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Effect of the *IGF-I* gene polymorphism on growth, body size, carcass and meat quality traits in Coloured Polish Merino sheep

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Abstract. Insulin-like growth factor I, encoded by the *IGF-I* gene, plays a role in cell growth and differentiation, embryogenesis, metabolism regulation, skeletal growth, and protein synthesis. The aims of this study were to investigate the polymorphism in the 5′ flanking region of the *IGF-I* gene and evaluate associations between the single-nucleotide polymorphism (SNP) in this gene and growth, body size, carcass and meat quality traits in Coloured Polish Merino sheep. In total 78 live and post mortem traits were investigated. Polymorphism in the *IGF-I* gene was identified with the use of the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method in 305 Coloured Polish Merino sheep. In association studies, traits of interest were analysed with the use of the MIXED and GENMOD procedures of the SAS statistical package. Two alleles named A and B, and two *IGF-I* genotypes – AA and AB – were detected. The A allele and the AA genotype were predominant, with the frequencies of 91.6 and 83.3 %, respectively. The *IGF-I* genotype was found to have a highly significant effect on fore shank weight ($P = 0.006$), kidney fat class ($P = 0.002$) and EUROP fat class ($P = 0.005$). Furthermore, the *IGF-I* genotype significantly affected external fatness of carcass class ($P = 0.038$), drip loss ($P = 0.049$), and subjective assessment of meat colour ($P = 0.043$), and it tended to be associated with *longissimus dorsi* (*LD*) muscle width ($P = 0.063$) and flavour (0.067). Concluding, the *IGF-I* gene could be considered as a candidate gene of selected carcass and meat quality traits in sheep.

1 Introduction

Insulin-like growth factor I (IGF-I) is a polypeptide hormone that is encoded by the *IGF-I* gene and is similar in molecular structure to insulin. IGF-I participates in the somatotrophic axis together with growth-hormone-releasing hormone (GHRH), growth hormone (GH), insulin-like growth factor II (IGF-II), and their associated binding proteins (BPs) and receptors (GHRHR, GHR, IGF-IR and IGF-IIR). Moreover, it interacts with insulin-like growth factor I receptors (IGF-IR) in target tissues (Jones and Clemmons, 1995) and with proteins binding IGF in blood (IGF-BP), which can modulate action of this hormone (e.g. Lackey et al., 1999; Schams et al., 1999). IGF-I is also believed to mediate a wide spectrum of biological responses such as cell growth and differentiation, embryogenesis, metabolism regulation, skeletal growth, and protein synthesis (Baxter, 1986; Clemmons et al., 1987; Froesch et al., 1985). For these reasons, the *IGF-I* gene is considered as a functional candidate for a number of production traits, i.e. growth, carcass and meat quality traits in livestock.

Growth traits are important attributes in sheep breeding, as they, among other factors, affect breeder's profit. Lamb body weight is one of the factors that regulate incomes from meat

production. Therefore, the improvement of these traits should be one of the most important aims in sheep production. Growth traits, like other quantitative traits, are controlled by several genes, e.g. the *IGF-I* gene, and environmental factors. However, there have been only a few association analyses of the *IGF-I* gene polymorphism with growth traits in sheep. For example, Hajihosseinlo et al. (2013), Tahmoorespur et al. (2009), Negahdary et al. (2013) and Gholibeikifard et al. (2013) observed significant effects of the *IGF-I* gene polymorphism on several growth traits in Makooei and Baluchi sheep. Moreover, Hajihosseinlo et al. (2013) investigated the association of nucleotide substitution in the *IGF-I* gene with several body size traits in sheep. It should be pointed out that the abovementioned studies were conducted only in Iranian sheep breeds. Therefore, there is a lack of information about these associations in sheep breeds in other countries, especially in Europe. To our knowledge, until now only Proskura and Szwczuk (2014) had investigated effects of single-nucleotide polymorphism (SNP) in the third exon of the *IGF-I* gene on growth traits in Pomeranian Coarsewool sheep in Poland and there was no association study involving the *IGF-I* gene and body size traits in countries other than Iran, nor any subjective assessment of body muscle and fat class in sheep. Interestingly, in cattle, several authors have found significant associations between the *IGF-I* gene polymorphism and growth traits (e.g. De la Rosa Reyna et al., 2010; Ge et al., 2001; Li et al., 2004). Moreover, Zhang et al. (2008) reported significant effects of nucleotide substitution in the *IGF-I* gene on several body size traits in goats. Additionally, Gao et al. (2009) showed that the *IGFBP-3* gene polymorphism was associated with chosen body measurements in Chinese beef cattle.

Despite the fact that the *IGF-I* gene plays a role in the growth of an organism, it could also be directly or indirectly associated with other traits, i.e. carcass and meat quality traits, as SNPs in this gene could be markers in linkage with causative mutations in other genes. For example, Behzadi et al. (2015) investigated effects of the *IGF-I* gene polymorphism on carcass traits in sheep. Moreover, Sun et al. (2014) studied correlations between the *IGF-I* gene expression and carcass and net meat weight in Hu sheep. Siadkowska et al. (2006) and Curi et al. (2005) observed associations between the *IGF-I* gene polymorphism and several carcass traits in cattle. In the case of meat quality traits, there have only been a few association analyses in sheep. Behzadi et al. (2015) investigated effects of the *IGF-I* gene on the level of triglycerides and cholesterol in blood. Su et al. (2014) analysed relationships between the *IGF-I* gene expression and diameter and density of muscle fibre as well as muscle tenderness in Hu sheep. In pigs, Wang et al. (2009, 2010) observed effects of the *IGFBP-3* and *IGFBP-5* gene polymorphisms on meat colour and pH of various meat cuts, respectively.

Among many factors affecting growth, body size, carcass and meat quality traits, effects of many candidate genes still need to be investigated, especially in sheep. Identification of associations between candidate gene polymorphisms and the abovementioned attributes can provide useful markers for selection of economically favourable traits. However, little is known about the effect of the *IGF-I* gene polymorphism on growth, body size, carcass and meat quality traits in sheep, especially in European breeds. Moreover, to our knowledge no previous association studies between *IGF-I* genotypes and many important carcass and meat quality traits in sheep have been conducted. Therefore, the aims of the present study were to investigate the polymorphism in the 5′ flanking region of the *IGF-I* gene and to evaluate the associations between SNP in this gene and a wide spectrum of growth, body size, carcass and meat quality traits in Coloured Polish Merino sheep.

2 Materials and methods

In total, 305 purebred Coloured Polish Merino lambs of both sexes were investigated. The Coloured Polish Merino is a sheep breed used for its wool and meat. It is included in the Programme of Farm Animal Genetic Resources Conservation supervised by the National Research Institute of Animal Production (NRIAP) in Poland. Sheep were kept indoors, but they also grazed on a pasture six times a week at the NRIAP Experimental Station Kołuda Wielka. Lambs were sired by nine different rams and were produced during a period of 3 years – from 2011 to 2013. Suckling lambs were fed dry granulated mash and meadow hay ad libitum. Procedures involving animals were approved by the local animal research ethics committee and the local veterinary service.

Growth data (body weight on the second, 30th, 56th and 78th days of life) were collected for 277 male and female lambs. Moreover, the average daily gains between the second and 30th, 30th and 56th, and 56th and 78th days were calculated.

In total, 305 male and female lambs were ultrasonically (USG) scanned according to the procedure described by Krupiński et al. (2009) using an Aloka SSD-900 device with a UST-5818-5 transducer (B-5MHz). In brief, USG examination of a cross section of the *longissimus dorsi* (*LD*) muscle was performed on the right body side perpendicularly to the spine axis behind the last thoracic vertebrae. Measurements of width, depth and area of the *LD* muscle and fat depth over *LD* muscle were performed on USG images with the use of MultiScan ver. 18.03 software.

In total, 106 ram lambs were chosen for slaughter during the 3 experimental years. Before slaughter, the following body measurements were carried out on lambs: withers height, chest depth, body length, shoulder width, hip width and leg depth. The measurements were taken while the lambs were in a standing position, according to the methodology of Kawęcka (2013). Subjective evaluations of muscling as well as fatness with the use of a five-point system (ranges

from one to five with a 0.5 precision, with a value of five being the meatiest or fattiest class) were undertaken in the lumbar part of the spine behind the last rib near the kidney according to the methodology of Jarrige (1988). Slaughters, carcass cutting and leg dissection took place three or four times a year from March to April according to the methodology of Nawara et al. (1963) and Krupiński et al. (2009) in the abattoir of the NRIAP Experimental Station Kołuda Wielka. Mean age at slaughter was 105 days (SD 4.2, range 92–119 days). In brief, lambs were weighed twice: 1 day before slaughter (final weight) and on the day of slaughter (pre-slaughter weight). Then animals were electrically stunned, exsanguinated and skinned. Subsequently, carcasses were weighed (hot carcass weight) and maintained in a chilling room, where the temperature was held at approximately 4 °C for 18 h. After chilling carcasses were weighed again (cold carcass weight) and the cold carcass dressing out was calculated by dividing the cold carcass weight by the pre-slaughter weight and multiplying by 100. In the next step, carcasses were visually graded for muscle and fat class on a nine-point scale (with the value of nine being the meatiest or fattiest class) according to the methodology implemented at ram evaluation stations in Germany as described by Krupiński et al. (2009). Conformation and muscling were assessed on each of the three parts of carcass separately: on the fore part of the carcass consisting of middle neck and shoulder, on the full loin part including rib and loin, and on the leg part. The evaluation of fatness involved two traits: kidney fat class and external fatness of carcass class. Carcasses were also categorised in terms of muscle and fat class according to the EUROP grading system as described by Krupiński et al. (2009). Subsequently, the following dimensions of carcasses were measured according to the methodology of Nawara et al. (1963): carcass length, breast depth, breast width, rump width, rump girth, leg depth, leg length index and loin width. Then carcasses were halved, and the right carcass side was weighed and taken for further examination. The kidney, kidney fat and the rest of the diaphragm were removed. At first, the right carcass side was divided into three parts: the fore part of the carcass, the full loin part, and the leg part, and each part was weighed. Subsequently, these three parts were divided into the following cuts: scrag, middle neck, shoulder, breast and flank, rib, loin, tenderloin, leg, fore shank, and hind shank, according to the methodology of Nawara et al. (1963). All cuts were weighed. On the surface, where a rib was cut from a loin, width, depth and the area of the eye of loin and fat depth over the eye of loin were measured. Moreover, fat depth over the ribs in the thickest point of this layer was recorded. The leg was dissected for three tissues: muscles, bones and fat, which were weighed separately and a yield of each tissue in the leg was calculated.

Furthermore, loin muscles from the right carcass side were vacuum-packed and transported to the Institute of Agricultural and Food Biotechnology (Poland), where meat quality analyses were undertaken. The pH measurements were performed on samples of *longissimus lumborum* (*LL*) muscle 24 h *post mortem* (pH24h) using a German Mettler Toledo 1140 pH meter with an integrated Mettler Toledo electrode (ISO 2917, 2001). Results were averaged from the three measurements per one sample. For an instrumental evaluation of meat colour, steaks (thickness approximately 10 mm each) were cut crosswise in the direction of the *LL* muscle fibres and exposed to daylight or electric light for 15 min. The values of reflectance coordinates – L^* (lightness), a^* (redness) and b^* (yellowness) – were gathered using a Konica Minolta chronometer CR-400. Drip loss value was measured on the *LL* muscle. Before, the test samples were weighed, individually packed in plastic bags, held at 4 °C for 48 h and weighed again. The value of drip loss was defined as a percentage weight loss calculated from the difference between initial and final weight of the sample. Water, intramuscular fat and total protein content percentages were determined on minced samples of the *LL* muscles according to the methods described in ISO 1442 (2000), 1444 (2000) and PN-75/A-04018 (2000), respectively, using a Kjeltec system 1002 distilling unit and a Soxtherm device manufactured by Gerhardt Analytical Systems. Water holding capacity was investigated in minced *LL* muscle samples according to the method devised by Grau and Hamm (1952) with later modifications made by Pohja and Ninivaara (1957). Cooking loss was analysed on samples of the *longissimus dorsi* (*LD*) muscle. First of all, the samples were weighed, then packed in plastic bags, heated in water until they reached 75 °C in the centre of the sample and were weighed again at the end of the analysis. The value of cooking loss was calculated from a difference in sample weights recorded before and after the process of heating and was expressed as a percentage. Furthermore, shear force was determined in cooked and cooled-down samples of lamb loin. For this purpose cylinder-shaped samples (2.5 cm diameter) of the *LD* muscle were extracted parallel to the direction of muscle fibres and were subjected to shear force ($N\,cm^{-2}$) measurement with the use of a Zwick Roell ZO with a 0.5 kN head and Warner Bratzler device with a blade speed of 100 mm min^{-1}. Moreover, subjective visual evaluations of meat colour and marbling were performed on the *LL* muscle by a team of five people using an eight-point scale for colour, where one is related to the lightest and eight is related to the darkest colour, and a four-point scale for marbling, with a score of one relating to minor marbling and four relating to the greatest marbling. Subjective sensory evaluation of aroma, succulence, tenderness and flavour were performed on boiled *LD* muscle using a five-point scale for each trait according to the methodology of Baryłko-Pikielna (1975), where one was related to bad levels and five was related to very good levels of the trait.

Total genomic DNA was purified from the blood using a MasterPure™ DNA Purification Kit for Blood Version II (Epicentre, USA). Polymorphism in the 5′ flanking region of the ovine *IGF-I* gene was detected with the use of the polymerase chain reaction restriction fragment length poly-

morphism (PCR-RFLP) method. A fragment of the ovine *IGF-I* gene was amplified using primers reported by Ge et al. (1997). DNA amplifications were performed in a Mastercycler Pro (Eppendorf AG, Germany) in 20 µL reaction volume containing 50 ng of genomic DNA, 5 pmol of each primer (forward and reverse), 200 µM of each dNTP and 1 U DreamTaq DNA polymerase (Thermo Scientific, USA) in a one-fold DreamTaq reaction buffer. The temperature profile of the reaction consisted of denaturation at 94 °C for 2 min, followed by 30 amplification cycles, including denaturation at 94 °C for 30 s, annealing at 66 °C for 30 s and extension at 72 °C for 30 s, with a final 5 min extension step at 72 °C. The PCR reaction products were digested with 4 U of *Hae*II (NEB, UK) restriction enzyme (Yilmaz et al., 2005) for 2 h at 37 °C. Digested PCR products were separated on 2 % agarose gels in 1 × TBE (Tris-borate-EDTA) buffer for 90 min at 120 V and visualised by Midori Green (Nippon Genetics, Germany) staining. PCR products representing the AA and AB genotypes were cleaned up with the use of an ExoSAP-IT® Affymetrix (USA) and sequenced in both directions in Genomed, Poland. Allele and genotype frequencies in the *IGF-I* locus were calculated. Furthermore, observed and expected heterozygosity as well as the Hardy–Weinberg equilibrium test calculations were made in the Arlequin 3.5.1.2 (Excoffier and Lischer, 2010).

An association analysis was performed between *IGF-I* genotypes and growth traits using the MIXED procedure of the SAS software package (SAS, 2008). The following mixed effect model I was applied:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m + e_{ijklm}, \quad (1)$$

where Y_{ijklm} is the performance of the nth individual lamb for each trait of interest, μ is the general mean for each trait of interest, a_i is the fixed effect of the ith *IGF-I* genotype ($i =$ AA, AB), b_j is the fixed effect of the jth sex ($j =$ male, female), c_k is the fixed effect of the kth litter size ($k = 1$, single; 2, twin and only one lamb born with triplet litter size was also included), d_l is the fixed effect of the lth year of observation ($l = 2011, 2012, 2013$), f_m is the random effect of the mth sire ($m =$ sire 1, 2, … 9) and e_{ijklm} is the random error. For all variables two-way interactions between fixed effects were tested in the model; however, they did not have a significant effect on investigated traits and were therefore excluded from the final model.

An association analysis between *IGF-I* genotypes and body size traits as well as *IGF-I* genotypes and slaughter traits was undertaken with the use of the model I described above without the effect of the sex because only male lambs were investigated. Moreover, final weight and cold carcass weight were found to be significant and were included in the final models as a covariate for live and post mortem attributes, respectively. Weight was not included as the covariate in the model where the Y variable was the final weight, pre-slaughter weight, a tissue proportion or dressing out. In the case of final and pre-slaughter weight, slaughter age was found to be significant and was included as the covariate in the model. Furthermore, final weight was fitted in the model as the covariate for hot and cold carcass weight. For all variables, two-way interactions between fixed effects were tested in the model but were not significant for almost all of the traits, with the exception of withers height, chest depth, leg depth (body measurement), scrag weight, and hot and cold carcass weight; therefore, they were included in the models only for the abovementioned traits, but not for all other characteristics.

Moreover, an association of *IGF-I* genotypes with USG measurements of the *LD* muscle was also estimated with the use of the model I described above; however, slaughter age was included as the covariate. For all variables, two-way interactions between fixed effects were tested in the model but did not have a significant effect on investigated traits, with the exception of fat depth over the *LD* muscle; therefore, it was fitted in the model only for this trait. An association analysis between *IGF-I* genotypes and meat quality traits was performed with the use of the model I described above without the effect of sex and litter size. For all variables, two-way interactions between fixed effects were tested in the model but did not show any significant effect on investigated traits; therefore, they were not included in the final model. In the case of each model, when a genotype was shown to be statistically significant, the significance of deviations was verified with the Tukey–Kramer test.

Associations of *IGF-I* genotypes with subjective assessment of live animal and carcass traits were analysed with the use of the GENMOD procedure of the SAS software package (SAS, 2008). The generalised linear model included the fixed effects of *IGF-I* genotype, litter size, year of observation and sire. Moreover, final weight and cold carcass weight were found to be significant and were fitted in the model as the covariates for live and post mortem attributes, respectively.

3 Results

3.1 Identification of alleles

PCR-RFLP analysis of polymorphism in the 5′ flanking region of the *IGF-I* gene in Coloured Polish Merino sheep revealed two alleles, named A and B, and only two genotypes: AA and AB. The *Hae*II digested allele A amplicon, while allele B remained undigested. Sequence analysis showed that two SNPs differed allele A from allele B. The first polymorphism covered G/C transversion, while the second covered G/A transition, at positions 85 and 87, respectively (nucleotide positions are relative to the first nucleotide in the sequence; GenBank no. LC151296.1).

Table 1. *IGF-I* allele and genotype frequencies (%) in Coloured Polish Merino sheep.

Allele/genotype ($n = 305$)		Frequency (%)
Allele	A	91.6 %
	B	8.4 %
Genotype	AA	83.3 %
	AB	16.7 %

3.2 Allele and genotype frequencies

IGF-I allele and genotype frequencies in the investigated breed are shown in Table 1. The A allele was predominant (91.6 %), while the B allele occurred with the frequency of 8.4 %. The most frequent group was AA homozygotes (83.3 %), while 16.7 % of lambs carried the AB genotype. The value of observed heterozygosity (H_o) was equal to 0.167, and was higher than the value of expected heterozygosity (H_e), which amounted to 0.153. The population was in the Hardy–Weinberg equilibrium ($P = 0.247$).

3.3 Effect of the *IGF-I* gene polymorphism on traits measured in live animals

Mixed-model association analyses of the *IGF-I* genotypes were performed for body weights, average daily gains, body measurements and ultrasound *LD* muscle measurements. Effects of the *IGF-I* gene polymorphism on these traits in Coloured Polish Merino sheep are presented in Tables 2–4. None of the analyses showed any significant effect of the *IGF-I* genotype on the abovementioned characteristics; however, the *IGF-I* gene polymorphism tended to be associated with *LD* muscle width ($P = 0.063$). AB heterozygous lambs had wider *LD* muscle (0.14 cm) than in AA homozygotes (Table 4). Results of the generalised linear model analyses of the *IGF-I* genotype effect on body muscle and fat class are presented in Table 7. No associations between the *IGF-I* gene polymorphism and these traits were found.

3.4 Effect of the *IGF-I* gene polymorphism on carcass traits

Tables 5 and 7 show the results of association analyses between the *IGF-I* gene polymorphism and carcass traits in Coloured Polish Merino sheep. *IGF-I* genotype was associated with fore shank weight ($P = 0.006$). Heterozygous lambs had heavier fore shank (4.3 %) than AA homozygous animals. Furthermore, the *IGF-I* genotype was found to have highly significant effects on kidney fat class ($P = 0.002$) and EUROP fat class ($P = 0.005$) and a significant effect on external fatness of carcass class ($P = 0.038$). No significant associations between *IGF-I* genotype and other investigated carcass traits were detected.

3.5 Effect of the *IGF-I* gene polymorphism on meat quality traits

Results of association analyses of the *IGF-I* gene polymorphism with meat quality traits in Coloured Polish Merino sheep are presented in Table 6. The *IGF-I* gene polymorphism was significantly associated with drip loss ($P = 0.049$). Meat from AA homozygous lambs was characterised by lower average drip loss (−0.86 %) than meat from heterozygotes AB. Moreover, the *IGF-I* gene polymorphism had a significant effect on the subjective assessment of meat colour ($P = 0.043$). AA homozygous ram lambs had meat characterised by the higher average value of this trait (0.34 point) when compared to meat form heterozygotes AB. Furthermore, the *IGF-I* genotype tended to be associated with the flavour ($P = 0.067$).

4 Discussion

In the present study, polymorphism in the 5′ flanking region of the *IGF-I* gene and its association with growth, body size, carcass and meat quality traits in Coloured Polish Merino sheep were analysed. Two alleles, named A and B, and only two genotypes, AA and AB, were detected. The A allele was predominant (91.6 %), which consequently resulted in the higher frequency of the AA homozygotes (83.3 %) than the AB heterozygotes. These results are consistent with the study of Yilmaz et al. (2005), who also reported a high frequency of the A allele and the AA genotype (89 and 77 %, respectively) in Polypay sheep. Conversely, Tahmoorespur et al. (2009) and Nazari et al. (2016) detected lower frequencies of the AA genotype (45 and 28 %) and identified the BB homozygotes (9 and 34 %) in Baluchi and Zandi sheep, respectively. Furthermore, Hajihosseinlo et al. (2013) and Kazemi et al. (2011) identified three genotypes in this locus in Makooei (AA – 52 %, AG – 42 % and GG – 6 %) and Zel (AA – 47 %, AB – 47 % and BB – 6 %) sheep, respectively. He et al. (2012) found two SNPs in the 5′ regulatory region of the *IGF-I* gene in four sheep breeds in China. Dorset and Texel sheep were characterised by very high AA genotype frequencies (100 and 93.8 %, respectively) and a lack of the BB homozygotes. Conversely, percentage frequency of the AA genotype in Hu sheep was low (37.9 %) and moderate in Small-Tailed Han sheep (64.6 %) when compared to Dorset and Texel sheep in the study of He et al. (2012). Moreover, Scatà et al. (2010) identified two SNPs (g.855G > C and g.857G > A) in the 5′ UTR of the *IGF-I* gene in Gentile di Puglia, Altamurana and Sarda sheep breeds. Proskura and Szewczuk (2014) analysed the g.271C > T in the third exon of the *IGF-I* gene and they found that the T allele was predominant in Pomeranian Coarsewool sheep (79.5 %). Similarly, Scatà et al. (2010) also observed high frequencies of the T allele in Gentile di Puglia (61.3 %) and Sarda (54.5 %), but slightly lower in Altamurana sheep (43.1 %). Conversely, Gholibeikifard et al. (2013) reported a very low frequency of

Table 2. Results of association analysis between *IGF-I* genotypes and growth traits – LSM ± SE (standard error) – in Coloured Polish Merino sheep.

Trait	Unit	LSM* ± SE		*P* value
		AA	AB	
N		227	50	
Body weight on second day of life	kg	5.11 ± 0.10	5.08 ± 0.13	0.783
Body weight on 30th day of life	kg	12.80 ± 0.18	12.61 ± 0.28	0.527
Body weight on 56th day of life	kg	19.30 ± 0.32	19.04 ± 0.45	0.545
Body weight on 78th day of life	kg	26.56 ± 0.46	26.21 ± 0.60	0.511
Average daily gain between second and 30th days of life	g	255.36 ± 3.95	250.10 ± 7.22	0.505
Average daily gain between 30th and 56th days of life	g	249.69 ± 6.75	247.59 ± 8.89	0.789
Average daily gain between 56th and 78th days of life	g	328.00 ± 8.13	324.75 ± 12.44	0.796
N		90	16	
Final weight	kg	34.64 ± 0.54	33.77 ± 1.16	0.487
Pre-slaughter weight	kg	32.40 ± 0.51	31.80 ± 1.07	0.601

* LSM is least squares mean.

Table 3. Results of association analysis between *IGF-I* genotypes and body size traits (LSM ± SE) in Coloured Polish Merino sheep.

Trait	Unit	LSM* ± SE		*P* value
		AA	AB	
N		90	16	
Withers height	cm	57.9 ± 0.48	59.5 ± 0.98	0.128
Chest depth	cm	25.5 ± 0.37	25.8 ± 0.70	0.671
Body length	cm	57.2 ± 0.47	56.9 ± 0.80	0.771
Shoulder width	cm	19.21 ± 0.13	19.0 ± 0.28	0.425
Hip width	cm	15.5 ± 0.14	15.6 ± 0.23	0.854
Leg depth	cm	20.4 ± 0.16	20.1 ± 0.34	0.396

* LSM is least squares mean.

the T allele (5 %) in Baluchi sheep and they did not find TT homozygotes. Niżnikowski et al. (2015) investigated polymorphism in the third exon of the *IGF-I* gene in different sheep breeds in Poland and they detected the T allele only in Charollais ewes. To summarise, allele and genotype frequencies in the *IGF-I* locus vary between different sheep breeds; therefore, studies covering other sheep breeds should be continued.

The *IGF-I* locus was characterised by a low genetic diversity in the investigated Coloured Polish Merino sheep population. The value of observed heterozygosity (0.167) was slightly higher than the value of expected heterozygosity (0.153). Moradian et al. (2013) also observed two alleles in the *IGF-I* locus in Makooei sheep; nevertheless, values of H_o and H_e were higher: 0.42 and 0.40, respectively. The Coloured Polish Merino sheep population was in the Hardy–Weinberg equilibrium ($P = 0.247$), indicating no selection for investigated *IGF-I* locus. Similarly, Negahdary et al. (2013), who investigated polymorphism of the 5′ flanking region of the *IGF-I* gene, reported that the population of Makooei sheep was in the Hardy–Weinberg equilibrium ($P > 0.05$). Conversely, Nazari et al. (2016), who studied the *IGF-I* locus in Zandi sheep, showed that this population was not in the Hardy–Weinberg equilibrium ($P < 0.01$).

The *IGF-I* gene affects many important processes in an organism, growth being among them (Akers, 2006; Burgos and Cant, 2010). Circulating IGF-I concentration has an impact on fetal and neonatal size and postnatal growth in several species (Baker et al., 1993; Breier et al., 1988; Duclos et al., 1999; Yakar et al., 2002; Zapf and Froesch, 1999). However, association analysis did not show significant effects of the *IGF-I* gene polymorphism on growth traits in Coloured Polish Merino sheep. Similarly, Nazari et al. (2016) did not find significant associations between SNP in the 5′ flanking region of the *IGF-I* gene and growth traits in Zandi sheep. Also, Proskura and Szewczuk (2014) did not show any relationships between the C/T substitution (g.271C > T) in the *IGF-I* gene and growth traits in Pomeranian Coarsewool sheep in Poland. Moreover, Gholibeikifard et al. (2013) did not observe the effects of SNP, located in the third exon of the *IGF-I* gene, on growth traits in Baluchi sheep. Conversely, Tahmoorespur et al. (2009) showed significant associations of the *IGF-I* gene polymorphism in the 5′ flanking region with average daily gain (ADG) from birth to weaning in Baluchi sheep. Heterozygous lambs had higher ADG from birth to weaning than homozygotes AA and BB. Furthermore, Hajihosseinlo et al. (2013) observed significant effects of nucleotide variation in the 5′ flanking region of the *IGF-I* gene with several growth traits in Makooei sheep: birth weight (BW), weaning weight (WW), 6-month weight (SW) and average daily gains from birth to weaning (GBW). Additionally, a significant association of polymorphism in the 5′ flanking region of the *IGF-I* gene with BW, WW, SW, breeding value estimated for body weight in the sixth month of life (EBV 6MW), GBW and average daily gains from the

Table 4. Results of association analysis between *IGF-I* genotypes and live ultrasound measurements of the *LD* muscle (LSM ± SE) in Coloured Polish Merino sheep.

Trait	Unit	LSM* ± SE		*P* value
		AA	AB	
N		254	51	
LD muscle depth	cm	2.24 ± 0.03	2.27 ± 0.05	0.530
LD muscle width	cm	4.90 ± 0.05	5.04 ± 0.07	0.063
LD muscle area	cm^2	7.83 ± 0.19	8.04 ± 0.26	0.341
Fat depth over *LD* muscle	cm	0.184 ± 0.004	0.183 ± 0.005	0.855

* LSM is least squares mean.

sixth month to the ninth month of life (GSN) were found in Makooei sheep (Negahdary et al., 2013). AG heterozygous Makooei sheep had higher values of the traits EBV 6MW, 6MW and WW than homozygotes. Conversely, AA homozygous sheep had higher BW and GBW (Negahdary et al., 2013). Furthermore, Sun et al. (2014), who investigated the effects of different factors on the level of the *IGF-I* gene expression and its associations with growth traits, observed a positive correlation of this gene's expression with body weight in Hu sheep. Other authors analysed the effects of the *IGF-I* gene polymorphisms with growth traits in other livestock species. For example, Ge et al. (2001) showed associations between variation in the *IGF-I* gene and growth traits in Angus cattle and suggested that this polymorphism could have an impact on gene transcription and consequently on animal phenotype. Szewczuk et al. (2013) reported effects of two SNPs in the *IGF-I* gene on body weight in the second month of life in calves and average daily gains in the periods from the first to the second months and from the second to the third months of life and for the whole rearing period. Zhang et al. (2008) investigated polymorphism of the *IGF-I* gene and its association with growth and body size traits in Nanjiang Huang goats. They found a significant effect of G/C substitution on birth weight, body weight at 6 months, body weight at 12 months, heart girth at 2 months, body length at 6 months, wither height at 6 months, wither height at 12 months and heart girth at 12 months in the goats. Concluding, there are analyses that confirm associations of the *IGF-I* gene polymorphisms with growth traits in sheep and other livestock species; however, there are other reports that are opposite to these findings. The growth of animals is a complex process that is under the control of several genes and environmental factors. Moreover, the effects of the *IGF-I* gene polymorphism on growth traits seem to depend on sheep breed. Therefore, before introducing DNA tests in sheep breeding, additional analyses of this gene's polymorphism and its association with growth traits in different sheep breeds should be undertaken.

The IGF-I hormone plays a crucial role in the postnatal linear growth of animals (e.g. Yakar et al., 2002; Zapf and Froesch, 1999). In this study an association of the *IGF-I* gene polymorphism with body size traits and subjective assessment of body muscle and fat class in Coloured Polish Merino sheep was investigated; however, the effects of *IGF-I* genotypes on these traits were not significant. Conversely, Hajihosseinlo et al. (2013), who investigated associations of the *IGF-I* gene polymorphism in the 5′ flanking region with such body size traits as height and length of body, wither height, chest width and rump length in Makooei sheep, found a significant effect of this gene's genotypes on sheep body length. Moreover, Chelongar et al. (2014) observed a significant effect of SNP in the first exon of the *IGF-I* gene on fat-tail fat thickness (the thick rump). AA homozygotes were superior in terms of this trait, whereas GG male lambs had the lowest fat thickness. The associations of nucleotide variation in the first exon of the *IGF-I* gene with tail length and width (rump length and width) in Makooei sheep was not significant (Chelongar et al., 2014). Associations between the *IGF-I* gene polymorphism and body size traits were also investigated in other livestock species. For example, Zhang et al. (2008) found the significant effects of G/C substitution in the fourth intron of the *IGF-I* gene on heart girth at 2 months, body length at 6 months, wither height at 6 months, wither height at 12 months and heart girth at 12 months in Nanjiang Huang goats. Gao et al. (2009) showed that the polymorphism in the *IGFBP-3* locus was associated with rump width and heart girth at 24 and 36 months in Chinese beef cattle. Furthermore, Mullen et al. (2011) reported that SNP in the *IGF-I* gene (*IGF1i6*; G allele) was positively associated ($P < 0.05$) with body condition score in Holstein-Friesian dairy cattle, while Lynch et al. (2010) showed a relationship between *IGF1i2* and body condition score at calving in a cohort of 241 dairy cows. IGF-I protein affects longitudinal bone growth by promoting osteoblast division and proliferation. Furthermore, it regulates muscle growth by enhancing myocyte differentiation and multiplication and has an impact on cartilage growth by increasing chondrocyte colony formation (Duclos et al., 1999; Yakar et al., 2002; Zapf and Froesch, 1999). It should be pointed out that there is limited information about effects of the *IGF-I* gene polymorphism on body size traits, especially in sheep. Moreover, to our knowledge it was the first association analysis of *IGF-I* genotypes and subjective

Table 5. Results of association analysis between *IGF-I* genotypes and carcass traits (LSM ± SE) in Coloured Polish Merino sheep.

Trait	Unit	LSM* ± SE		*P* value
		AA	AB	
N		90	16	
Hot carcass weight	kg	13.97 ± 0.11	13.91 ± 0.16	0.685
Cold carcass weight	kg	13.60 ± 0.10	13.56 ± 0.15	0.796
Cold carcass dressing out	%	43.72 ± 0.32	43.38 ± 0.51	0.484
Carcass parts				
Right carcass side weight	kg	6.67 ± 0.02	6.61 ± 0.04	0.170
Fore part of the carcass weight	g	2629 ± 13.7	2607 ± 28.0	0.453
Full loin part weight	g	1711 ± 9.9	1690 ± 20.4	0.339
Leg part weight	g	2214 ± 10.3	2212 ± 18.1	0.884
Carcass dimensions				
Carcass length	cm	55.7 ± 0.27	56.0 ± 0.43	0.468
Breast depth	cm	23.4 ± 0.09	23.6 ± 0.18	0.232
Breast width	cm	21.9 ± 0.13	21.6 ± 0.24	0.278
Rump width	cm	22.4 ± 0.09	22.3 ± 0.16	0.760
Rump girth	cm	59.0 ± 0.14	59.1 ± 0.28	0.773
Leg depth	cm	17.0 ± 0.12	17.3 ± 0.21	0.185
Leg length index	cm	24.7 ± 0.20	24.7 ± 0.27	0.867
Loins width	cm	11.9 ± 0.06	12.0 ± 0.13	0.718
Carcass cuts				
Scrag weight	g	376.2 ± 4.77	365.8 ± 9.30	0.304
Middle neck weight	g	531.8 ± 7.09	514.0 ± 13.00	0.187
Shoulder weight	g	1050.3 ± 7.13	1042.4 ± 13.00	0.556
Fore shank weight	g	**307.1 ± 2.21**[a]	**320.9 ± 4.6**[b]	**0.006**
Breast and flank weight	g	1031.6 ± 7.88	1039.3 ± 15.91	0.647
Rib weight	g	470.2 ± 4.35	460.0 ± 9.02	0.297
Loin weight	g	515.3 ± 6.27	500.9 ± 13.01	0.304
Tenderloin weight	g	58.2 ± 0.88	58.5 ± 1.59	0.846
Leg weight	g	1816.2 ± 9.36	1806.4 ± 16.16	0.551
Hind shank weight	g	400.3 ± 3.10	405.1 ± 5.91	0.441
Eye of loin				
Width	mm	55.1 ± 0.35	54.6 ± 0.73	0.461
Depth	mm	29.6 ± 0.25	30.4 ± 0.50	0.137
Area	cm^2	12.40 ± 0.16	12.42 ± 0.34	0.955
Fat depth over eye of short loin	mm	2.29 ± 0.09	2.26 ± 0.17	0.861
Leg				
Muscle tissue yield	%	71.74 ± 0.29	71.78 ± 0.56	0.934
Fat tissue yield	%	12.64 ± 0.28	12.26 ± 0.55	0.509
Bone tissue yield	%	15.12 ± 0.15	15.52 ± 0.33	0.253
Other				
Fat depth over ribs	mm	4.56 ± 0.16	4.09 ± 0.33	0.199

* LSM is least squares mean. Values with different superscripts [a,b] within a row are significantly different ($P < 0.05$). Values in bold were applied to highlight highly significant effect of the *IGF-I* gene on carcass traits.

assessment of body muscle and fat class in sheep. Therefore, similar association analyses should be undertaken in other sheep breeds.

IGFs, their receptors and BPs play a crucial role in muscle growth and differentiation (Oksbjerg et al., 2004). In this study effects of the *IGF-I* gene polymorphism with ul-

Table 6. Results of association analysis between *IGF-I* genotypes and meat quality traits (LSM ± SE) in Coloured Polish Merino sheep.

Trait	Unit	LSM* ± SE		*P* value
		AA	AB	
N		90	16	
Muscle pH 24 h post mortem	pH units	5.68 ± 0.01	5.66 ± 0.02	0.629
Reflectance coordinates				
L^*, lightness	units	39.75 ± 0.29	39.57 ± 0.66	0.800
a^*, redness	units	12.86 ± 0.17	12.69 ± 0.28	0.532
b^*, yellowness	units	2.31 ± 0.19	2.08 ± 0.35	0.528
Water content	%	73.35 ± 0.18	73.20 ± 0.43	0.752
Intramuscular fat content	%	1.91 ± 0.06	1.81 ± 0.14	0.500
Total protein content	%	23.59 ± 0.16	23.85 ± 0.38	0.523
Sensory evaluations				
Aroma	1–5	4.46 ± 0.02	4.46 ± 0.05	0.944
Succulence	1–5	4.48 ± 0.03	4.50 ± 0.06	0.822
Tenderness	1–5	4.29 ± 0.03	4.25 ± 0.08	0.572
Flavour	1–5	4.43 ± 0.03	4.31 ± 0.06	0.067
Subjective visual evaluations				
Colour	1–8	**3.95 ± 0.06**[a]	**3.61 ± 0.15**[b]	**0.043**
Marbling	1–4	2.02 ± 0.05	2.01 ± 0.11	0.982
Water holding capacity	%	34.27 ± 0.33	33.28 ± 0.77	0.238
Warner Bratzler shear force	N cm^{-2}	45.28 ± 1.63	44.13 ± 3.90	0.788
Drip loss	%	**2.47 ± 0.24**[a]	**3.33 ± 0.43**[b]	**0.049**
Cooking loss (CL, %)	%	15.53 ± 0.47	15.07 ± 0.99	0.662

* LSM is least squares mean. Values with different superscripts [a,b] within a row are significantly different ($P < 0.05$). Values in bold were applied to highlight significant effects of the *IGF-I* gene on meat quality traits.

Table 7. Results of association analysis between *IGF-I* genotypes and subjective assessment of live animal and carcass traits in Coloured Polish Merino sheep ($n = 106$).

Trait	Unit	*P* value
Live animal traits		
Muscle class	1–5	0.107
Fat class	1–5	0.383
Carcass traits		
Conformation and muscle class of the fore part of carcass	1–9	0.373
Conformation and muscle class of the full loin part of carcass	1–9	0.115
Conformation and muscle class of the leg part of carcass	1–9	0.190
Kidney fat class	1–9	**0.002**
External fatness of carcass class	1–9	**0.038**
EUROP conformation class	EUROP	0.916
EUROP fat class	1, 2, 3L, 3H, 4L, 4H, 5	**0.005**

Values in bold are significant or highly significant.

trasound measurements of *LD* muscle in Coloured Polish Merino sheep was not significant; however, it tended to be associated with the width of this muscle. Additionally, *LD* muscle dimensions were also measured post mortem, but no significant associations between the *IGF-I* gene polymorphism and these traits were showed. No other paper was found in the literature concerning association between *IGF-I* genotypes and ultrasound *LD* muscle measurements in sheep. In-

terestingly, in cattle the SNP in the *IGF-I* gene tended to be associated with the ultrasound *LD* area ($P = 0.06$) (Curi et al., 2005). As the current study was the first association analysis of *IGF-I* genotypes with ultrasound *LD* muscle measurements in sheep, similar analysis in other sheep breeds should be undertaken in order to investigate possible breed effects on these traits.

In this study effects of *IGF-I* genotypes on carcass traits in Coloured Polish Merino sheep were investigated. Among 40 traits, significant associations between *IGF-I* genotypes and fore shank weight, EUROP fat class, kidney fat class and external fatness of carcass class in Coloured Polish Merino ram lambs were found. Similarly, Behzadi et al. (2015) investigated effects of the *IGF-I* gene polymorphism on fat-related traits in Mehraban sheep. They observed a tendency for an association between SNP in this gene and dorsal fat thickness ($P = 0.07$); however, effects of this nucleotide variation in abdominal fat and fat-tail weight were not significant. Furthermore, Siadkowska et al. (2006) reported correlations between polymorphism in the 5′ non-coding region of the *IGF-I* gene and lean and fat weight of valuable cuts and a percentage of fat of valuable cuts in Polish Holstein-Friesian cattle. Moreover, concerning the *IGF2* gene polymorphism, CT heterozygous Polish Holstein-Friesian bulls had more fat in valuable cuts in the 15th month of life (Zwierzchowski et al., 2010). Also in cattle, Curi et al. (2005) observed a significant association of *IGF-I*/*Sna*BI polymorphism in the regulatory region of the *IGF-I* gene with subcutaneous back fat assessed by ultrasonography. In pigs, Wang et al. (2006) investigated effects of polymorphism of the *IGF-I* receptor gene (*IGF-IR*) on back-fat thickness and lean percentage estimated by ultrasonography, and they found less back-fat thickness ($P < 0.05$) and a greater lean percentage ($P < 0.01$) in AA homozygous Yorkshire pigs than BB individuals. To summarise, the results of the abovementioned research showed that polymorphisms in the *IGF-I* gene, as well as *IGF2* and *IGF-IR*, were associated with several fat traits of sheep, cattle and pig carcasses. Indeed, the *IGF-I* gene was shown to be involved in fat cell development in transgenic mice (Rajkumar et al., 1999). Rajkumar et al. (1999) observed enhanced expression of this gene during differentiation of precursor cells into mature fat cells. Furthermore, Anderson et al. (1988) found that circulating IGF-I protein was negatively correlated with carcass fat percentage, fat accretion rate and fat thickness in Simmental crossbred bulls. Additionally, Davis and Simmen (1997) found associations between lower plasma IGF-I protein concentrations and higher marbling scores as well as dorsal fat thickness in Angus bulls. Concluding, the *IGF-I* gene should be considered as a potential candidate gene of fat-related carcass traits in sheep.

As mentioned before, among several carcass and carcass cut weights, *IGF-I* genotype was only associated with fore shank weight in Coloured Polish Merino lambs. Contrary to our results, other authors showed an effect of this gene's polymorphism on carcass weights. For example, Behzadi et al. (2015) reported a tendency of the *IGF-I* gene polymorphism to be associated with carcass weight ($P = 0.07$). Moreover, Sun et al. (2014) found that an expression of the *IGF-I* gene was positively correlated with carcass weight in Hu sheep. Siadkowska et al. (2006) observed a correlation between polymorphism in the 5′ non-coding region of the *IGF-I* gene and cold carcass weight in Polish Holstein-Friesian cattle. Curi et al. (2005) found significant association of *IGF-I*/*Sna*BI polymorphism in the regulatory region of the *IGF-I* gene with hot carcass weight, but the effect of this SNP on carcass yield was insignificant in beef cattle. Zwierzchowski et al. (2010) reported effects of the *IGF2* gene polymorphism on cold carcass weight and percentage meat content in valuable cuts at the age of 11 months in Polish Holstein-Friesian cattle. It should be pointed out that there is a dearth of information about the association of the *IGF-I* gene polymorphism with carcass traits in sheep. To our knowledge, until now only Behzadi et al. (2015) had investigated effects of SNP in this gene on carcass traits in Iranian sheep. In the current study we reported results of association analyses in Polish sheep breeds involving new carcass traits, which have not been investigated before. These results suggest that the *IGF-I* gene should be considered as a potential candidate gene of carcass traits in sheep, and similar studies involving other sheep breeds in different countries should be continued.

In this study, association analysis between the *IGF-I* gene polymorphism and meat quality traits was undertaken. A significant effect of *IGF-I* SNP on natural drip loss in Coloured Polish Merino sheep was found. Meat from AA male lambs had lower natural drip loss than meat from heterozygous individuals. Moreover, an association of this nucleotide variation in the *IGF-I* gene with subjective meat colour was observed. In this case, meat from AA homozygous ram lambs was slightly darker than meat from heterozygotes. Little is known about associations of the *IGF-I* gene polymorphism with meat quality traits in sheep. To our knowledge, this study is the first that presents the effect of *IGF-I* genotypes on several new meat quality traits in sheep. Other authors have shown association analyses of the *IGF-I* gene polymorphism with various meat quality traits in sheep, but with different traits than investigated in this study. For example, Behzadi et al. (2015) found significant effects of nucleotide variation in the first exon of the *IGF-I* gene with the level of triglycerides and cholesterol in blood. Su et al. (2014) analysed relationships of the *IGF-I* gene expression in *LD* muscle with meat quality traits in Hu sheep. They observed that the expression of this gene was positively and significantly ($P<0.01$) correlated with muscle fibre diameter and muscle fibre shear stress, and negatively and significantly ($P < 0.01$) correlated with muscle fibre density. Furthermore, Davis and Simmen (1997) showed that lower plasma IGF-I protein concentrations were associated with higher marbling scores in Angus cattle. Wang et al. (2009) observed an effect of the *IGFBP-3* gene polymorphism on meat colour in pigs. More-

over, Wang et al. (2010) found an association between the *IGFBP-5* gene and pH of various meat cuts in pigs. SNPs in the *IGF-I* gene may have a direct or indirect effect on meat quality traits in sheep, cattle and pigs. There is also a possibility that SNPs detected in the *IGF-I* gene are not causative mutations for the investigated effects on meat quality traits, but are markers in linkage with the causative mutations in the other genes. As the results of this study constitute the first information gathered on the *IGF-I* gene polymorphism association with several meat quality parameters in sheep, further investigation should be carried out to evaluate potential breed-specific effects of SNPs in this gene on lamb quality.

5 Conclusion

In the present study, information about the *IGF-I* gene polymorphism and its effect on a wide range of growth, body size, carcass and meat quality traits in Coloured Polish Merino sheep were reported. Associations between *IGF-I* genotype and fore shank weight ($P = 0.006$), kidney fat class ($P = 0.002$), EUROP fat class ($P = 0.005$), external fatness of carcass class ($P = 0.038$), natural drip loss ($P = 0.049$) and subjective assessment of meat colour ($P = 0.043$) were found. Therefore, the *IGF-I* gene polymorphism could be used as a marker for an improvement of these traits in Coloured Polish Merino sheep. Moreover, in the case of several investigated traits, the present study provided the first information about the effect of this marker in sheep. Concluding, the results suggest that the *IGF-I* gene could be considered as a candidate gene of certain carcass and meat quality traits in sheep; however, further investigations are recommended in other sheep breeds in the world in order to evaluate potential breed- and/or flock-specific effects of this gene's polymorphism on sheep production traits. Furthermore, the *IGF-I* gene is well known for its role in many processes in an organism, which is a result of its pleiotropic function; therefore, before using *IGF-I* polymorphism in a production system, association analyses regarding other traits, which were not investigated in this study, i.e. reproductive and milk production attributes, should be carried out to confirm that selection will not have negative consequences for the breed.

Data availability. The original data of the paper will be available upon request to the corresponding author.

Competing interests. The authors declare that they have no conflict of interest.

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