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POLYMORPHISMS IN CODING AND NON-CODING REGIONS OF RABBIT (*ORYCTOLAGUS CUNICULUS*) MYOGENIN (*MyoG*) GENE

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Abstract: In animal breeding, selection based on growth is very often used, as this trait affects the profitability of animal production. Identification of polymorphisms within the genes affecting the growth process seems to be very important. Therefore, we decided to analyse rabbit myogenin (*MyoG* gene) for potential polymorphic sites and their association with growth and carcass traits in Termond White (TER), Belgian Giant Grey (BGG) and crossbred New Zealand White×Belgian Giant Grey (NZW×BGG) rabbits. We found three single nucleotide polymorphisms (SNPs) – in 5' upstream sequence g.68679476 C>T, in exon 1 – silent mutation g.68680096 T>C and g.68680097 G>A resulting in change of GTG triplet (valine) into ATG triplet (methionine). Association analysis showed that GG genotype weaning weight was statistically higher compared to GA in TER population (P=0.005), and that the hind parts for GG genotypes were heavier compared to those of GA (P=0.024), but association analysis of dissectible parts showed this was caused by higher bone weight (P=0.015). For g.68679476 C>T in NZW×BGG population, the CC genotypes for fore (678±35) and hind part (615±29) weights were heavier compared to CT (588±16 and 549±13, respectively); moreover, association analysis of dissectible parts showed that weight of dissectible meat in hind part. Unfortunately, we did not find similar associations for other analysed breeds. For g.68679476 C>T in NZWxBGG *musculus longissimus lumborum* pH leg after 24 h chilling (pH24L) were statistically lower for CC genotypes compared to CT (P=0.027). For g.68680097 G>A in Termond White population L* value on the hind leg after 24 h chilling (L*24H) was higher for GA genotypes compared to GG (P=0.03), while for g.68679476 C>T for *musculus longissimus lumborum* L* value after 24 h (L*24L) CC genotypes had higher value compared to CT (P=0.016) in BGG population. Moreover, in BGG population CT genotypes had higher weaning weight compared to CC (P=0.018). Our results show that SNPs within the *MyoG* gene may influence growth traits in some rabbit breeds, but the evolutionary conserved sequence may not be favourable for changes within coding sequences. For a better understanding thereof, additional analysis is required.

Key Words: rabbits, myogenin, single nucleotide polymorphism, growth traits, slaughter traits.

INTRODUCTION

Myogenin (myogenic factor 4; *MyoG*) is a muscle-specific basic-helix-loop-helix (bHLH) transcription factor, encoded by *MyoG* gene. During the development of an organism, proper growth is very important, which is linked with the number of myofibres (te Pas *et al.*, 2000). This occurs during prenatal or early postnatal development of muscle fibres. The *MyoD* genes family is involved in this process. In addition to *MyoG*, other members of this family include myogenic factor 5 (*MYF5*) and myogenic factor 6 (*MYF6*). This family belongs to the myogenic regulatory factors (MRFs). The central position of *MyoG* amongst the *MyoD* family is due to its being expressed in all myogenic cell lines (Anton *et al.*, 2002). *MyoG* is considered one of the expression control genes, encoding proteins responsible for the formation or apoptosis of muscle fibres. In mice, animals without both copies of this gene die in perinatal period due to the lack of mature secondary skeletal muscle fibres in their body. Hasty *et al.* (1993) generated mouse targeted

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mutation within myogenin gene, which causes severe reduction of all skeletal muscles and death after birth. Thus, it was considered that mutations within the myogenin gene might be associated with meat content in livestock.

Rabbit *MyoG* gene (ENSOCUG00000011548) is located on chromosome 16. It consists of three exons coding 225 aa protein. Because previous reports stated that myogenin is essential for functional skeletal muscles development (Hasty *et al.*, 1993), we decided to analyse coding and non-coding regions of rabbit *MyoG* gene for polymorphisms and their potential influence on growth traits in Termond White, Belgian Giant Grey and crossbreed rabbits.

MATERIALS AND METHODS

Animals

The experiment was conducted under standardised conditions at the Experimental Station of the Department of Genetics and Animal Breeding, University of Agriculture in Krakow. In the present study, we analysed data from 380 animals: 130 Termond White (TER); 60 Belgian Giant Grey (Flemish Giant) rabbits (BGG); and 190 crossbreed of the F2 generation of New Zealand White×Belgian Giant Grey (NZW×BGG). The crossbreed population is part of an experiment conducted at the Department of Genetics and Animal Breeding focused on the possibility of increasing the slaughter weight of NZW rabbits by mating them with a larger breed (BGG). Termond White rabbits are a medium sized breed used in meat industry as pure breed or crossbreed animal components. Pure breed animals were under the control of the National Centre for Animal Breeding and for further breeding only animals with highest score were kept. Crossbreed population were derived from 48 litters and for the experiment we chose a subpopulation from the animals with the highest, medium and lowest slaughter weight. The animals were kept in a heated room, furnished with water supply (nipple drinkers), lighting (14L:10D), and exhaust ventilation. Water and feed were available *ad libitum*; the pelleted commercial diet contained 15% crude protein, 16.1% crude fibre and 3.5% crude fat.

Carcass traits

The rabbits were weaned at 5th week of life and slaughtered at week 12 (84th day BW). After 24 h fasting, the slaughter body weight (SW) was recorded and the animals were subsequently slaughtered. The experiment was conducted under a permit from the Local Ethics Commission (agreement no 267/2018). The rabbits were stunned, immediately bled, pelted and eviscerated. After slaughter, the hot carcass weight (HCW) was recorded, and after 24 h storage at 4°C, the chilled carcass weight (CCW) was recorded. The fore part (FP), the intermediate part (IP) and the hind part (HP) were weighed and dissected. The fore part meat (MF), the fore part bone (BF), the fore part dissectible fat (FF), the intermediate part meat (MI), the intermediate part bone (BI), the intermediate part dissectible fat (FI), the hind part meat (MH), the hind part bone (BH) and the hind part dissectible fat (FH) were weighed. Dressing-out percentages (%) were calculated – both dressing-out percentage hot (DPH)=[HC/SW]×100 and dressing-out percentage chilled (DPC)=[CC/SW]×100.

Colour and pH measurement

The lightness [L*], redness [a*] and yellowness [b*] of meat were determined using Konica Minolta CM – 600d spectrophotometer. The pH values in meat were determined using Consort C561 pH-metre. The colouring and pH were recorded 45 min after slaughter and 24 h after chilling on *musculus longissimus lumborum* (loin) and *musculus biceps femoris* (hind leg).

Blood collection and DNA extraction

DNA was extracted using a GeneMATRIX kit (EURx) from 300 µL of blood collected after slaughter into tubes containing EDTA.

Primer design and sequencing

Primers for sequencing were designed in the Primer3 program, using the rabbit *MYOG* (Figure 1) sequence ENSOCUT00000011544.4, and genome assembly OryCun 2.0 (Carneiro *et al.*, 2014) available in the ENSEMBL

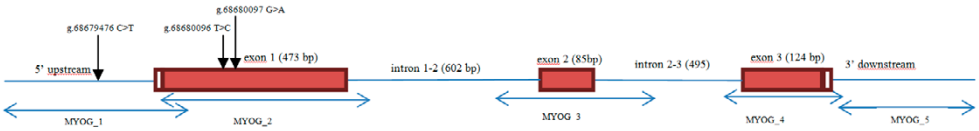

Figure 1: Rabbit *MyoG* gene structure. Boxes corresponds to exons, coloured part of exons corresponds to coding sequences while blank part corresponds to untranslated region. MYOG_1 to MYOG_5 corresponds to different amplicons studied.

database. Primers were designed to amplify part of 5' upstream sequence, exon 1, part of intron 1-2, exon 2, part of intron 2-3, exon 3 and part of 3' downstream sequence (Table 1). DNA samples from 20 random rabbits representing the highest and the lowest slaughter weight representing all analysed breeds were sequenced. Polymerase chain reaction (PCR) products were purified using Exo-BAP (EURx); PCR sequencing was performed for forward and reverse strands with a BigDye terminator v3.1 sequencing kit (Applied Biosystems) on an ABI 3500xl automatic sequencer (Applied Biosystems).

Sequence analysis and genotyping

All sequences were visually inspected in FinchTV. BLAST tool was used to confirm obtained sequences. Quality of the sequences was inspected using CodonCode Aligner (CodonCode Corporation, www.codoncode.com). Single nucleotide polymorphisms (SNPs) were identified by aligning reference sequence of OryCun 2.0 and aligned sequencing readings in MEGA6 (Tamura *et al.* 2013). The identified SNPs were analysed using PCR-HRM (High Resolution Melt) on LightCycler 96 (Roche). Fifteen ng of DNA was added to 2.6 µL H_20; 10 µL of MasterMix 2xconc; 2.4 µL of $MgCl_2$, 25 nM; 1 µL of Primer mix, 20×conc for final volume of 20 µL and thermal profile: pre-incubation 95°C for 600 s, followed by 50 cycles of 3-step incubation: 95°C for 10 s, 60°C for 15 s, and 72°C for 20 s, followed by high resolution melting (95°C for 60 s, 40°C for 60 s, 65°C for 1 s and 97°C for 1 s). To confirm genotypes from PCR-HRM, five samples representing each group were sequenced.

Statistical analysis

The chi-square test for Hardy-Weinberg equilibrium was applied to assess deviation of the number of observed *vs.* expected genotypes. Genotype distribution was tested for Hardy-Weinberg equilibrium (P>0.05).

Table 1: Primer pairs designed for sequencing and High Resolution Melt (HRM) analysis.

	Primers (5' – 3')	lenght (bp)	Ta	Method
MYOG_1	F:GGTCCTTCCTATCCCTGGAC R:GGACGGGCAGGTAGTTCTC	643	58	Sequencing of part of 5' upstream, 5'UTR and part of exon 1
MYOG_2	F:GCTTCTACGACGGGGAGAAC R:GACAGCCCAGCTCTGATGA	519	58	Sequencing of part of exon 1 and part of intron 1_2
MYOG_3	F:GGGCACTGGCTGTTAGGAAT R:GTTGCAGCACTGACTCAGCA	523	58	Sequencing of part of intron 1_2, exon 2 and intron 2_3
MYOG_4	F:CCTTCTGCCCTGGAGACC R:AGGAAGAGACCGGAACAGGA	564	58	Sequencing of part of intron 2_3, exon 3 (whole coding part and part of 3' downstream sequence)
MYOG_5	F:CAACACACAGCCTCTAATTCCA R:ACATCATCATGGACGCCTTT	458	58	Sequencing part of 3' downstream sequence
g.68679476 C>T	F:GGAGGTGGGTGAGCTACTCTT R:CCCCTCTCATCTGCTCCTTT	126	60	PCR-HRM
g.68680097 G>A	F:GCGTGCAAGGTGTGTAAGAG R:CGAAGGCCTCGTTCACTTT	78	60	PCR-HRM

Analysis of variance using the MIXED procedure of SAS (2014) was performed to evaluate the effect of genotype within breed. The following linear model was used:

$$Y_{ij}=\mu+G_i+\beta M_{ij}+\varepsilon_{ij}$$

where: Y_{ij}, analysed traits; μ, overall mean; G_i, effect of i-th genotype (i=1, 2); βM_{ij}, linear regression of litter size (from 3 to 13 individuals); ε_{ij}, residual effect. The significance of differences was determined by the Tukey-Kramer test. The effect of sex was non-significant (P>0.05), so we omitted this factor in the linear model.

Bioinformatics analysis

Estimation of the likelihood of SNP impact on protein was analysed in PANTHER (Tang and Thomas, 2016), using cSNP PANTHER-PSEP (position-specific evolutionary preservation). The latter measures the length of time (in millions of years) that a position in current protein has been preserved by tracing back to its reconstructed direct ancestors. The longer a position has been preserved, the more likely it is to produce a deleterious effect. SIFT (Sorting Intolerant from Tolerant) classifies an amino acid change as tolerated or deleterious to protein function. SIFT takes into account protein conservation with homologous sequences and the severity of the amino acid change (Vaser *et al.*, 2016)

RESULTS

Sequence analysis revealed three SNPs – in 5' upstream sequence (g.68679476 C>T, ss2095231721) and two localised in exon 1 (g.68680096 T>C, ss2095231722 and g.68680097 G>A, ss2095231723). The latest one resulted in the change of GTG triplet (valine) into ATG triplet (methionine) in the protein sequence (V80M substitution, Figure 2). The g.68679476 C>T and g.68680097 G>A polymorphisms were evaluated using PCR-HRM methods (Table 1). Table 2 presents allele and genotype frequencies. For statistical analysis, we found that for g.68679476 C>T linear regressions of litter size were significant for NZWxBGG, while for g.68680097 G>A regressions for litter size were significant for NZWxBGG and TER population. Table 3 presents traits for which statistical differences were found.

For g.68679476 C>T in Belgian Giant Grey population (statistical information available at Supplementary Tables 1 and 2), CT genotypes had statistically higher fore fat weight (FF) and L* measured on loin after 24 h (16.0±2.0 and 60.1±0.7 g, respectively) compared to TT genotypes (9±3 and 57±1 g, respectively). In NZW×BGG population, CC

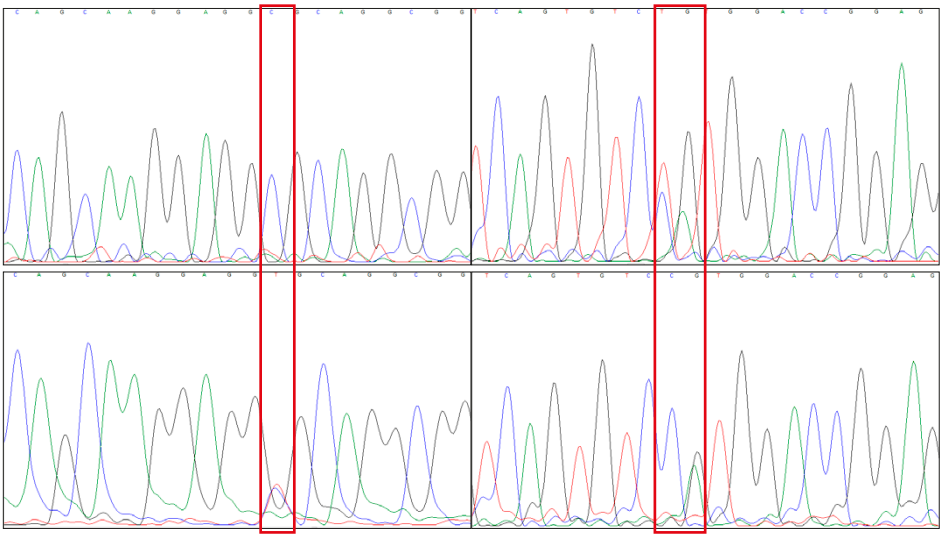

Figure 2: Polymorphisms found with rabbit *MyoG* gene.

genotypes showed statistically higher weight of fore part (FP) and hind part (HP) (678±35 and 615±29 g, respectively) compared to CT genotypes (588±16 and 549±13 g, respectively). Association analysis of dissectible parts showed that weight of meat and bone in fore part (MBF), meat weight (MH) and fat weight (FH) in hind part were heavier for CC genotypes (656±34, 476±25, 7±1 g, respectively) compared to CT genotypes (566±16, 416±12, 2±1 g, respectively). Another important meat quality trait for which significant differences were reported is pH measured on *musculus longissimus lumborum* after 24 h chilling – for CC genotypes (5.5±0.1), the values were lower compared to CT (5.7±0.1) (Table 3). For g.68680097 G>A in NZW×BGG breed (statistical information available at Supplementary Table 3 and 4), the GA genotypes had statistically higher b* measured on hind leg after 24 h (4.7±0.4) compared to GG genotypes (3.5±0.2).

For g.68680097 G>A, in Termond White rabbits we found higher weight in the 5th week (W5) for GG genotypes compared to GA (886±16 and 817±18 g, respectively). Moreover, hind part (HP) weights for GG genotypes (526±8 g) were heavier compared to GA (496±9 g). However, we did not find significant differences for dissectible meat weight in hind part (supplementary Table 2), but for bone weight (BH), where GG genotypes (122±3 g) had heavier dissectible bones in hind leg compared to GA (111±3 g). Also in TER population we found L* value measured on *musculus biceps femoris* after 24 h chilling to be lower for GG genotypes (54.5±0.4) compared to GA (55.8±0.4) (Table 3).

DISCUSSION

Growth traits are crucial in animal husbandry (pigs, chicken, rabbits). Many studies have been carried out to identify polymorphisms within myogenin (*MyoG*) gene that may be used in the selection process. In pigs, Soumillion *et al.* 1997 reported significant effect of genotypes deducted from *MspI* digestion site within *MyoG* gene on birth weight, growth rate and lean weight in pigs. Those results were further confirmed by analysis of polymorphisms in *MyoG* gene (Kapelanski *et al.*, 2005, Anton *et al.*, 2006). Analyses of this gene were also performed within the *Bovidea* family, but with contradictory results. Some authors reported association with some important economically relevant body measurement (Bhuiyan *et al.*, 2009, Xue *et al.*, 2011, Sun and Han, 2017), while others reported lack of association between SNPs and growth traits (Abu El-Magd *et al.*, 2015).

As mentioned, *MyoG* factor plays an important role in myoblast differentiation to myotubes. Absence of this protein causes severe deficiency of muscle fibres (Hasty *et al.*, 1993). The combination of these results suggests that *MyoG* can directly or indirectly mediate livestock growth. As reported by Blasco *et al.* (2018), the selection process for growth traits was successful, but with some negative effects, e.g. leading to poorer carcass yield. It therefore seems reasonable to analyse sequences of candidate genes for growth trace, and to analyse the identified SNPs for their influence on growth and carcass traits, which may help in selection (Ramírez *et al.*, 2004). In our experiment we used large breed (BGG) rabbits, which to the best of our knowledge were not under selection process (Pałka *et al.*, 2018), a commonly used medium breed (TER) known for their high growing rate, and NZW×BGG crossbreeds. Results obtained for all genetic groups showed no association with growth and slaughter weight, although in NZW×BGG population we

Table 2: Frequency of identified single nucleotide polymorphisms in rabbit *MyoG* gene.

		Allele frequency %		Genotypes frequency %			
Polymorphism		C	T	CC (n)	CT (n)	TT (n)	*P*-value
g.68679476 C>T	TER	50	50	0	100 (130)	0	
	BGG	16	84	1.67 (1)	28.33 (17)	70 (42)	0.625
	NZWxBGG	50	50	24 (46)	52 (99)	24 (45)	0.66
g.68680097G>A		G	A	GG (n)	GA (n)	AA (n)	
	TER	87	13	73 (95)	27 (35)	0	0.076
	BGG	83	17	67 (40)	33 (20)	0	0.121
	NZW×BGG	84	16	71 (135)	26 (50)	3 (5)	0.885

n: number of observations; TER, Termond White; BGG, Belgian Giant Grey; NZW×BGG, crossbreeds of New Zealand White and Belgian Giant Grey.
If *P*-value<0.05, not consistent with Hardy-Weinberg equilibrium.

Table 3: Association analysis between g.68679476 C>T and g.68680097 G>A polymorphism and rabbit carcass traits within each breed studied.

	TER			BGG			NZWx BGG					
	g.68680097 G>A			g.68679476 C>T			g.68679476 C>T			g.68680097 G>A		
Trait[1]	GG	GA	*P*-value	CT	TT	*P*-value	CC	CT	*P*-value	GG	GA	*P*-value
W5(g)	886^{b}±16[2]	817^{a}±18	0.005	945^{b}±53	809^{a}±19	0.018						
FP(g)							678^{b}±35	588^{a}±16	0.026			
HP(g)	526^{b}±8	496^{a}±9	0.024				615^{b}±29	549^{a}±13	0.042			
MBF(g)							656^{b}±34	566^{a}±16	0.021			
FF(g)				16^{b}±2	9^{a}±3	0.045						
BI(g)	43^{b}±2	36^{a}±2	0.007				49^{b}±3	41^{a}±1	0.033			
MH(g)							476^{b}±25	416^{a}±12	0.036			
BH(g)	122^{b}±3	111^{a}±3	0.015									
FH(g)							7^{b}±1	2^{a}±1	<0.001			
pH24L							5.5^{a} ±0.1	5.7^{b}±0.1	0.027			
L*24H	54.5^{a}±0.4	55.8^{b}±0.4	0.03									
b*24H							2.6^{a}±0.4	3.9^{b}±0.2	0.008	3.5^{a}±0.2	4.7^{b}±0.4	0.007
b*45L							-2.3^{b}±0.6	-2.3^{a}±0.3	0.031			
L*24L				60.1^{b}±0.7	57.0^{a}±1.0	0.016						
b*24L							0.7^{a}±0.7	2.9^{b}±0.3	0.008			

[1]W5(g), weight at 5 week of age(g); FP, fore part weight (g); HP, hind part weight (g); MBF(g), weight of fore part (meat+bones) (g); FF, dissectible fat in fore part (g); BI, bones in intermediate part (g); MH, meat in hind part (g); BH, bones in hind part (g); FH, dissectible fat in hind part (g). H, hind leg (*m. biceps femoris*); L, *m. longissimus lumborum.* 45, measured 45 min after slaughter; 24, measured after 24 h chilling.

[2]least square means are reported with their standard error.

[3]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 different letters differ significantly at $P<0.05$.

found an association with dissectible meat weight in hind leg. This finding may be interesting as hind leg with loin is considered the most valuable part of the rabbit carcass. However, the overall effect of identified SNPs is very weak. Interesting findings were for g.68679476 C>T in TER population, where all animals were heterozygotes (CT). This may be explained by the fact that for breeding, and therefore as parents for the experimental TER population, the animals kept were those with the highest scores from the National Centre for Animal Breeding controls. We hypothesise that the highest score (where weight is also included) may be associated with heterozygosity in g.68679476 C>T. In a commercial swine study, Kapelanski *et al.* (2005) and Anton *et al.* (2006) found heterozygote in *MyoG/MspI* restriction site to have the highest frequencies. We hypothesise that the selection process could favour heterozygosity in the *MyoG/MspI* restriction site in the swine population and that a similar process could be present in Termond White rabbits.

The most interesting finding may be SNPs within the coding region, as they may alter translation and code new protein. We analysed g.68680097 G>A substitution using a bioinformatics tool. cSNP PANTHER-PSEP analysis showed that V80M substitution is possibly damaging. Polyphen-2 analysis showed that mutation is predicted to be probably damaging. Another *in silico* analysis in SIFT showed that this substitution can be considered as tolerant with a score of 0.47 (damaging score is ≤0.05). Similar substitution was found in human *MyoG* protein sequence: V80M and PolyPhen-2 analysis predict this mutation to be benign. On the corresponding position in cattle, substitution V80G were identified and are also counted as tolerated (rs475006823 from ENSEMBL database). Results from bioinformatics analyses from different species are contradictory for V80 position in the amino acids chain. One possible explanation may be that both valine and methionine amino acids have a hydrophobic side chain, so this substitution may have limited influence on protein function. It can be concluded that g.68680097 G>A may be a conservative missense variant. This polymorphism is located close to MyoDbHLHdomain (Figure 3). The DNA-binding domain conserved motif known as myogenic recognition motif (MRM) has been identified and described as essential for activation of muscle-specific transcription (Olson, 1993). Multiple sequence alignment in Clustal Omega with myogenin sequence, V80M mutant and sequence of basic/helix-loop-helix/leucine zipper (b/HLH/Z) (PDB: 1AN4_A) showed that substitution occurs at the beginning of this conserved motif (Figure 3). In addition to *MyoG,* other muscle-specific genes are *MyoD*, *myf5* and *myf6.* This group

of genes encode bHLH proteins known for their role in myogenesis. Moreover, the evolutionary conservative structure of this protein transcription factor had been mentioned (Tseng *et al.*,1999). The mouse model showed that *MyoG* null mice had drastic reduction of skeletal muscle fibres, which led to perinatal lethality. Surprisingly, this was not reported for null *MyoD*, *myf5* and *myf6* mice (Hasty *et al.*, 1993). High similarity was reported between vertebrates (4 myogenic bHLH proteins) and invertebrates (one bHLH protein). It may be concluded that the mechanism regulating muscle gene expression is extremely old and thus highly conserved (Olson and Klein, 1994). Therefore, mutations disrupting bHLH structure may be lethal and only benign ones may be conserved in population. It must be stated that Kuang *et al.*, (2014) found that *MyoG* mRNA expression was statistically higher in Zika rabbits (high yield meat hybrid) compared to Californian (meat hybrid), therefore selection for growth rate may positively influence expression, or there may be other SNPs that can alter expression rate.

For *MyoG* gene, results presented for other livestock species showed that SNPs might be useful for the selection. Reports have shown that SNPs within this gene in swine (Kapelański *et al.*, 2005; Kim *et al.*, 2009), cattle (Xue *et al.*, 2011), sheep (Sun and Han, 2017) and chickens (Wei *et al.*, 2016) influence growth traits. However most of the SNPs analysed were synonymous, intronic or 3' region. Only Sun and Han (2017), in sheep, reported Gly37Arg substitution which is located outside bHLH. Association with meat weight in hind part and meat and bone weight in fore part of carcass SNP identified in 5' upstream sequence was found only in the NZW x BGG population, while for SNP within exon the hind part weight differed significantly only in the TER population, but it appears to be associated with dissectible bone weight, not dissectible meat weight (Table 3). These outcomes may explain our weak association results with growth traits in rabbits.

In addition to high yield, meat quality is very important to consumers, and thus one of most important aspects for the industry (Kim *et al.*, 2009). Parameters such as pH and colour values (L*, a*, b*) are some of most important indicators of meat quality. The pH values after 24 h found in our study are very similar to those of Dalle Zotte et al. (2016) and Mattioli *et al.* (2016). The meat appeared to be less acidified only for the Belgian Giant Grey population. Bolet *et al.* (2000) reported that pH and L* values could be good predictors of meat quality. L* values were found to be useful as indicators of PSE and DFD meat in pork (Przybylski and Hopkins, 2016). For the TER population, we found differences for L* value on *musculus biceps femoris* after 24 h storage for g.68680097 G>A and for BGG L*value on *musculus longissimus lumborum* after 24 h chilling. The L* and pH* values obtained in our study were in agreement with results from other studies (Kozioł *et al.*, 2015, Dalle Zotte et al., 2016)

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CLUSTAL O(1.2.4) multiple sequence alignment

myog_rabbit     MELYETSPYFYQEPRFYDGENYLPVHLQGFEPPGYERTELSLSPEARGPLEDKGLGTPEH   60
myog_V80M       MELYETSPYFYQEPRFYDGENYLPVHLQGFEPPGYERTELSLSPEARGPLEDKGLGTPEH   60
pdb|1AN4|A      ------------------------------------------------------------   0

myog_rabbit     CPGQCLPWACKVCKRKSVSVDRRRAA--TLREKRRLKKVNEAFEALK----RSTLLNPNQ   114
myog_V80M       CPGQCLPWACKVCKRKSVSmDRRRAA--TLREKRRLKKVNEAFEALK----RSTLLNPNQ   114
pdb|1AN4|A      -------------------MDEKRRAQHNEVERRRRDKINNWIVQLSKIIPDSSMESTKS   41
                                  :*.:* *   .  *:** .*:*: :  *.     *:: . :.

myog_rabbit     RLPKVEILRSAIQYIERLQALLSSLNQEERDLRYRGGAGPQPGQVPSECSSHSASCSPEW   174
myog_V80M       RLPKVEILRSAIQYIERLQALLSSLNQEERDLRYRGGAGPQPGQVPSECSSHSASCSPEW   174
pdb|1AN4|A      GQSKGGILSKASDYIQELRQSNHR-----------------------------------   65
                   *  ** .* :**:.*:

myog_rabbit     GNALEFGPNPGDHMLAADPTDAHNLHSLTSIVDSIAVEDVSVAFPDETMPN    225
myog_V80M       GNALEFGPNPGDHMLAADPTDAHNLHSLTSIVDSIAVEDVSVAFPDETMPN    225
pdb|1AN4|A      -------------------------------------------------     65
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Figure 3: Alignment of animo acid sequences of non-mutant rabbit *MyoG*(myog_rabbit), with g.68680097 G>A (myog_V80M) substitution and human HLH (helix-loop-helix) region (pdb|1AN4|4).

CONCLUSION

Analysis of the impact of *MyoG* SNPs on the growth and carcass traits was presented. More research should be conducted, particularly on meat type breeds, in order to fully understand the influence of the SNPs identified on specific traits. We have proven that *MyoG* SNPs influence NZW×BGG meat traits – i.e. weight of dissectible meat from hind leg and pH after 24 h on *musculus longissimus lumborum*. Moreover, another important quality trait – L* value differed for g.68679476 C>T in BGG population and g.68680097 G>A in TER population.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1: Association analysis between g.68679476 C>T polymorphism and growth and carcass traits[2].

	BGG[3]			NZWxBGG		
Trait[1]	CT	TT	*P*-value	CC	CT	*P*-value
BW(g)	84±4	76±5	0.026	70±3	66±1	0.161
W5(g)	893±43	991±60	0.196	945^{b}±53	809^{a}±19	0.018
W12(g)	3268±104	3288±148	0.912	2973±119	2817±42	0.222
SW(g)	3380±103	3381±146	0.993	3192±149	2951±8	0.151
HC(g)	1715±63	1771±94	0.623	1682±85	1509±39	0.073
CC(g)	1653±61	1700±86	0.659	1642±82	1461±37	0.051
LIV(g)	97±3	90±4	0.172	87±7	77±3	0.202
FP(g)	737±26	750±37	0.777	678^{b}±35	588^{a}±16	0.026
IP(g)	303±15	323±21	0.434	349±22	324±10	0.313
HP(g)	614±23	627±32	0.736	615^{b}±29	549^{a}±13	0.042
DPW (%)	50.6±0.4	51.5±0.6	0.219	53±1	51±1	0.111
DPC (%)	48.7±0.5	50.2±0.8	0.13	51.7±1	49±1	0.051
MBF(g)	721±25	741±36	0.657	656^{b}±34	566^{a}±16	0.021
FF(g)	16^{b}±2	9^{a}±3	0.045	14±3	11±2	0.517
MI(g)	245±12	263±16	0.319	279±17	256±8	0.216
BI(g)	45±2	47±3	0.548	49^{b}±3	41^{a}±1	0.033
FI(g)	14±2	13±3	0.812	18±4	16±2	0.631
MH(g)	461±19	472±27	0.742	476^{b}±25	416^{a}±12	0.036
BH(g)	150±5	154±6	0.659	128±7	115±3	0.076
FH(g)	2±1	1±1	0.388	7^{b}±1	2^{a}±1	0.000

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

[2]least square means are reported with their standard error.

[3]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 different letters differ significantly at $P<0.05$.

Supplementary Table 2: Association between rabbits g.68679476 C>T polymorphism and pH and colour[2].

	BGG[3]			NZW×BGG		
Trait[1]	CT	TT	*P*-value	CC	CT	*P*-value
pH45H	6.7±0.1	6.6±0.1	0.26	6.7±0.1	6.7±0.1	0.578
pH24H	6±0.1	6.1±0.1	0.212	5.8±0.1	5.9±0.1	0.138
pH45L	6.9±0.1	6.8±0.1	0.152	6.7±0.1	6.8±0.1	0.556
pH24L	6±0.1	5.9±0.1	0.295	5.5^{a} ±0.1	5.7^{b}±0.1	0.027
L*45H	53.2±0.6	52.1±0.9	0.331	57.9±0.8	57.3±0.4	0.479
a*45H	3.8±0.3	3±0.4	0.137	10.7±0.5	10.9±0.2	0.612
b*45H	0.8±0.4	0.6±0.5	0.734	0.9±0.7	0.6±0.3	0.707
L*24H	58.1±0.7	56.8±0.9	0.239	58.3±0.75	58.3±0.34	0.965
a*24H	4.5±0.4	4.3±0.5	0.706	12.6±0.7	12.2±0.3	0.606
b*24H	4.7±0.4	4±0.5	0.295	2.6^{a}±0.4	3.9^{b}±0.2	0.008
L*45L	62.8±1.1	62.1±1.5	0.743	61.8±1.3	61.8±0.6	0.993
a*45L	1.9±0.8	3.7±1.1	0.224	7.6±0.7	7.9±0.3	0.639
b*45L	–1.8±0.8	0.8±1.1	0.06	$–2.3^{b}$±0.6	$–2.3^{a}$±0.3	0.031
L*24L	60.1^{b}±0.7	57^{a}±1	0.016	57.5±1	58.1±0.5	0.597
a*24L	7.3±0.5	8.2±0.7	0.31	12.4±0.8	12.6±0.4	0.8
b*24L	5.4±0.5	6±0.7	0.481	0.7^{a}±0.7	2.9^{b}±0.3	0.008

[1]H, hind leg (*m. biceps femoris*); L, *m. longissimus lumborum.* 45, measured 45 min after slaughter; 24, measured after 24 h chilling.
[2]least square means are reported with their standard error.
[3]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 different letters differ significantly at $P<0.05$.

Supplementary Table 3: Association analysis between g.68680097 G>A polymorphism and growth and carcass traits[2].

	TER			BEL			NZW×BGG		
Trait[1]	GG	GA	*P*-value	GG	GA	*P*-value	GG	GA	*P*-value
BW(g)	65±1	66±1	0.435	80±5	81±4	0.806	68±1	64±2	0.557
W5(g)	886^{b}±16	817^{a}±18	0.005	945±53	881±42	0.356	831±14	797±22	0.193
W12(g)	2747±42	2689±47	0.335	3294±123	3227±97	0.668	2665±32	2664±51	0.984
SW(g)	2824±47	2698±54	0.106	3399±122	3333 ±96	0.673	2880±64	2897±111	0.893
HC(g)	1504±26	1429±30	0.084	1726±75	1711±60	0.698	1466±36	1485±62	0.792
CC(g)	1459±26	1389±32	0.123	162±72	1650±57	0.892	1422±35	1442±61	0.778
LIV(g)	76±3	74±3	0.709	94±4	92±3	0.706	81±3	84±6	0.615
FP(g)	611±13	598±15	0.543	738±31	736±24	0.954	572±15	576±25	0.911
IP(g)	322±9	295±10	0.071	305±18	306±14	0.961	313±9	324±15	0.546
HP(g)	526^{b}±8	496^{a}±9	0.024	618±27	608±21	0.755	538±13	542±22	0.876
DPW (%)	53±0.1	53±0.1	0.461	51±1	51±0.1	0.817	51±0.1	51±1	0.548
DPC (%)	52±0.1	51±0.1	0.19	49±1	49±1	0.514	49±0.1	50±1	0.486
MBF(g)	578±10	562±12	0.343	722±31	722±24	0.988	553±14	571±26	0.546
FF(g)	33±4	36±5	0.715	16±3	14±2	0.611	12±1	12±2	0.93
MI(g)	248±6	231±7	0.093	247±14	248±11	0.961	247±7	269±12	0.133
BI(g)	43^{b}±2	36^{a}±2	0.007	44 ±3	45±2	0.622	43±1	42±2	0.736
FI(g)	31±3	28±3	0.603	14±2	13±2	0.621	15±1	17±1	0.379
MH(g)	401±6	381±8	0.065	465±22	457±17	0.779	410±10	434±19	0.258
BH(g)	122^{b}±3	111^{a}±3	0.015	151±6	148±4	0.699	114±3	111±6	0.7
FH(g)	3±1	4±1	0.412	2±1	2±1	0.969	4±1	2±1	0.217

[1]BW(g), birth weight (g); W5(g), weight at 5 wk of age(g); W12(g), weight at 12 wk of age(g); SW, slaughter weight (g); HC, hot carcass weight (g); CC, chilled carcass weight (g);LIV(g), weight of liver(g); FP, fore part weight (g); IP, intermediate part (loin) weight (g); HP, hind part weight (g); DPW, dressing-out percentage warm (%); DPC, dressing-out percentage cold (%); MBF(g), weight of fore part (meat+bones)(g); FF, dissectible fat in fore part (g); MI, meat in intermediate part (g); BI, bones in intermediate part (g); FI, dissectible fat in intermediate part (g); MH, meat in hind part (g); BH, bones in hind part (g); FH, dissectible fat in hind part (g).
[2]least square means are reported with their standard error.
[3]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 different letters differ significantly at $P<0.05$.

Supplementary Table 4: Association between g.68680097 G>A polymorphism and pH and colour[2].

	TER			BGG			NZWxBGG		
Trait	GG	GA	*P*-value	GG	GA	*P*-value	GG	GA	*P*-value
pH45H	6.5±0.1	6.7±0.1	0.238	6.6±0.1	6.7±0.1	0.506	6.7±0.1	6.8±0.1	0.537
pH24H	5.9±0.1	5.9±0.1	0.983	6.1±0.1	6.1±0.1	0.417	5.8±0.1	5.8±0.1	0.684
pH45L	6.5±0.1	6.6±0.2	0.561	6.9±0.1	6.8±0.1	0.142	6.8±0.1	6.7±0.1	0.858
pH24L	5.8±0.1	5.8±0.1	0.763	6±0.1	6±0.1	0.811	5.7±0.1	5.7±0.1	0.482
L*45H	52.4±0.5	53.6±0.5	0.122	53.5±0.8	52.2±0.7	0.22	57.4±0.3	57.5±0.5	0.849
a*45H	3.4±0.2	3.5±0.2	0.748	3.6±0.4	3.7±0.3	0.836	10.9±0.2	10.6±0.4	0.551
b*45H	1±0.3	0.9±0.3	0.81	0.8±0.4	0.7±0.3	0.815	0.8±0.2	0.8±0.4	0.177
L*24H	54.5[a]±0.4	55.8[b]±0.4	0.03	57.7±0.8	57.4±0.7	0.79	58.1±0.3	58.9±0.5	0.122
a*24H	4.6±0.3	4.5±0.3	0.924	4.8±0.5	4.5±0.4	0.645	12.5±0.3	12.4±0.5	0.861
b*24H	4.2±0.3	4.1±0.3	0.731	4.9±0.4	4.2±0.3	0.176	3.5[a]±0.2	4.7[b]±0.4	0.007
L*45L	60.2±0.6	58.7±0.7	0.125	62.8±1.3	61.8±1	0.556	61.4±0.4	61.2±0.8	0.886
a*45L	1±0.4	1.4±0.4	0.512	1.8±1	3.2±0.8	0.27	8.1±0.3	8.1±0.5	0.998
b*45L	–1.8±0.5	–1.8±0.6	0.969	–1.5±1.1	–1±0.9	0.753	–2.6±0.2	–2.5±0.3	0.761
L*24L	55.7±0.5	55.9±0.5	0.808	60.1±0.9	58.3±0.7	0.137	57.3±0.3	58.4±0.6	0.116
a*24L	5.8±0.4	5.9±0.4	0.847	6.9±0.6	8.1±0.5	0.122	12.9±0.3	12.5±0.5	0.52
b*24L	4±0.3	4.1±0.4	0.798	5.5±0.6	5.9±0.5	0.524	2.27±0.3	3.2±0.5	0.131

[1]H, hind leg (*m. biceps femoris*); L, *m. longissimus lumborum*; 45, measured 45 min after slaughter; 24, measured after 24 h chilling.
[2]least square means are reported with their standard error.
[3]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 different letters differ significantly at $P<0.05$.