 2005). Bovine gene coding for this receptor (*IGF1R*) is located on chromosome 21 and consists of 21 exons and sometimes very long introns (50–150 kbp). The gene contains a short (46 bp) 5' UTR sequence and a lot longer (840 bp) 3' UTR sequence [http://www.ncbi.nlm.nih.gov/gene/281848].

To date in the literature there have been several records of the structure and function of the bovine IGF-IR. Based on the analogy to the human gene it is a single amino acid chain synthesized as a pre-pro-receptor containing 1367 amino acids, which is then a subject to a series of post-translational modifications [Ullrich et al. 1986]. It was shown that IGF-IR is structurally similar to insulin receptor (IR) (70% of amino acid sequence homology) [Riedemann and Macaulay 2006].

It was proven that the IGF-I/IGF-IR system has a significant influence on body mass development. The main regulator of the fetal growth is the system of insulin-like growth factors. The fetal growth is correlated with the level of IGF-II in blood serum while antenatal growth is correlated with the levels of growth factor and its main mediator (IGF-I) [Frago and Chowen 2005]. IGF-I prevents lipolysis (increases lipogenesis), regulates transport and utilisation of glucose in muscles and inhibits gluconeogenesis in the liver [Kaplan and Cohen 2007]. According to Fernandez et al. [2001] IGF-I/IGF-IR participates in glucose level regulation in muscles, where dusruption in expression and function of type 1 receptor for IGF-I resulted in diabetes type 2. The IGF-I/IGF-IR system is described as pleyotropic, i.e. it is also important in increasing muscle mass. A mature receptor with full activity of tyrosine kinase binds IGF-I and directly influences regulation of mitogenesis and miogenesis as well as osteogenesis [Grochowska and Zwierzchowski 2000]. Numerous publications prove that application of exogenic IGF-I may cause hipetrophy [Adams and McCue 1998] and in many cases it restored the initial muscle mass [Musaro et al. 2001]. It is connected to a whole

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signaling cascade starting with IGF-IR/IRS activated by locally produced IGF-I.

Several publications presented association research focused on the relation between *IGF1R* and the parameters connected to meat production traits of cattle [Curi et al. 2005, Akis et al. 2010, Szewczuk et al. 2013, Szewczuk 2016]. Therefore it may be useful to identify further polymorphisms in the coding parts of the bovine insulin-like growth factor 1 receptor gene.

The aim of this study was to determine the frequency of the alleles and genotypes of the polymorphic site in exon 12 (*bIGF1R/e12/Msp*I) and an attempt to to estimate the potential relation between genetic variants and selected meat production traits features (mass at birth, standard mass in 210th day of life, daily mass gains between birth and 210th day of life) in Hereford (HH) and Limousin (LM) cattle.

MATERIAL AND METHODS

The research was conducted on two herds of beef cattle: Hereford (HH) and Limousin (LM) counting 141 and 161 individuals, respectively. The breeding and feeding conditions were similar for all of the animals.

Calves were kept with their mothers for 6–7 months after birth. During this time they had free access to grassland and mother's milk and were fed with crushed barley, oat and triticale seeds. Summer feeding was based on grassland, which was used from the beginning of May to the end of October. In winter the animals were kept in pen, which included shelters screened with straw. They feeding was based on corn and weeds ensilage, haylage and hay supplemented with vitamins and minerals. Cows in advanced gestation additionally received concentrates B-1. The animals had free access to water.

The samples of blood were collected from external jugular vein into test tubes with EDTA as anticoagulation agent. The isolation was carried out using MasterPure Genomic DNA Purification Kit (Epicentre Technologies) and procedure described by the manufacturer. The polymorphism in the gene *IGF1R/e12/Msp*I (rs41640706) was identified by PCR-RFLP (Restriction Fragment Length Polymorphism) using starter sequences developed by Szewczuk et al. [2013].

During PCR a 164 bp long DNA fragment was amplified, then it was subjected to restriction enzyme *Msp*I (cutting the sequence C/CGG) in 37°C for 3 hours. Electrophoresis was conducted in 2% agarose gel in TBE buffer with ethidium bromide, in 120 V for 30 minutes, using pUC19/*Msp*I (Fermentas) ladder. Restriction fragments were visiualized in UV light in Vilber Lourmat transilluminator and saved. In order to find the genetic structure of the population the frequencyies of alleles and genotypes were calculated.

The next step was to statistically analyse the relations between the *IGF1R/e12/Msp*I system genotypes and selected parameters connected to growth and development of cattle (mass at birth (BTW), mass in 210th day of life (WWT_{210}), daily gains from birth to 210th day of life (ADG) using Statistica 12.5 PL programme. The data about the selected parameters were obtained from the breeding documentation of the farm collected by The Polish Association of Beef Cattle Breeders and Producers [PZHiPBM] The mean values ($\bar{x}$) and standard deviation (SD) were calculated. One way variance analysis (ANOVA) was carried out for the main effects. In cases of significant influence of a particular factor the significance of differences between the means were calculated using the Duncan's multiple range tests.

RESULTS

Out of three expected genotypes only two were identified: *GG* (113 and 51 bp) and *AG* (164, 113 and 51 bp). There was no *AA* individuals recorded. In the analysed herds of beef cattle (HH and LM) the *GG* homozygotes were the most frequent (0.7305; 0.9068, respectively) and the heterozygotes were less frequent (0.2695; 0.0932). The *G* and *A* allele frequencies in HH cattle were, respectively, 0.8652 and 0.1348, and in LM cattle, respectively, 0.9534 and 0.0466 (Table 1).

Table 1. Frequency of genotypes and alleles of the polymorphism *IGF1R/e12/Msp*I

Tabela 1. Frekwencja genotypów i alleli dla układu polimorficznego *IGF1R/e12/Msp*I

Breed Rasa	Genotype Genotyp	n	Frequency – Frekwencja	
			Genotype Genotyp	Alleles Allele
Hereford	*GG*	103	0.7305	*G* = 0.8652
	AG	38	0.2695	*A* = 0.1348
Limousin	*GG*	146	0.9068	*G* = 0.9534
	AG	15	0.0932	*A* = 0.0466

Table 2 presents mean mass at birth, in 210th day of life and daily mass gains with respect to *IGF1R* genotypes.

It was concluded that in both analysed herds the highest mass at birth was a feature of individuals with *GG* genotype (34.39–34.81 kg). The heterozygotes were lighter (32.80–33.76 kg). However, there were no significant differences between homozygotes and heterozygotes in both breeds analysed.

The Hereford and Limousin *GG* homozygotes in their 210th day of life had significantly ($P \leq 0.01$) higher body mass compared to heterozygotes (differences: +18.08 kg and +33.31 kg).

Mean daily gains of mass are very important in describing a correct breeding practice. It was concluded that the highest daily gains during the whole analysed period were recorded in *GG* individuals of both breeds ($P \leq 0.01$), where the highest value of the feature was recorded for HH cattle (1096.3 g).

DISCUSSION

Keeping calves healthy and letting them develop correctly is important for an effective breeding and cattle use. Mistakes made during the process may have negative consequences in cows later life, such as decreased heifers' and cows' condition, their health and yielding and therefore negatively affect profitability of production.

Table 2. Mean birth weight, at 210 day of life and daily gains in association with *IGF1R* genotype (standard deviation in parentheses)

Tabela 2. Średnie masy ciała po urodzeniu, w 210 dniu życia oraz przyrosty dobowe w zależności od genotypów *IGF1R* (w nawiasie podano odchylenia standardowe)

Breed Rasa	Genotype Genotyp	n	BWT, kg	ADG, g	WWT_{210}, kg
Hereford	*GG*	103	34.39 (4.22)	1096.3^{A} (131.33)	264.60^{A} (27.60)
	AG	38	33.76 (2.73)	1013.1^{A} (69.53)	246.52^{A} (14.13)
Limousin	*GG*	146	34.81 (4.87)	1072.8^{A} (104.11)	268.44^{A} (29.35)
	AG	15	32.80 (4.88)	944.07^{A} (141.45)	235.13^{A} (37.13)

BWT – birth weight, ADG – average daily gains between birth and weaning; WWT_{210} – weaning weight adjusted to 210 days of age.
BWT – masa przy urodzeniu, ADG – przyrosty dobowe od urodzenia do odsadzenia, WWT_{201} – masa przy odsadzeniu w 210 dniu.
Means within a column and proper breed marked with identical letters differ significantly; capital letters – $P \leq 0.01$.
Średnie w obrębie kolumny i rasy oznaczone takimi samymi literami różnią się istotnie; duże litery – $P \leq 0{,}01$.

Correct growth and development of calves is determined by genetic and environmental factors, such as mother's genotype, bull's influence, cattle age and body mass during the first calving, number of calvings, season of calving [Swali and Wathes 2006, Szewczuk et al. 2011, Szewczuk 2016]. Body mass appropriate for the age and breed as well as correct daily mass gains are important especially in case of beef cattle, thus genomic selection becomes increasingly popular in cattle breeding [WCHiRZ, 2017].

So far in the literature only two papers involved research on the polymorphic system *IGF1R*/*e12*/*Msp*I in relations to body mass and daily gains in cattle. They concerned two breeds: Angus [Szewczuk et al. 2013] and Montbeliard [Szewczuk 2016]. The present research concerned Limousin and Hereford breeds and therefore comparison of the results with the findings of other authors is difficult. Thus it was only possible to observe certain patterns.

Szewczuk et al. [2013] were the first ones to research *Bos taurus* in terms of the polymorphism in the coding region of *IGF1R* gene. It was found that similarly to the present research, the individuals with *GG* genotype had signifficantly ($P \leq 0.05$) higher body mass in 210th day of life (+5.06 kg) compared to heterozygotes *AG*. The authors found that the polymorphism in 3'UTR did not significantly affect the growth features. In the paper by Szewczuk [2016] the authors researched mother's genotype influence on body mass at birth of the calves after I and II calvings. The findings were that mean body mass of calves was the highest in the offspring of mothers with *GG* genotype, regardless of the number of calving. The lowest body mass was recorded in the offspring of *AA* mothers in the first calving and *AG* mothers in the second calving ($P \leq 0.05$; $P \leq 0.01$). According to Gaafa et al. [2011] the numer of calvings significantly influences calves' mass at birth. Cows in their first calving as well as those giving birth to twins tend to have smaller offspring.

CONCLUSIONS

Analysis of the relation between *IGF1R*/*e12*/*Msp*I genotypes and body mass at birth, in 210th day of life and daily mass gains revealed a trend in development of the mean values of the selected features. Regardless of the breed, the *GG* genotype determines highest body mass and daily gains compared to potentially worse *AG* genotype. Because of significant influence of IGF-I/IGF-IR system on growth and development of muscle mass it is worth to research insulin-like growth factor 1 receptor (*IGF1R*), which may be an ideal candidate gene for a meat use marker of beef cattle due to it being a main mediator in IGF-I signalling to the inside of the target cell.

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UDZIAŁ POLIMORFIZMU W EKSONIE 12 GENU *IGF1R* W KSZTAŁTOWANIU CECH MIĘSNOŚCI BYDŁA RAS HEREFORD I LIMOUSINE

STRESZCZENIE

Celem badań była charakterystyka genetyczna stada krów ras: hereford (n = 141) i limousine (n = 161) na przykładzie polimorfizmu zlokalizowanego w eksonie 12 genu kodującego receptor dla insulinopodobnego czynnika wzrostu typu I (układ *bIGF1R*/*e12*/*Msp*I) oraz próba oszacowania ewentualnego związku pomiędzy wariantami genetycznymi a wybranymi cechami użytkowości mięsnej. Identyfikacja genotypów poszczególnych osobników prowadzona była przy użyciu PCR-RFLP. W analizowanych stadach najczęściej występował genotyp homozygotyczny *GG*. Nie zidentyfikowano osobników o genotypie *AA*. Analiza statystyczna wykazała, że genotyp *GG* powiązany był z wyższymi wartościami ocenianych cech w stosunku do heterozygot, a różnice te w większości przypadków zostały potwierdzone statystycznie.

Słowa kluczowe: bydło mięsne, IGF1R, polimorfizm, przyrosty dobowe