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Association of polymorphisms in the *GH* and *GHR* genes with growth and carcass traits in rabbits (*Oryctolagus cuniculus*)

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Abstract: In rabbits, growth and carcass traits are important for the breeding programme. An increasing number of annotated polymorphisms demands validation of their influence on those traits before they can be implemented in breeding practice. Therefore, the aim of this study was to investigate *GH* c.-78C>T, *GHR* c.106G>C polymorphisms in the population of Belgian Giant Grey, Termond White, and a crossbreed between New Zealand White and Belgian Giant Grey (NZW × BGG) rabbits. In total 379 animals were genotyped and association analyses with growth traits and carcass traits were conducted. Our results demonstrated that *GH* c.-78C>T showed an association with growth weight in Belgian Grey and NZW × BGG rabbits. Meat weight in intermediate and hind parts for *GH* c.-78C>T statistically differed between Belgian Giant Grey and crossbred rabbits. *GHR* c.106G>C showed an association with meat weight in the intermediate part and dressing percentage in Termond White. *TT/CC* haplotype in Belgian Giant Grey had significantly higher meat weight in hind part, while in crossbred rabbits *CC/CC* haplotype was characterised by the lowest meat weight in intermediate and hind parts. Results from our study confirm that *GH* c.-78C>T, *GHR* c.106G>C polymorphisms constitute good molecular markers for growth and carcass traits.

Keywords: molecular markers; rabbits growth; GH; GHR; association analysis; SNPs

Growth, as a biological phenomenon, is controlled by complex mechanisms, acting in para-, endo- and autocrine ways. They play a key role in growth regulation, together with growth hormone (GH) and growth hormone receptor (GHR), among others. All those proteins play a role as factors in a series of events which can be described as a somatotropic axis (Renaville et al. 2002). GH plays a key role in postnatal growth and it regulates many biological functions, such as muscle mass deposition. It acts by binding with GHR, which causes dimerization and initiates a signalling cascade, activating the JAK-STAT pathway resulting in the expression of genes such as *IGF-2* (Frank 2001).

In recent years, our knowledge of the genetic basis of physiological processes in both humans and animals has expanded. Therefore, current animal husbandry can be described as a result of the environment and nutrition that interact with the genetic value of animals. Successful implementation of genomic selection in dairy cattle leads to the increase in annual rates of genetic gain by 50–100% for lowly heritable traits like female fertility and herd life (Weller et al. 2017). Those results can be encouraging for conducting further investigations into major gene polymorphisms, and the influence thereof on major traits in other animals. Rabbit is one of the species that play an

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important role as a meat supplier. Meat from rabbits exhibits a desirable protein content as well as essential amino acid proportions. Moreover, the effectiveness in dietary manipulation, combined with a promising improvement of oxidative stability of rabbit meat with its "functional" properties, qualify rabbit meat as one of the most precious sources of meat (Pla et al. 2004; Dalle Zotte and Szendro 2011; Dalle Zotte et al. 2016; Martins et al. 2018). Besides commercial breeds, there are many valuable local breeds (Nagy et al. 2011; Chodova et al. 2014), e.g. the Belgian Giant Grey – a large breed, but there is insufficient information about their growth and carcass traits.

Molecular markers can be used to enhance selection accuracy, and therefore they improve genetic gain for important economic traits, such as slaughter weight and carcass weight. So far limited research has been conducted when single nucleotide polymorphisms (SNPs) were identified within candidate genes for important traits. For growth traits, polymorphism associations were found within the growth hormone (*GH*) gene (Fontanesi et al. 2012), the growth hormone receptor (*GHR*) gene (Zhang et al. 2012).

With the view of the importance of confirming the impact on economic traits, and a possibility of excluding their negative effects on other economic traits, we decided to analyse the effect of SNPs within *GH*, *GHR* on growth traits and carcass traits in three rabbit breeds: Termond White, Belgian Giant Grey and F2 crossbreed between New Zealand White and Belgian Giant Grey.

MATERIAL AND METHODS

Animals. In the present study, we analysed data from 379 animals: 190 crossbreeds of the F2 generation of New Zealand White × Belgian Giant Grey (NZW × BGG); 129 Termond White (TER) and 60 Belgian Giant Grey (BGG) rabbits (bucks to does 1 : 1). Animals were kept in a heated hall, furnished with water supply (nipple drinkers), lighting (14 h light : 10 h darkness), and exhaust ventilation. Water and feed were available *ad libitum.* Animals were fed a pelleted commercial diet, containing 15% crude protein, 16.1% crude fibre, and 3.5% crude fat.

Growth traits. Litters were weighed after birth (BW). The rabbits were weaned at week 5 of life (W5), and slaughtered at week 12 of life (W12).

Slaughter traits. The animals were slaughtered after 24-hour fasting, under a permission from the II Local Ethics Committee in Krakow (No. 37, 30th May 2016). Slaughter body weights (SW) were recorded. The rabbits were stunned and immediately bled, pelted and eviscerated. *Post-mortem* data was recorded, including hot carcass weight without head (HCW), and chilled carcass weight (CCW) after a 24-hour storage at 4°C. Weights of the fore part – FP (cut behind the last rib), the intermediate part – IP (cut at the last lumbar vertebra) and the hind part – HP (includes back legs and sirloin) were recorded, and all carcass parts were dissected. Weights of the fore part meat (MF), fore part bone (BF), fore part dissectible fat (FF), intermediate part meat (MI), intermediate part bone (BI), intermediate part dissectible fat (FI), hind part meat (MH), hind part bone (BH), and hind part dissectible fat (FH) were recorded. Hot (DPH) and cold (DPC) dressing percentages (%) were calculated:

$$DPH = (HCW/SW) \times 100, DPC = (CCW/SW) \times 100$$

Blood collection and DNA extraction. DNA was extracted using a GeneMATRIX kit (EURx) from 300 µl of blood collected at slaughter into tubes containing EDTA.

PCR and RFLP conditions. Genotyping of *GH* polymorphism c.-78C>T (Fontanesi et al. 2012), *GHR* polymorphism c.106G>C (Zhang et al. 2012), was carried out using a polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP). For the analysis, DNA fragments were amplified using GoTaq®G2 Hot Start Polymerase (Promega, USA). About 80 ng of template DNA were added to the Master Mix and filled with nuclease-free water to a target volume of 15 µl. For *GH* polymorphism primers and PCR-RFLP conditions were prepared according to Fontanesi et al. (2012). For SNP within the *GHR* gene, we developed a PCR-RFLP method where for *C* allele enzyme *Hin*fI digest 525 bp amplicon, giving 256, 162 and 107 bp, while for *G* allele, 363 and 162 bp (see Table 1). PCR were carried out using a T100 thermocycler (BioRad, USA) with three steps: initial denaturation at 95°C for 2 min, followed by 34 cycles, denaturation at 95°C for 30 s, annealing at 60°C for *GH* and *GHR* and extension at 72°C for 45 s with final extension cycles at 72°C for 5 min. PCR products were visualised on 1% agarose gel and digested

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Table 1. Summary of single nucleotide polymorphisms, primer pairs designed to sequence the fragments and fragment length used to check their variability in the population

Gene	Polymorphism	Primers (5'-3')	Enzyme	Fragment size (bp)	Source
GH	c.-78 C>T	GTATAGTGGGATGGGGTTGG TTACGCTCCCATTCAGAAGC	*Bsh*1236I	T: 231 C: 169, 62	Fontanesi et al. 2012a
GHR	c.106 G>C	CATTTTTCTCCACCAAGTCCA TTTGGCCTAGCTTAGCCTTT	*Hin*fI	G: 363, 162; C: 256, 162, 107	designed for this experiment

GH = growth hormone, GHR = growth hormone receptor

with *Bsh*1236I (Thermo Scientific, USA) for *GH* c.-78C>T, *Hin*fI (EURx) for *GHR* c.106G>C. Digested PCR products were visualised on 4% agarose gel with 100 bp DNA ladder. Allele frequencies and genotypes are presented in Table 2.

Statistical analysis. Associations between SNPs and quantitative traits were investigated in the analysis of variance using the MIXED procedure of SAS software (Version 9.4, 2014), specifically, the following models:

$$Y_{ijk} = \mu + G_i + S_j + (G \times S)_{ij} + \beta M_{ijk} + e_{ijk} \text{ – growth traits}$$

$$Y_{ijk} = \mu + G_i + S_j + (G \times S)_{ij} + \beta N_{ijk} + e_{ijk} \text{ – slaughter traits}$$

where:

Y_{ijk} = studied traits
μ = overall mean of the trait
G_i = fixed effect of i^{-th} genotype (i = 1, 2, 3)
S_j = fixed effect of j^{-th} gender (j = 1, 2)
$(G \times S)_{ij}$ = interaction between genotype and gender
βM_{ijk} = linear regression of litter size
βN_{ijk} = linear regression of the day of slaughter
e_{ijk} = residual effect

The significance of differences was determined using the Tukey-Kramer test. Haplotype analysis was performed using Haploview software (Barrett et al. 2005).

RESULTS

Frequencies of genotypes and alleles for all the analysed breeds are presented in Table 2. Allele frequencies for *GH* gene were between 0.28 and 0.85 for *C* allele, and from 0.15 to 0.72 for *T* allele. For *GHR*, *G* allele frequencies were between 0.35 and 0.77, and for allele *C* between 0.23 and 0.65. For *GH* c.-78C>T the frequency of *TT* genotypes in NZW × BGG was the highest (54.21%) while in BGG we did not identify this genotype at all and in the TER rabbit population *TT* genotypes were low at 3.1%.

Association analysis. In Table 3, the association analysis between SNPs and traits is shown for *GH* gene, in Table 4 for *GHR*. For *GH* c.-78C>T, we found that in Belgian Giant Grey rabbits, *CT* genotypes had statistically lower birth weight compared to *CC*

Table 2. Frequency of identified single nucleotide polymorphisms in rabbit *GH* and *GHR* genes

Polymorphism	Breed	Allele frequency (%)		Genotypes frequency (%)			*P*-value
		C	*T*	*CC* (*n*)	*CT* (*n*)	*TT* (*n*)	
	TER	0.68	0.32	44.96 (58)	45.74 (59)	9.30 (12)	0.58
GH c.-78C>T	BGG	0.85	0.15	52.46 (42)	13.11 (18)	0	0.17
	NZW × BGG	0.28	0.72	10.53 (20)	35.26 (67)	54.21 (103)	0.07
		G	*C*	*GG* (*n*)	*GC* (*n*)	*CC* (*n*)	
	TER	0.77	0.23	57.36 (74)	39.53 (51)	3.10 (4)	0.17
GHR c.106G>C	BGG	0.35	0.65	16.67 (10)	36.67 (22)	46.67 (28)	0.13
	NZW × BGG	0.63	0.37	41.05 (78)	43.68 (83)	15.26 (29)	0.37

n = number of observations, TER = Termond White, BGG = Belgian Giant Grey, NZW × BGG = crossbreeds of New Zealand White and Belgian Giant Grey, GH = growth hormone, GHR = growth hormone receptor
if $P < 0.05$ – not consistent with Hardy–Weinberg equilibrium

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Table 3. Association analysis between *GH* c.-78 C>T polymorphism and growth and carcass traits (values are means ± standard deviation)

Trait	TER				BGG			NZW × BGG			
	CC (38)	*CT* (39)	*TT* (9)	*P*-value	*CC* (32)	*CT* (8)	*P*-value	*CC* (20)	*CT* (67)	*TT* (103)	*P*-value
BW (g)	69 ± 12	65 ± 8	64 ± 11	0.256	87^{a} ± 14	65^{a} ± 13	0.000	66 ± 14	71^{a} ± 16	65^{a} ± 12	0.043
W5 (g)	856 ± 215	831 ± 170	856 ± 160	0.683	950^{a} ± 160	704^{a} ± 112	0.000	708^{ab} ± 197	861^{a} ± 201	833^{b} ± 168	0.143
W12 (g)	2659 ± 478	2722 ± 300	2775 ± 249	0.116	3324 ± 473	3019 ± 196	0.080	2639 ± 465	2823 ± 372	2740 ± 450	0.161
SW (g)	2677 ± 412	2772 ± 305	2811 ± 239	0.081	3447^{a} ± 439	3069^{a} ± 261	0.02	2725^{a} ± 455	2946^{a} ± 458	2788 ± 487	0.028
HC (g)	1421 ± 248	1488 ± 164	1486 ± 134	0.122	1770^{a} ± 283	1553^{a} ± 104	0.04	1291^{a} ± 258	1535^{ab} ± 281	1427^{b} ± 246	0.015
CC (g)	1370 ± 247	1436 ± 160	1439 ± 128	0.275	1722^{a} ± 263	1495^{a} ± 102	0.02	1256^{a} ± 247	1484^{ab} ± 273	1383^{b} ± 248	0.021
LIV (g)	69 ± 8	73 ± 16	76 ± 15	0.170	96 ± 16	87 ± 14	0.142	77 ± 13	79 ± 25	80 ± 24	0.697
FP (g)	553^{a} ± 98	600 ± 76	614^{a} ± 57	0.022	762 ± 115	680 ± 57	0.088	501^{a} ± 102	596^{ab} ± 110	560^{b} ± 103	0.029
IP (g)	308 ± 75	312 ± 51	309 ± 40	0.971	319 ± 66	274 ± 28	0.076	276 ± 60	320 ± 58	302 ± 59	0.032
HP (g)	509 ± 90	524 ± 49	515 ± 47	0.825	641^{a} ± 93	542^{a} ± 40	0.007	476^{a} ± 95	555^{ab} ± 91	522^{b} ± 89	0.017
DPW (%)	52.9 ± 1.4	53.7 ± 1.9	52.8 ± 1.7	0.056	50.8 ± 2.3	50.7 ± 2.4	0.963	47.2^{ab} ± 2.7	51.6^{a} ± 2.7	51.27^{b} ± 2.6	0.000
DPC (%)	51 ± 1.8	51.8 ± 1.7	51.2 ± 1.7	0.088	49.4 ± 2.6	48.8 ± 2.4	0.553	45.9^{ab} ± 2.4	49.9^{a} ± 2.7	49.39^{b} ± 2.3	0.000
MBF (g)	530^{a} ± 78.5	572 ± 62.7	578^{a} ± 41.4	0.021	747 ± 112	662 ± 54.4	0.051	487^{a} ± 97.7	582^{a} ± 107	546.10 ± 101	0.031
FF (g)	22.7 ± 21.2	28.2 ± 17.9	36.3 ± 26.2	0.119	14.4 ± 9.2	17.3 ± 10	0.47	7.7 ± 10	9.5 ± 10.3	12.05 ± 8.7	0.127
MI (g)	247 ± 54.6	244 ± 35	239 ± 28.1	0.442	259^{a} ± 51.8	220^{b} ± 21.9	0.048	226 ± 47.9	263^{a} ± 51.2	242.2^{a} ± 47.2	0.041
BI (g)	37.9^{a} ± 14	42.1^{a} ± 9.7	38 ± 6.8	0.046	46 ± 9.9	41.8 ± 6.2	0.275	38.1 ± 8.3	42.4 ± 8.4	43.05 ± 9.3	0.725
FI (g)	22.7^{a} ± 12.6	25.3^{b} ± 13.4	32.3^{ab} ± 14.6	0.045	14 ± 8.5	12 ± 5	0.537	10.9^{a} ± 10.2	11.9^{b} ± 7.6	16.22^{ab} ± 9.7	0.026
MH (g)	393 ± 71.5	400 ± 39.1	393.8 ± 39.9	0.416	484^{a} ± 77.8	406^{a} ± 33.8	0.011	366^{a} ± 69.2	435^{ab} ± 72.6	401.55^{b} ± 72.8	0.040
BH (g)	112 ± 21.3	121 ± 14.8	117 ± 15.4	0.352	155^{a} ± 19.4	133^{a} ± 14.1	0.006	106 ± 28.8	112 ± 30.9	111.02 ± 20.3	0.768
FH (g)	4.3 ± 3.7	2.7 ± 3.2	3.9 ± 4.4	0.255	1.96 ± 2.5	2.13 ± 4.4	0.537	0.44^{a} ± 1.3	3.65^{a} ± 3.2	4.63 ± 5.1	0.016

BW = birth weight, W5 = weight at 5 weeks of age, W12 = weight at 12 weeks of age, SW = slaughter weight, HC = hot carcass weight, CC = chilled carcass weight, LIV = weight of liver, FP = fore part weight, IP = intermediate part (loin) weight, HP = hind part weight, DPW = dressing out percentage warm, DPC = dressing out percentage cold, MBF = weight of fore part (meat + bones), FF = dissectible fat in fore part, MI = meat in intermediate part, BI = bones in intermediate part, FI = dissectible fat in intermediate part, MH = meat in hind part, BH = bones in hind part, FH = dissectible fat in hind part, TER = Termond White, BGG = Belgian Giant Grey, NZW × BGG = crossbreeds of New Zealand White and Belgian Giant Grey

$^{a–c}$values within the same trait and polymorphism marked by the same superscript differ at $P < 0.05$

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genotypes. In terms of slaughter weight of the Belgian Giant Grey rabbit, *CC* genotypes were characterised by higher slaughter weight than *CT* (3447 ± 439 g and 3069 ± 261 g, respectively), while in NZW × BGG *CT* genotypes had higher slaughter weight than *CC* (2946 ± 458 g and 2725 ± 455 g, respectively). The analysis of carcass cuts showed that weights of the fore, intermediate, and hind part statistically differed in NZW × BGG – *CT* genotypes had higher weight compared to *CC*. Moreover, *TT* genotypes of Termond White had higher fore part weight compared to *CC* (614 ± 57 g and 553 ± 98 g, respectively) and Belgian Giant Grey had higher hind part weight in *CC* than in *CT* genotypes (641 ± 93 g and 542 ± 40 g, respectively). In NZW × BGG, for meat in intermediate part, dissectible fat in intermediate part, and meat in hind part, the *CT* genotypes were statistically higher than *TT*, and in the weight of fore part (meat + bones) and dissectible fat in hind part, the *CT* genotypes were higher than *CC*. The weight of bones in intermediate part for *CT* genotype (42.1 ± 9.7 g) of Termond White was higher than that of *CC* (37.9 ± 14 g). Dissectible fat weight in intermediate part for *TT* genotype (32.3 ± 14.6 g) was higher than in *CC* and *CT*. In Belgian Giant Grey the weights of meat in hind part and bones in hind part for *CC* genotypes (484 ± 77.8 g and 155 ± 19.4 g, respectively) were higher than those of *CT* genotypes (406 ± 33.8 g and 133 ± 14.1 g, respectively).

For *GHR* c.106G>C in the Termond White population, we used only two genotypes in the analysis: *GG* and *GC*. The *GC* genotypes had higher

Table 4. Association analysis between *GHR* c.106 G>C polymorphism and growth and carcass traits (values are means ± standard deviation)

Trait	TER			BGG				NZW × BGG			
	GG (54)	*GC* (31)	*P*-value	*GG* (6)	*GC* (14)	*CC* (20)	*P*-value	*GG* (78)	*GC* (83)	*CC* (29)	*P*-value
BW (g)	63[a] ± 10	69[a] ± 10	0.007	76 ± 12	80 ± 12	84 ± 22	0.522	68 ± 13	66 ± 15	68 ± 13	0.636
W5 (g)	862 ± 167	860 ± 171	0.927	1078[a] ± 89	920 ± 197	811[a] ± 146	0.018	860 ± 214	829 ± 182	816 ± 193	0.292
W12 (g)	2717 ± 299	2783 ± 303	0.280	3338 ± 337	3307 ± 367	3200 ± 519	0.586	2706 ± 420	2613 ± 441	2620 ± 407	0.379
SW (g)	2762 ± 289	2830 ± 298	0.338	3374 ± 400	3390 ± 444	3357 ± 466	0.868	2752 ± 468	2652 ± 505	2703 ± 471	0.463
HC (g)	1457[a] ± 159	1520[a] ± 161	0.026	1706 ± 258	1739 ± 269	1699 ± 283	0.964	1421 ± 238	1355 ± 268	1374 ± 291	0.366
CC (g)	1410[a] ± 155	1492[a] ± 149	0.031	1634 ± 246	1691 ± 241	1651 ± 278	0.974	1376 ± 241	1311 ± 264	1332 ± 280	0.387
LIV (g)	72 ± 17	75 ± 15	0.833	84 ± 18	95 ± 17	96 ± 13	0.174	77 ± 20	78 ± 21	75 ± 21	0.954
FP (g)	599 ± 77	626 ± 67	0.147	720 ± 115	743 ± 97	745 ± 124	0.822	556 ± 102	531 ± 109	537 ± 120	0.591
IP (g)	304 ± 44	321 ± 52	0.129	293 ± 53	320 ± 73	301 ± 55	0.887	303 ± 60	288 ± 61	291 ± 71	0.234
HP (g)	506[a] ± 52	544[a] ± 43	0.002	621 ± 87	628 ± 80	604 ± 109	0.677	516 ± 88	497 ± 96	502 ± 97	0.498
DPW (%)	52.7[a] ± 1.9	53.7[a] ± 1.7	0.014	50.4 ± 3	51.1 ± 1.5	50.5 ± 2.7	0.985	51.7 ± 2.8	50.8 ± 2	50.5 ± 3.8	0.153
DPC (%)	51[a] ± 1.8	52[a] ± 1.5	0.018	48.3 ± 3	49.9 ± 1.7	49 ± 3.2	0.821	50 ± 2.3	49.1 ± 2	49 ± 3.6	0.196
MBF (g)	568 ± 60.8	590 ± 51	0.126	714 ± 108	725 ± 97.7	731 ± 121	0.903	542 ± 98.1	519 ± 108	524 ± 116	0.471
FF (g)	31 ± 23.6	36.1 ± 26	0.437	6.5[a] ± 10.5	18.6[a] ± 9.5	14.4 ± 8.1	0.045	11 ± 8.4	10.4 ± 8.8	8.4 ± 10.5	0.653
MI (g)	238 ± 31.8	249 ± 35.8	0.152	241 ± 44.7	256 ± 56.2	246 ± 45.7	0.966	245 ± 51.9	233 ± 50.7	234 ± 58.7	0.281
BI (g)	38.5 ± 8.8	40.4 ± 7.9	0.341	48 ± 7.3	47 ± 11.5	42.2 ± 7	0.147	42 ± 8.4	40 ± 9.8	41 ± 10.8	0.304
FI (g)	27.6 ± 14.1	31.4 ± 15.2	0.287	3.5[ab] ± 4.4	17[b] ± 8.5	13.1[a] ± 5.3	0.007	18.1[a] ± 12	14 ± 9.6	11.5[a] ± 7.8	0.014
MH (g)	389[a] ± 43.3	415[a] ± 37.4	0.010	464 ± 77.6	471 ± 68.2	458 ± 87.8	0.792	401 ± 73.9	383 ± 76.7	392 ± 78.5	0.320
BH (g)	113[a] ± 16.3	126[a] ± 14	0.002	157 ± 12.4	154 ± 18.8	144 ± 23	0.267	108 ± 19	105 ± 24.9	101 ± 29.2	0.618
FH (g)	3.8 ± 4.5	3.2 ± 3.4	0.652	0.3 ± 0.5	2.8 ± 3.8	1.8 ± 2.4	0.244	4.3 ± 5.9	5 ± 6	5.8 ± 11	0.561

BW = birth weight, W5 = weight at 5 weeks of age, W12 = weight at 12 weeks of age, SW = slaughter weight, HC = hot carcass weight, CC = chilled carcass weight, LIV = weight of liver, FP = fore part weight, IP = intermediate part (loin) weight, HP = hind part weight, DPW = dressing out percentage warm, DPC = dressing out percentage cold, MBF = weight of fore part (meat + bones), FF = dissectible fat in fore part, MI = meat in intermediate part, BI = bones in intermediate part, FI = dissectible fat in intermediate part, MH = meat in hind part, BH = bones in hind part, FH = dissectible fat in hind part, TER = Termond White, BGG = Belgian Giant Grey, NZW × BGG = crossbreeds of New Zealand White and Belgian Giant Grey

[a,b]values within the same trait and polymorphism marked by the same superscript differ at $P < 0.05$

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hot carcass weight and chilled carcass weight (1520 ± 161 g and 1492 ± 149 g, respectively) than the *GG* genotypes (1457 ± 159 g and 1410 ± 155 g, respectively). Moreover, animals with *GC* genotypes had higher values of hind part weight (544 ± 43 g) and meat in hind part (415 ± 37.4 g) and bones in hind part (126 ± 14 g) compared to *GG* genotypes (506 ± 52 g, 389 ± 43.3 g and 113 ± 16.3 g, respectively). In Belgian Giant Grey rabbit, statistical differences in body weight at 5 weeks of age were found between *GG* genotypes and *CC* genotypes. In *GG* genotypes the weight of dissectible fat in intermediate part (3.5 ± 4.4 g) and in fore part (6.5 ± 10.5 g) was statistically lower compared to *CC* and *GC* genotypes. In the population of NZW × BGG dissectible fat weight in intermediate part was higher in *GG* genotypes (18.1 ± 12 g) than in *CC* genotypes (11.5 ± 7.8 g).

Table 5 shows information about identified haplotypes in all analysed breeds. Because *haplo9* in Termond White and *haplo2* and *haplo3* in BGG were only one observation, we excluded them from further analysis. In our study in the population of Termond White *haplo8* (*TT/GG*) (34%), and in Belgian Giant Grey and NZW × BGG crossbreeds *haplo6* (*TT/GC*) (33% and 32%, respectively) were identified. In Table 6 we document the association analysis of *GH* and *GHR* haplotypes of Belgian Giant Grey, in Table 7 for Termond White rabbits, in Table 8 for NZW × BGG crossbreeds.

Table 5. Sequences and frequencies of defined haplotypes in *GH* and *GHR* genes

Haplotype	Haplotype sequence	Frequencies (%)		
		TER	BGG	NZW × BGG
haplo1	*CC/GG*[1]	4		
haplo2	*CT/GG*	22	3	6
haplo3	*CT/GC*	20	3	14
haplo4	*CC/GC*	5		5
haplo5	CC/CC			3
haplo6	*TT/GC*	13	33	32
haplo7	*TT/CC*		30	7
haplo8	*TT/GG*	34	12	23
haplo9	*CT/CC*	1	18	11

GH = growth hormone, GHR = growth hormone receptor, TER = Termond White, BGG = Belgian Giant Grey, NZW × BGG = crossbreeds of New Zealand White and Belgian Giant Grey

[1]*CC/GG* – for *GH* c.-78C>T genotype is *CC* and for *GHR* c.106G>C genotype is *GG*

DISCUSSION

The association analysis between traits and polymorphisms should contain as much information as

Table 6. Association analysis of *GH* and *GHR* haplotypes identified in Belgian Giant Grey rabbits (values are means ± standard deviation)

Traits	Haplotypes			
	haplo6	*haplo7*	*haplo8*	*haplo9*
BW (g)	81^{ab} ± 13	99^{ab} ± 8	76^{b} ± 11	62^{b} ± 14
W5 (g)	965^{a} ± 178	873^{b} ± 125	1078^{b} ± 88	717^{ab} ± 130
W12 (g)	3345 ± 385	3337 ± 645	3338 ± 336	2995 ± 216
SW (g)	3445 ± 462	3554^{a} ± 464	3374 ± 399	3062^{a} ± 300
HC (g)	1767 ± 284	1802 ± 318	1706 ± 257	1544 ± 119
CC (g)	1722^{a} ± 250	1761^{a} ± 304	1634 ± 246	1486^{ab} ± 116
LIV (g)	97^{ab} ± 17	101 ± 11	177^{b} ± 20	159^{a} ± 8
FP (g)	756 ± 100	788 ± 138	720 ± 115	681 ± 64
IP (g)	327 ± 78	322 ± 58	293 ± 53	271 ± 33
HP (g)	639^{a} ± 83	652^{b} ± 115	621 ± 87	533^{ab} ± 43
DPW (%)	51.1 ± 1.7	50.4 ± 2.8	50.4 ± 3	50.6 ± 2.79
DPC (%)	50 ± 1.8	49.3 ± 3.3	48.3 ± 3	48.7 ± 2.77
MBF (g)	739 ± 98.3	773 ± 136	714 ± 108	667 ± 56.9
FF (g)	17.1 ± 8.7	14.7 ± 8.9	6.5 ± 10.5	14 ± 7.6
MI (g)	263 ± 59	263 ± 48.9	241 ± 44.7	220 ± 25.9
BI (g)	47.6 ± 12.5	43 ± 7.3	48 ± 7.3	41 ± 7
FI (g)	16.8^{a} ± 9.2	15.2^{b} ± 05.4	3.5^{abc} ± 4.4	10^{c} ± 3.4
MH (g)	482^{a} ± 68.9	494^{b} ± 94.2	464 ± 77.6	404^{ab} ± 39.1
BH (g)	155^{a} ± 20.2	155^{c} ± 22.7	157^{b} ± 12.4	129^{abc}± 12.4
FH (g)	2.18 ± 2.8	2.44 ± 2.5	0.25 ± 0.5	0.83 ± 2

BW = birth weight, W5 = weight at 5 weeks of age, W12 = weight at 12 weeks of age, SW = slaughter weight, HC = hot carcass weight, CC = chilled carcass weight, LIV = weight of liver, FP = fore part weight, IP = intermediate part (loin) weight, HP = hind part weight, DPW = dressing out percentage warm, DPC = dressing out percentage cold, MBF = weight of fore part (meat + bones), FF = dissectible fat in fore part, MI = meat in intermediate part, BI = bones in intermediate part, FI = dissectible fat in intermediate part, MH = meat in hind part, BH = bones in hind part, FH = dissectible fat in hind part

[a–c]values within the same trait and polymorphism marked by the same superscript differ at $P < 0.05$

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Table 7. Association analysis of *GH* and *GHR* haplotypes identified in Termond White rabbits (values are means ± standard deviation)

Traits	Haplotypes					
	haplo1	*haplo2*	*haplo3*	*haplo4*	*haplo6*	*haplo8*
BW (g)	72 ± 19	64 ± 10	65 ± 7	64 ± 1	72 ± 10	60 ± 9
W5 (g)	933 ± 311	912 ± 189	808 ± 115	715 ± 127	915 ± 192	826 ± 140
W12 (g)	2748^{b} ± 680	2735 ± 308	2705 ± 242	2373^{c} ± 435	2862^{a} ± 211	2742^{c} ± 258
SW (g)	2702 ± 607	2775 ± 278	2758 ± 254	2508^{a} ± 421	2891^{a} ± 220	2792 ± 254
HC (g)	1456 ± 349	1488^{c} ± 160	1479^{c} ± 136	1292^{abc} ± 273	1561^{a} ± 121	1463 ± 136
CC (g)	1407 ± 345	1441 ± 155	1427 ± 136	1236^{a} ± 274	1510^{ab} ± 115	1415^{b} ± 130
LIV (g)	66^{a} ± 6	67a ± 11	72 ± 16	71 ± 17	79^{a} ± 15	75 ± 16
FP (g)	576 ± 148	594 ± 85	602 ± 60	521^{a} ± 98	628^{a} ± 53	609 ± 61
IP (g)	304 ± 73	323 ± 52	299 ± 44	259 ± 77	330 ± 48	304 ± 35
HP(g)	527 ± 124	523 ± 49	526 ± 43	456^{b} ± 99	552^{ab} ± 35	502^{a} ± 46
DPW (%)	53.8 ± 0.8	53.6 ± 1.8	53.7^{a} ± 2.3	52.3^{a} ± 2.3	54^{b} ± 0.9	52.4^{a} ± 1.8
DPC (%)	51.9^{b} ± 1	51.9 ± 1.7	51.7 ± 1.9	49.1^{ab} ± 2.7	52.3^{a} ± 0.9	50.7 ± 1.7
MBF (g)	545 ± 118.9	569 ± 70.2	578 ± 52.1	509^{a} ± 84.2	587^{a} ± 32.7	574 ± 45.8
FF (g)	31.3 ± 30.9	25.8 ± 14.7	23.6^{b} ± 12.1	12.5^{a} ± 13.4	41.6^{ab} ± 28.1	34.8 ± 26.3
MI (g)	250 ± 58.8	249 ± 35.9	239 ± 31.5	205 ± 53	250 ± 35.3	237 ± 23.9
BI (g)	33^{b} ± 3.5	45.6^{ab} ± 10.2	39.8 ± 9.2	31^{a} ± 1.4	40.5 ± 6	37.2^{a} ± 7.7
FI (g)	20.7^{b} ± 12.4	27.9 ± 14.3	21.1^{a} ± 8.7	22 ± 16	38.8^{ab} ± 12.8	29.8 ± 15.4
MH (g)	408 ± 90.4	405 ± 41.5	399 ± 29.9	354^{b} ± 99	423^{ab} ± 38.6	382^{a} ± 34.9
BH (g)	114 ± 3	116 ± 14.1	124^{a} ± 15	110^{ab} ± 19.6	125^{b} ± 12.9	115 ± 16.7
FH (g)	4.7 ± 5	2.1 ± 3.3	2.9 ± 3.1	2.7 ± 2.3	4.5 ± 3.6	3.8 ± 4.9

BW = birth weight, W5 = weight at 5 weeks of age, W12 = weight at 12 weeks of age, SW = slaughter weight, HC = hot carcass weight, CC = chilled carcass weight, LIV = weight of liver, FP = fore part weight, IP = intermediate part (loin) weight, HP = hind part weight, DPW = dressing out percentage warm, DPC = dressing out percentage cold, MBF = weight of fore part (meat + bones), FF = dissectible fat in fore part, MI = meat in intermediate part, BI = bones in intermediate part, FI = dissectible fat in intermediate part, MH = meat in hind part, BH = bones in hind part, FH = dissectible fat in hind part

$^{a–c}$values within the same trait and polymorphism marked by the same superscript differ at $P < 0.05$

possible about the influence on the analysed traits in different breeds. Our experiments were conducted in order to analyse the influence of SNPs within *GH*, *GHR* genes on growth and carcass traits of medium-sized breed of broiler rabbits – Termond White, large breed – Belgian Giant Grey, and the crossbreed between New Zealand White and Belgian Giant Grey. In beef cattle, Gill et al. (2010) found an association between *GH* and for instance the eye muscle length. According to Fontanesi et al. (2012), *GH* genotype *CT* showed significantly higher body weight at 70 days of age (2778.83 ± 31.76 g) compared to *CC* and *TT* (2720.04 ± 33.91 g and 2693.94 ± 36.18 g, respectively). In our research results for *GH* c.-78C>T SNP seem to be most interesting. In Belgian Giant Grey, *CC* genotypes had statistically higher birth weight, weight at 5 weeks of age and slaughter weight compared with *CT*. Results for the crossbreed NZW × BGG were consistent with findings reported by Fontanesi et al. (2012), namely, the animals of *CT* genotype had significantly higher slaughter weight compared to *TT*. Similar significance was also found for birth weight. In Termond White, the weight of fore part (meat + bones) significantly differed as well as the weight of dissectible bones and fat in intermediate part. We did not find any associations between growth traits and other carcass traits. For Belgian Giant Grey and NZW × BGG, statistically significant differences were found between hot carcass weight and chilled carcass weight. The weights of the fore, intermediate (IP), and hind (HP) part differed statistically in NZW × BGG, while in Belgian Giant Grey a statistically significant difference in the hind part occurred only between *CT* and

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Table 8. Association analysis of *GH* and *GHR* haplotypes identified in New Zealand White × Belgian Giant Grey crossbred rabbits (values are means ± standard deviation)

Trait	Haplotypes							
	haplo2	*haplo3*	*haplo4*	*haplo5*	*haplo6*	*haplo7*	*haplo8*	*haplo9*
BW (g)	69^{ab} ± 5	78 ± 21	73 ± 18	59^{ab} ± 3	63 ± 12	70 ± 9	67^{b} ± 9	69 ± 18
W5 (g)	945 ± 287	889^{a} ± 199	816^{c} ± 247	573^{abd} ± 93	808^{b} ± 189	849 ± 156	819^{c} ± 135	829^{d} ± 171
W12 (g)	2935^{a} ± 98	2798 ± 428	2787 ± 551	2305^{ab} ± 135	2622 ± 442	2647 ± 540	2828 ± 441	2733^{b} ± 307
SW (g)	3148^{ab} ± 100	2896 ± 531	2825 ± 517	2402^{a} ± 85	2676^{b} ± 499	2738 ± 628	2851 ± 447	2800 ± 401
HC (g)	1645^{a} ± 107	1473^{b} ± 308	1384 ± 296	$1076^{a–e}$ ± 32	1386^{bc} ± 243	1378 ± 372	1447^{d} ± 209	1478^{e} ± 234
CC (g)	1600^{ab} ± 106	1420 ± 296	1344 ± 291	1056^{acd} ± 34	1340^{b} ± 247	1341 ± 364	1403^{c} ± 216	1424^{d} ± 223
LIV (g)	86 ± 25	81 ± 2	90 ± 21	64 ± 2	84 ± 25	19 ± 5	19 ± 5	18 ± 3
FP (g)	648^{ab} ± 45	577^{b} ± 125	552 ± 143	420^{abcd} ± 21	545^{b} ± 99	537 ± 156	565^{c} ± 97	569^{d} ± 100
IP (g)	354^{ab} ± 35	308 ± 69	299^{c} ± 52	218^{acde} ± 16	291^{b} ± 58	298 ± 90	307^{d} ± 51	319^{e} ± 57
HP (g)	604^{ab} ± 65	535 ± 112	493 ± 101	407^{ac} ± 29	507^{b} ± 89	505 ± 123	529 ± 76	536^{c} ± 81
DPW (%)	52.2^{a} ± 2.7	50.7^{c} ± 2.5	48.8 ± 3.2	44.8^{acd} ± 0.6	51.3^{d} ± 2.5	49.9 ± 3.7	50.9 ± 2.9	52.8 ± 3.1
DPC (%)	50.8^{a} ± 2.8	48.9^{c} ± 2.6	47.4 ± 3.2	44^{acd} ± 0.4	49.5^{d} ± 2.1	48.6 ± 3.9	49.3 ± 2.4	50.9 ± 3.17
MBF (g)	627^{a} ± 39.6	568^{b} ± 120	534 ± 133.7	$409^{a–e}$ ± 20.4	536^{c} ± 100	525 ± 152	556^{d} ± 96	551^{e} ± 99.4
FF (g)	16.4^{ab} ± 6.1	5.9^{a} ± 7.8	17.2 ± 13.1	0.00^{bc} ± 0.0	11.2 ± 5.8	9.4 ± 8	13.6^{c} ± 10.1	10.9 ± 12.2
MI (g)	296^{abc} ± 33.1	252 ± 56.8	245 ± 48.3	183^{bde} ± 15.2	236^{c} ± 48.7	238 ± 69.1	244^{ad} ± 41.4	261^{e} ± 50.4
BI (g)	41.6^{a} ± 5.6	42.4 ± 9.3	37.6 ± 5.8	31^{ab} ± 5.6	42 ± 9.1	45 ± 12.3	44.7^{b} ± 8.5	42.4 ± 10.3
FI (g)	15.8^{a} ± 6.8	11.9 ± 8.3	16 ± 6^{b}.8	2.7^{abc} ± 4.6	14.6 ± 10.2	13.6 ± 10.6	16.8^{c} ± 8.5	12.5 ± 7.2
MH (g)	488^{abc} ± 67.6	411 ± 85.7	381 ± 9.5	320^{bd} ± 28.6	391^{c} ± 75.4	388 ± 99	413^{a} ± 61.8	422^{d} ± 63.4
BH (g)	110^{a} ± 5.5	117 ± 31	107 ± 23.5	85^{a} ± 15.8	110 ± 21	109 ± 22	113 ± 19	99 ± 40.9
FH (g)	4.6 ± 10.3	3.1 ± 5.1	0.8 ± 1.8	0.0 ± 0.0	4.9 ± 4	4.7 ± 4.8	5.2 ± 7	7.7 ± 17.2

BW = birth weight, W5 = weight at 5 weeks of age, W12 = weight at 12 weeks of age, SW = slaughter weight, HC = hot carcass weight, CC = chilled carcass weight, LIV = weight of liver, FP = fore part weight, IP = intermediate part (loin) weight, HP = hind part weight, DPW = dressing out percentage warm, DPC = dressing out percentage cold, MBF = weight of fore part (meat + bones), FF = dissectible fat in fore part, MI = meat in intermediate part, BI = bones in intermediate part, FI = dissectible fat in intermediate part, MH = meat in hind part, BH = bones in hind part, FH = dissectible fat in hind part

$^{a–e}$values within the same trait and polymorphism marked by the same superscript differ at $P < 0.05$

TT genotypes. Hot and cold dressing percentage exhibited statistically significant differences in NZW × BGG. Moreover, in NZW × BGG, the weights of dissectible meat in *CT* genotypes were statistically higher compared to *TT* genotypes for intermediate part and for hind part. In the Belgian Giant Grey population, *CC* genotypes had higher meat weight in intermediate part and in hind part compared to *CT* genotypes. These results can confirm the hypothesis that the *GH* c.-78 C>T SNP can be used as a marker for growth and carcass parameters in rabbits. We noticed that only for crossbreeds where one of the components was New Zealand White, our results are in agreement with Fontanesi et al. (2012), who used commercial rabbits that were mostly selected from New Zealand White. In the other breed – Belgian Giant Grey – *CC* and *TT* genotypes had the highest values of growth and carcass traits. In NZW × BGG, the weight of dissectible fat showed statistically significant differences between *CT* and *TT* genotypes in intermediate part, and between *CT* and *CC* genotypes in hind part. Moreover, many authors reported correlations between *GH* polymorphisms and fat-related traits in farm animals (Franco et al. 2005; Barendse et al. 2006; Bahrami et al. 2014), therefore it should also be taken into consideration when growth traits are the main selection criteria.

For *GHR* c.106 G>C, Zhang et al. (2012) reported lack of correlation between genotypes for 70-day weight, however, for 84-day weight significant differences were found between *GG*–*GC* (2613 ± 20 g and 2525 ± 24 g, respectively) and *GC*–*CC*

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(2525 ± 24 g and 2632 ± 43 g, respectively), while in the panel of meat male line Fontanesi et al. (2016) found in 70-day weight that *GG* genotypes had higher weight at this age. We did not find any correlation with W12 body weight in any of the analysed breeds. For Termond White we revealed that *GC* genotypes had higher hot carcass weight and chilled carcass weight compared to *GG* genotypes. Interestingly, for Termond White *CG* genotypes, the weight of meat in hind part and bones in hind part was statistically higher than in *GG* genotypes. Therefore, the hind part weight of *CG* genotypes was statistically higher compared to *GG*. Hot and cold dressing percentage was found to statistically differ between *CG* genotypes and *GG* genotypes. In Belgian Giant Grey, dissectible fat weight in the fore part and in the intermediate part for *GG* genotypes was statistically lower compared to *CG*. While their crossbreed NZW × BGG – *GG* genotypes had higher dissectible fat weight in the intermediate part compared to *CC*.

For *GH* c.-78 C>T Fontanesi et al. (2012) stated that *CT* genotypes had the highest final weight while for *GHR* c.106 G>C Zhang et al. (2012) found that 84-day weight, eviscerated weight, semi-eviscerated weight, eviscerated slaughter rate, and semi-eviscerated slaughter weight were the highest in *CC* genotypes. In our study the highest slaughter weight was found in *haplo7* (*TT/CC*) for Belgian Giant Grey (Table 6), *haplo6* (*TT/GC*) for Termond White (Table 7) and *haplo2* (*CT/GG*) for NZW × BGG crossbreeds (Table 8) and those values differed statistically. Those results compared with data presented in Tables 3 and 4 suggest that using additional molecular markers can lead to an improvement in growth performance (Fontanesi et al. 2012). According to Fontanesi et al. (2012) and Zhang et al. (2012), the *CT/GG* (*haplo2* in the present study) haplotype should be the most favourable. We confirm this hypothesis in the population of NZW × BGG crossbred rabbits. Slaughter weight, weight of meat in intermediate part and in hind part were the highest for *haplo2*, therefore selection based on SNPs identified within different genes may increase selection efficiency.

CONCLUSION

To conclude, the performed analyses showed that *GH*1 c.78 C>T, *GHR* c.106 G>C polymorphisms seem to constitute good markers for growth and carcass traits.

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