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Association between polymorphism within rabbit *IGF1* gene and slaughter weight in Termond White rabbits

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Abstract: Growth traits belong to the most important economic traits in livestock. One of the genes involved in vertebrate growth and development is insulin-like growth factor 1 (*IGF1*). Therefore, in our study we hypothesised that within the sequence of rabbit *IGF1* gene it is possible to identify a polymorphism that may influence growth, carcass or meat traits in rabbits. We identified 6 polymorphisms (g.89259430T>C; g.89259338C>G; g.89259328T>C; 89210029A>G; 89210349C>G and g.89194199C>T) within introns of the *IGF1* gene. One polymorphism, g.89194199C>T, was analysed using polymerase chain reaction high-resolution melting (PCR-HRM). We performed the association analysis on 370 animals (males to females 1 : 1) of different breeds: New Zealand White × Flemish Giant crossbreds (NZW × FG), Termond White (TW) and Flemish Giant (FG). Results showed that for growth and slaughter traits in TW populations a significant association ($P = 0.003$) was found for slaughter weight (SW) when *CT* genotypes showed significantly higher values compared to *CC* genotypes (2 878.0 g ± 107.0 and 2 678.0 g ± 34.0, respectively). For TW rabbits we found a significant association ($P = 0.009$) for dissected bone weight in hind leg (HB) when *CT* genotypes (127.0 g ± 5.8) had significantly higher values compared to *CC* genotypes (112.0 g ± 2.4). For carcass traits and physical characteristics of meat we found for *musculus longissimus lumborum* in TW rabbits that the b^* parameter value 45 min after slaughter was significantly higher ($P = 0.001$) for *CT* genotypes (1.88 ± 0.05) compared to *CC* genotypes (0.05 ± 0.02). We conclude that the use of identified SNP in breeding may be limited to some breeds.

Keywords: association analysis; growth traits; insulin like growth factor 1; meat traits oryctolagus cuniculus; SNP

Insulin-like growth factor 1 (IGF1) is also known as growth-promoting factor (somatomedin C). This gene encoding IGF1 is conserved across species – in humans, dogs, cattle and pigs the sequence of IGF1 is identical (Nixon et al. 1999). Along with insulin-like growth factor 2 (IGF2), they play a key role in cell differentiation, embryogenesis, growth and regulation of metabolism (Siadkowska et al.

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2006). IGF1 acts on its receptors – IGF1R (insulin-like growth factor 1 receptor) and influences growth and development, immunity cellular metabolism and reproduction (Cannata et al. 2010). Due to the role of IGF1 in growth regulation, cell proliferation and regulation of metabolic processes (Gorecki et al. 2007) this gene is considered as candidate marker for growth and meat traits. The analysis of sequences of *IGF1* gene in animals allowed identifying polymorphisms that were associated with mostly meat and growth traits. Many single nucleotide polymorphisms (SNPs), both in coding and non-coding regions, were identified e.g. C512T in the promoter region and rs29012855 in intron 3 were associated with growth traits in Angus only (Islam et al. 2009). Moreover, polymorphisms within *IGF1* gene were associated with the number of kids in goats (Li et al. 2022) and milk production traits in cattle (Siadkowska et al. 2007)

Rabbit (*Oryctolagus cuniculus*) meat is considered as healthy, with low fat content and it is known for its nutritional value. Europe is described as the second world producer of meat. Many rabbits are kept in a backyard farming system as a source of meat for the breeder's family or for a local market (European Commission 2017). As growth is decisively important in rabbit meat husbandry and this trait.is considered moderately to highly heritable (Blasco et al. 2018), molecular approaches were performed to identify polymorphisms that may be used in rabbit selection. So far there have been reports showing associations between SNPs in candidate genes for growth traits and traits like slaughter weight (Fontanesi et al. 2012, 2013) and body weight at 84 days of age (Wu et al. 2015). Currently, whole genome sequencing methods are used in search for the molecular background of meat traits (Sosa Madrid et al. 2020) as well as Angora long-hair trait (Fatima et al. 2023) or signatures of selection of fancy rabbits (Ballan et al. 2022). It must be stated that not only SNPs within coding regions were analysed but also in the non-coding part of genes like it was presented by Fontanesi et al. (2012) and synonymous SNPs (Wu et al. 2015).

Still, the costs of a commercial SNP platform, short generation intervals, and low economic value of paternal rabbits are considered as the main limitations to using genomic selection as the main evaluation method in meat-type rabbits (Mancin et al. 2021). It may be reasonable and cheaper to use marker-assisted selection (MAS) where sequences of candidate genes for important traits are analysed (Migdal et al. 2017). According to the European Commission (2017) backyard production (34%) is a significant source of rabbit meat. According to this report three times more rabbits are slaughtered at backyard farms and sold locally. It should be highlighted that Eastern European countries are known for many rabbit breeds (especially medium-sized ones) that are kept in backyard farming but also that are used as broiler rabbits. Researches conducted by Posta et al. (2024) on Debrecen White rabbits, by Szendro et al. (2012) on Pannon White rabbits, Chodova et al. (2014) on Czech genetic resources of rabbits (Moravian Blue, Czech White, Czech Solver, Czech Spotted, Moravian White Brown-Eyed, Czech Gold, and Czech Black Guard Hair) prove that breeding of rabbits is very popular in those countries. Similar papers focusing on reproduction, conservation status, growth and meat quality can be found for Slovak (Tomka et al. 2022) and Polish (Kowalska and Bielanski 2011, Migdal et al. 2019) rabbits.

We hypothesized that within the rabbit *IGF1* gene we can identify polymorphisms that may influence growth and meat quality traits in the population of the broiler breed Termond White, primitive breed Flemish Giant and crossbreds of New Zealand White and Flemish Giant rabbit.

MATERIAL AND METHODS

The animals

The experiment was performed under standardised conditions at the experimental station of the University of Agriculture in Krakow. A permit from the 2nd Local Institutional Animal Care and Use Committee (IACUC) in Krakow – agreement No.: 267/2018 and University of Agriculture permissions – 29/2016, 37/2016 and 2/2018 were obtained for the described experiment. For the analysis of the hypothesis we used data from 370 animals (males to females 1 : 1): 130 Termond White (TW) rabbits (from 20 sires), 40 Flemish Giant (Belgian Giant Grey) rabbits (FG) (from 8 sires) and 200 crossbred rabbits of the F2 generation of New Zealand White × Flemish Giant (NZW × FG) (18 sires). Crossbred rabbits were derived from 48 litters from the mating of NZW and

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FG rabbits. Subpopulations used in this experiment were rabbits with the highest, medium and lowest slaughter weight randomly chosen from the population. TW and FGrabbits were under the auspices of the National Centre for Animal Breeding. The animals were kept in a heated hall with exhaust air ventilation. Animals had continuous access to water (nipple drinkers) and feed (pelleted commercial diet contained 15% of crude protein, 16.1% of crude fibre, and 3.5% of crude fat). The lighting period was set at 14 L/10 D. From weaning (on the 35th day of age) to the 84th day of age, the animals were kept in wire cages intended for the commercial rearing of rabbits (2 rabbits per cage).

Carcass traits

The rabbits were weaned at 5 weeks of age and slaughtered at 12 weeks of age (BW12). At the 12th week of age animals were fasted (24 h) with continuous access to water. The slaughter body weight (SW) was recorded, and the animals were subsequently slaughtered. The rabbits were stunned, immediately bled, skinned and eviscerated. The hot carcass weight (HCW) was recorded, and storage at 4 °C for 24 h chilling followed. After chilling the chilled carcass weight (CCW) was recorded. Hot dressing-out percentage (%) (DPH) (Equation 1) and chilled dressing-out percentage % (DPC) (Equation 2) were calculated.

$$\mathrm{DPH} = \frac{HCW}{SW} \times 100 \quad (1)$$

$$\mathrm{DPC} = \frac{CCW}{SW} \times 100 \quad (2)$$

For dissection, the carcass was divided between the 7th and the 8th *thoracic vertebra*, and between the 6th and 7th *lumbar vertebra*. Fore part, intermediate part and hind part were dissected and the weight of meat, bone and fat from each part was recorded. All measurements were done using the Lucznik KS-205 electronic scales (GaleriaL Co. Ltd., Wroclaw, Poland; e = 0.1).

Colour and pH measurement

The colour of meat was determined using the Minolta CR-400 chroma meter (Minolta Co. Ltd., Osaka, Japan) calibrated with a white plate supplied by the manufacturer, and programmed to use a D65 standard illuminant, 10° observer with an illuminating/viewing aperture size of 11 mm, and a blooming time of 45 min. The average value from three measurements of lightness (L^*), redness (a^*), and yellowness (b^*) was recorded. The pH values of meat were determined using the Consort C561 pH meter (calibration was performed before analysis in 4.0 and 7.0 pH buffers) equipped with temperature probe with temperature compensation (Consort, Turnhout, Belgium). The colour and pH were recorded on the same part of the carcass 45 min after slaughter and 24 h after chilling on *m. longissimus lumborum* (loin – m.l.l.) and *m. biceps femoris* (hind leg – m.b.f.).

Blood collection and DNA isolation

Blood was collected during slaughter into tubes containing EDTA and stored at –20 °C until further analysis. DNA was isolated using a WIZARD Genomic Kit (Promega, Wisconsin, USA) from 300 µl of blood volume. Quality and quantity of DNA were analysed using SmartSpec^{TM} Plus spectrophotometer (BioRad, CA, USA)

Primer design and sequencing

Primers for sequencing were designed with the Primer3 software (v.0.4.0) (https://bioinfo.ut.ee/primer3/), using the sequences of the rabbit *IGF1* gene sequence NC_067377.1. Primers designed for the amplification of coding fragments and closest intronic part are presented in Table 1. DNA from 20 animals was used in the sequencing process. Animals were chosen from all analysed breeds based on slaughter weight at 12th weeks of age (the highest and the lowest body weight). PCR was performed using GoTaq G2 Hot Start polymerase (Promega, Madison, WI, USA). First, 50 ng of DNA was added to the master mix and filled with nuclease-free water up to a final volume of 15 ul. After visual inspection, PCR products were purified using ExoBAP (EURx, Gdansk, Poland); forward and reverse strands were sequenced with a BigDye Terminator (v3.1) sequencing kit (Thermo Fisher Scientific, Waltam, MA, USA) on an ABI 3500xL automatic sequencer (Applied Biosystems).

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Table 1. Sequences of primers used for sequencing and PCR- high-resolution melting genotyping

Name of starters	Amplified region of gene	Sequence 5'–>3'	Length (bp)	Ta	Used for
IGF1_1	Part of 5'upstream, exon1 part of intron 1_2	F: AGATGCTGATTGTACGCCCA R: GCATATCAGTCGAGCCCAGA	481	60	Sequencing
IGF1_2	Part of intron 1_2, exon2, part of intron 2_3	F: CCTGCCAGGACTCCAAGTAA R: CCCGCTGAAACTCTATGCTT	552	65	Sequencing
IGF1_3	Part of intron 2_3, exon 3, part of intron 3_4	F: ACGGAAGTCCAGTTCTGTGG R: TTCCATGTGGAGTGACCAAA	559	65	Sequencing
IGF1_4	Part of intron 3_4, exon 4, part of intron 4_5	F: CTTCCAGTGCCATTCCTGTCT R: ACCAGGCCTACTTGTCTTCA	415	60	Sequencing
IGF1_5	part of intron 4_5, exon5, part of 3' downstream	F: TCACACTGTACCCCATGAGTG R: GCTCTTGAGAGGCAGGGACT	507	65	Sequencing
g.89194199C>T	PCR HRM for g.89194199C>T	F: TGCACTCAATTACTATGGATCACTT R: AAGAGTGACATGAAATCAATGAGG	116	62	genotyping

bp = base pairs; Ta = annealing temperature

All chromatograms were visually inspected in FinchTV (v1.4.0) (Geospiza Inc., Seattle, WA, USA). The online sequence alignment BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to confirm obtained sequences with known sequence in the database. Quality of the sequences was inspected using CodonCode Aligner (CodonCode Corporation; www.codoncode.com). SNPs were identified by aligning the reference sequence of OryCun (2.0) and aligned sequencing reads in MEGAX (Kumar et al. 2018).

We tried to design a genotyping method for the identified SNPs. From all SNPs we were able to analyse one polymorphism g.89194199C>T using PCR-HRM. Primers are listed in Table 1. The PCR-HRM amplification reaction for g.89194199C>T polymorphism was performed in 10 µl volume using Quantum EVAGreen®HRM kit (Syngen Biotech, Wroclaw, Poland) according to the protocol using a MIC qPCR cycler (Bio Molecular Systems, Queensland, Australia).

Statistical analysis

Associations between SNP and traits were investigated within breed. The following linear model was fitted using the general linear models (GLM) procedure in SAS (2014):

$$Y_{ijk} = \mu + G_i + S_j + (G \times S)_{ij} + bN_{ijk} + e_{ijk} \quad (3)$$

where:

Y_{ijk} – post-slaughter traits or meat quality parameter;
μ – overall mean;
G_i – fixed effect of i-th genotype (i = *CC*, *CT*);
S_j – fixed effect of j-th gender (j = 1,2);
$(G \times S)_{ij}$ – the interaction between genotype and gender (ij = 1,2,3,4);
bN_{ijk} – linear regression on the day of slaughter;
e_{ijk} – residual effect.

The significance of differences between means (LSM) was determined by the Tukey-Kramer test, SAS 9.3 software (SAS Institute, Inc., Cary, NC, USA). To account for multiple testing, the Bonferroni correction was used. The correction factor was derived from the number of tested SNPs. The significance threshold ($P < 0.05$) was divided by the number of tests. Thus, the Bonferroni-corrected significance level of 0.05/3 = 0.016 was applied.

RESULTS

We identified SNPs within intron 1_2: g.89259430T>C, g.89259338C>G, g.89259328T>C, intron 2_3: g.89210349C>G, within intron 3_4:89210029A>G and within intron 4_5-g.89194199C>T. As for the g.89194199C>T substitution, we were able to design a genotyping method; in this case we analysed this SNP using a PCR-HRM method on 370 animals. Allele and genotype frequencies are presented in Table 2. Due to minor allele frequencies *TT* genotypes for TW and NZW × FG breeds were excluded from the analysis. For analysed samples *CC* were predominant genotypes in each breed. The range of PIC values

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Table 2. Allele and genotype frequencies for g.89194199C>T polymorphism within the rabbit *IGF1* gene

Polymorphism	Breed	Allele frequencies (%)		Genotypes frequencies (%)*			*P*-value**	MAF	He	PIC
		C	*T*	*CC*	*CT*	*TT*				
g.89194199C>T	TW	69.20	30.80	52.50 (63)	33.30 (50)	14.20 (17)	0.169	0.32	0.44	0.34
	FG	65.00	15.00	62.50 (25)	37.50 (15)	–	0.144	0.18	0.304	0.26
	NZW × FG	85.30	14.70	87.05 (148)	26.40 (45)	4.10 (7)	0.136	0.15	0.25	0.22

*Genotype frequencies and number of observations in brackets; **Hardy-Weinberg equilibrium

FG = Flamish Giant rabbits; He = expected heterozygosity; MAF = minor allele frequency; NZW × FG = crossbreds of New Zealand White and Flamish Giant rabbits; PIC = polymorphism information content; TW = Termond White rabbits

was between 0.22 to 0.34, which can be described as somewhat informative. For expected heterozygosity the polymorphic site showed medium values for each breed (Serrote et al. 2020)

Table 3 shows the results of growth and slaughter trait analysis. We found a significant association only for TW rabbits: SW was significantly (P = 0.003) higher for *CT* genotypes compared to *CC* genotypes (2 878 ± 107 and 2 678 ±34, respectively). Weight of dissected bone in the hind leg (HB) were significantly higher (P = 0.009) for *CT* genotypes (127 g ± 5.8) compared to *CC* genotypes (112.0 g ± 2.4).

Table 4 shows the analysis of associations of g.89194199C>T polymorphism with meat traits and physical characteristics of meat. For the *m. longissimus lumborum* muscle in the TW rabbit population, the *b** parameter 45 min after slaughter was found to be significantly higher (P = 0.001) for *CT* genotypes (1.88 ± 0.05) compared to *CC* genotypes (0.05 ± 0.02).

Table 3. Associations between g.89194199C>T polymorphism within *IGF1* and growth and slaughter traits

Traits	TW[2]			NZW × FG			FG		
	CC	*CT*	*P*-value	*CC*	*CT*	*P*-value	*CC*	*CT*	*P*-value
BW12	2 644.0 ± 38.0[3]	2 814.0 ± 110.0	0.080	2 614.0 ± 35.0	2 499.0 ± 84.0	0.346	3 243.0 ± 102.0	3 164.0 ± 83.0	0.684
SW	2 678.0 ± 34.0[a]	2 878.0 ± 107.0[b]	0.003	2 699.0 ± 42.0	2 488.0 ± 97.0	0.137	3 321.0 ± 99.0	3 285.0 ± 104.0	0.849
HCW	1 423.0 ± 19.0	1 513.0 ± 61.0	0.080	1 371.0 ± 22.0	1 260.0 ± 55.0	0.139	1 693.0 ± 62.0	1 710.0 ± 64.0	0.876
CCW	1 387.0 ± 20.0	1 444.0 ± 70.0	0.270	1 331.0 ± 22.0	1 223.0 ± 54.0	0.144	1 604.0 ± 59.0	1 645.0 ± 58.0	0.731
DPH	53.1 ± 0.2	52.5 ± 0.5	0.291	50.7 ± 0.3	50.5 ± 0.5	0.870	50.0 ± 0.6	51.1 ± 0.5	0.705
DPC	51.1 ± 0.2	51.2 ± 0.5	0.359	49.2 ± 0.2	49.1 ± 0.5	0.882	48.1 ± 0.6	50.0 ± 0.6	0.084
FMB	559.0 ± 6.9	572.0 ± 27.0	0.484	530 ± 9.0	482.0 ± 24.0	0.124	699.0 ± 26.0	706.0 ± 25.0	0.894
FF	33.0 ± 3.5	32.0 ± 7.5	0.904	11.0 ± 0.8	11.0 ± 1.8	0.869	13.0 ± 2.1	16.0 ± 2.6	0.457
IM	228.0 ± 4.8	247.0 ± 12.4	0.115	230.0 ± 4.2	223.0 ± 10.0	0.583	238.0 ± 10.0	254.0 ± 13.0	0.455
IB	38.0 ± 1.2	40.0 ± 3.1	0.548	41.0 ± 0.8	37.0 ± 1.6	0.167	45.0 ± 1.6	49.0 ± 3.0	0.321
IF	25.0 ± 2.0	24.0 ± 5.5	0.786	12.0 ± 0.8	15.0 ± 1.9	0.235	13.0 ± 2.1	15.0 ± 2.2	0.573
HM	389.0 ± 5.7	399.0 ± 17.0	0.427	387.0 ± 6.6	357.0 ± 18.0	0.176	448.0 ± 18.0	458.0 ± 16.0	0.773
HB	112.0 ± 2.4[a]	127.0 ± 5.8[b]	0.009	107.0 ± 1.9	94.0 ± 3.3	0.038	146.0 ± 4.4	145.0 ± 4.5	0.870
HF	3.0 ± 0.6	3.0 ± 1.0	0.972	4.8 ± 0.7	3.3 ± 0.7	0.519	2.2 ± 0.6	0.9 ± 0.9	0.336

[a,b]Values within a row and within breeds with different superscripts differ significantly at P < 0.016; means are least squares means + standard deviation

BW12 = body weight at the 12th week of age (g); CCW = chilled carcass weight (g); DPC = cold dressing-out percentage (%); DPH = warm dressing-out percentage (%); FF = dissectible fat in fore part (g); FG = Flemish Giant; FMB = meat and bones in fore part (g); HB = bones in hind part (g); HF = dissectible fat in hind part(g); HCW = hot carcass weight (g); HM = meat in hind part (g); IB = bones in intermediate part (g); IF = dissectible fat in intermediate part (g); IM = meat in intermediate part (g); NZW × FG = crossbreds of New Zealand White and Flemish Giant rabbits; SW= slaughter weight (g); TW = Termond White

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Table 4. Associations between g.89194199C>T polymorphism within *IGF1* and meat traits and physical characteristics of meat

Traits	TW			NZW × FG			FG		
	CC	*CT*	*P*-value	*CC*	*CT*	*P*-value	*CC*	*CT*	*P*-value
Musculus biceps femoris									
pH_{45}	6.57 ± 0.04	6.75 ± 0.08	0.117	6.73 ± 0.02	6.76 ± 0.07	0.776	6.67 ± 0.08	6.70 ± 0.08	0.799
L^*_{45}	53.00 ± 0.05	52.90 ± 0.06	0.282	57.60 ± 0.02	57.80 ± 0.05	0.757	53.2 ± 0.06	51.10 ± 0.06	0.106
a^*_{45}	3.41 ± 0.02	3.25 ± 0.04	0.716	11.40 ± 0.01	10.90 ± 0.02	0.231	3.65 ± 0.03	3.27 ± 0.04	0.529
b^*_{45}	0.05 ± 0.02[a]	1.88 ± 0.05[b]	0.001	1.30 ± 0.01	1.60 ± 0.02	0.448	0.74 ± 0.03	0.56 ± 0.03	0.760
pH_{24}	5.93 ± 0.05	5.97 ± 0.03	0.719	5.77 ± 0.02	5.76 ± 0.03	0.914	6.11 ± 0.02	6.05 ± 0.03	0.224
L^*_{24}	55.90 ± 0.33	55.10 ± 0.53	0.314	55.90 ± 0.18	55.10 ± 0.41	0.314	58.00 ± 0.69	57.30 ± 0.57	0.586
a^*_{24}	4.29 ± 0.23	4.21 ± 0.33	0.882	12.69 ± 0.16	11.85 ± 0.29	0.117	4.04 ± 0.34	5.13 ± 0.44	0.132
b^*_{24}	4.01 ± 0.24	4.17 ± 0.37	0.770	3.90 ± 0.12	3.18 ± 0.30	0.103	4.06 ± 0.31	5.35 ± 0.25	0.030
Musculus longissimus lumborum									
pH_{45}	6.57 ± 0.13	6.69 ± 0.08	0.664	6.82 ± 0.02	6.87 ± 0.04	0.535	6.81 ± 0.07	6.81 ± 0.05	0.996
L^*_{45}	61.70 ± 0.13	59.30 ± 0.08	0.246	61.30 ± 0.02	61.30 ± 0.04	0.968	62.60 ± 0.06	61.20 ± 0.05	0.461
a^*_{45}	0.33 ± 0.13	0.38 ± 0.08	0.947	8.08 ± 0.02	8.47 ± 0.04	0.523	2.27 ± 0.06	2.98 ± 0.05	0.627
b^*_{45}	−3.45 ± 0.50	−2.82 ± 0.83	0.586	−2.98 ± 0.14	−2.18 ± 0.33	0.080	−1.16 ± 0.72	−1.84 ± 1.16	0.682
pH_{24}	5.80 ± 0.03	5.80 ± 0.04	0.990	5.63 ± 0.02	5.65 ± 0.04	0.710	6.00 ± 0.05	5.97 ± 0.07	0.758
L^*_{24}	55.30 ± 0.38	56.60 ± 0.52	0.133	57.10 ± 0.23	57.60 ± 0.57	0.560	59.30 ± 0.75	58.70 ± 0.96	0.703
a^*_{24}	5.65 ± 0.34	6.09 ± 0.39	0.549	12.68 ± 0.20	12.84 ± 0.36	0.814	7.40 ± 0.37	8.61 ± 0.64	0.166
b^*_{24}	3.42 ± 0.29	5.00 ± 0.42	0.024	2.01 ± 0.16	2.06 ± 0.34	0.921	5.43 ± 0.38	6.64 ± 0.71	0.209
Physical characteristic of meat									
SF3	1.81 ± 0.09	1.90 ± 0.12	0.711	3.95 ± 0.12	4.16 ± 0.21	0.583	2.10 ± 0.13	2.15 ± 0.15	0.721
HAR	12.40 ± 0.46	13.60 ± 0.87	0.291	63.00 ± 1.57	58.30 ± 4.44	0.392	11.90 ± 0.71	13.10 ± 1.17	0.472
SPR	0.45 ± 0.01	0.48 ± 0.02	0.244	0.52 ± 0.01	0.51 ± 0.02	0.877	0.49 ± 0.01	0.48 ± 0.02	0.219
COH	0.42 ± 0.01	0.43 ± 0.01	0.657	0.43 ± 0.01	0.40 ± 0.02	0.102	0.45 ± 0.01	0.44 ± 0.01	0.204
CHE	2.42 ± 0.13	2.84 ± 0.27	0.185	14.61 ± 0.43	11.53 ± 1.37	0.046	2.74 ± 0.26	2.82 ± 0.36	0.927

[a,b]Values within a row and within breeds with different superscripts differ significantly at $P < 0.016$; means are least squares means + standard deviation

a^* redness parameter; b^* yellowness parameter; CHE = chewiness; COH = cohesiveness; FG = Flemish Giant; HAR = hardness; L^* lightness parameter; NZW × FG = crossbreds of New Zealand White and Flemish Giant rabbits; pH_{24} = pH value after 24 h of chilling; pH_{45} = pH value measured 45 min after slaughter; SF = shear force; SPR = springiness; ; TW = Termond White

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DISCUSSION

In our experiment we analysed coding and part of intronic sequences (about 10% of the whole gene) of the *IGF1* gene in rabbits. A polymorphism for which we were able to design a genotyping procedure was chosen from the identified polymorphisms. In this case using the PCR-HRM method, 370 animals were used to genotype and perform the analysis of associations with growth, carcass and meat quality traits in three different rabbit breeds. As a part of the somatotropin axis, IGF1 is associated with polygenic traits like growth and carcass traits. As Blasco et al. (2018) reported, the feed constitutes up to 45.3% of all costs in rabbit production. As growth is considered as one of the most important factors in rabbit breeding, it seems reasonable to analyse the most important candidate genes for this trait. El-Sabrout and Aggag (2017) analysed the expression of *IGF1* in the small intestine in rabbits weaned at different age; they found that the highest expression pattern was found in rabbits weaned at 33 days of age in contrast to 28 and 23 days of age, and also weaning weight and finishing weight were the highest in a group weaned at 33 days of age. These results support the statement that *IGF1* plays an important role in the development of body weight and we hypothesised that polymorphisms within the *IGF1* gene may have a positive impact on the finishing weight of rabbits. Fontanesi et al. (2012) identified SNP within the rabbit *IGF2* that influenced finishing weight in commercial rabbits. As SNP within the *IGF2* gene was associated with finishing weight in rabbits, we hoped to find similar results for identified SNP within *IGF1* as they are structurally related proteins and act in similar pathways (Baxter et al. 1988). However, the results presented in Table 3 and 4 confirmed our hypothesis only for TW rabbits where slaughter weight was significantly higher for *CT* genotypes compared to *CC* genotypes. Associations with finishing weight were found for the rabbit *MC4R* gene where c.101G>A polymorphism was identified (Fontanesi et al. 2013). Wu et al. (2015) found associations with synonymous SNP within the rabbit *PGAM2* gene with body weight at 84 days of age (BW84) and with average daily weight gain (ADG). In literature there are many reports on the influence of SNPs within the *IGF1* gene on economic traits in farm animals, e.g. in chickens (Bian et al. 2008; Sato et al. 2012; Bhattacharya et al. 2015). On the other side, there are reports on chicken breeds mentioned above where no associations with *IGF1* polymorphisms were found (Hosnedlova et al. 2020). For Chinese Simmental Beef cattle Duan et al. (2021) found SNPs within the *IGF1* gene to be the most promising for growth parameters. Islam et al. (2009) reported an association of SNP within the *IGF1* gene with fat deposition in the Angus population but not in hybrid and Charolais breeds, which may suggest a breed dependent effect. It was also reported that *IGF1* may be one of the genes to improve meat tenderness in Iberian pigs (Fernandez-Barroso et al. 2020). It must be added that SNPs within the *IGF1* gene influence the traits like litter size in goats (Naicy et al. 2016) and milk production in some cattle breeds (Kasarda et al. 2021). As it was mentioned above, the effect of SNPs may be breed dependent (Islam et al. 2009; Bhattacharya et al. 2015). Different breeds of animals and selection processes can be pointed out as reasons for differences between them – like indigenous breeds and meat type breeds. In an experiment of Chodova et al. (2014) genetic differences were highlighted for differences between 8 analysed breeds (small, medium and giant breeds and commercial hybrid). As in our study we did not find any trait in each breed that was influenced by analysed SNP, it could easily indicate its usefulness in selection (e.g. MAS). The analysis using GWAS performed on rabbits e.g. by Yang et al. (2020) in their study of the crossbred population of California and Kangda5 (commercial rabbit meat strain) did not point at the *IGF1* gene as a candidate for any of the analysed growth, carcass and meat quality traits. Similarly, Liao et al. (2021) did not find *IGF1* as a candidate gene for growth performance in rabbits. Neither did Ballan et al. (2022) find the *IGF1* gene as one of the genes associated with body weight and growth traits in fancy rabbits.

The analyses presented in Table 3 and 4 did not show any association between the g.89194199C>T polymorphism and traits in all analysed breed. We found for b^* parameter 45 min after slaughter a significantly higher ($P = 0.001$) value for *CT* genotypes (1.88 ± 0.05) compared to *CC* genotypes (0.05 ± 0.02) measured on the *m. longissimus lumborum* muscle in TW rabbits (Table 4). As meat colour is the first point of consumer judgement (Purslow et al. 2020), this parameter could be considered for further use.

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CONCLUSION

We identified 6 SNPs within the *IGF1* gene in rabbits and analysed one polymorphism – g.89194199C>T. We found these associations in Termond White rabbits: for *b** value on m.l.l. after 45 min from slaughter, dissected bone weight in HB and SW. Results obtained in our study showed the influence of SNP on economic traits like slaughter weight in one rabbit breed. This breed is a broiler breed and is often used as a parental component for hybrid rabbits. Therefore, it is possible that this SNP could be used in the selection of TW rabbits.

Conflict of interest

The authors declare no conflict of interest.

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