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ASSOCIATION ANALYSIS OF NUCLEOTIDE POLYMORPHISMS IN GROWTH HORMONE (GH) AND ITS RECEPTOR (GHR) WITH BODY WEIGHT IN CALIFORNIAN RABBITS

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Abstract: The objective of the present study was to evaluate the influence of the genotypes of two single nucleotide polymorphisms (SNPs) – c.78C>T located in the growth hormone gene (GH) and c.106C>G in the growth hormone receptor gene (GHR) on individual body weight (IBW) during the growing period at 35, 70 and 90 d of age on a total of 107 weaned Californian breed rabbits. The restriction fragments obtained revealed that 74.8% of the rabbits carrying c.78C>T SNP and 52.3% of the rabbits carrying c.106C>G SNP were heterozygous, which indicated a moderate level of genetic diversity in this Californian population. Association analysis based on a single-gene approach revealed that c.78C>T polymorphism in the GH gene had a significant effect ($P<0.05$) on the weight at 70 and 90 d of age. The highest IBW (2530.4±66.6 g) was observed in rabbits carrying the c.78C>T TT genotype, and detected individuals were significantly affected by the dominance effect. Significant differences were observed between individuals with homozygous c.106C>G CC genotype and those with heterozygous CG genotype. The highest IBW (2462.0±198.3 g) was observed in rabbits carrying the c.106C>G CC genotype and detected individuals were significantly affected by the additive effect. A total of nine combined genotypes of c.78C>T and c.106C>G SNPs was found in the study, of which only four major groups (CT/CC, CC/CG, CT/CG, and CT/GG) were concerned in the diplotype analysis. Significant differences were observed between individuals with CT/CC and CC/CG genotype combinations, and between those with the CC/CG and CT/GG diplotypes. However, the highest IBW at 90 d of age (2447.2±213.8 g) was observed in rabbits carrying the CT/CC genotype combinations. The highest coefficient of determination found for individual body weight at 90 d of age (R^2=10.8%) indicated a high effect of genotype combinations. In conclusion, the results obtained suggested that c.78C>T of GH gene and c.106C>G of GHR gene could be useful candidate genes to improve growth performance in Californian rabbits with potential application in rabbit breeding programmes.

Key Words: Californian rabbits, growth hormone gene, growth hormone receptor gene, SNPs, PCR-RFLP, body weight.

INTRODUCTION

With the advances in molecular genetic techniques in animal production, scientists could achieve accurate and effective selection assisted by candidate genes or genetic markers associated with traits of interest. This could provide opportunities to enhance response to selection, in particular for traits that are difficult to improve through conventional selection (such as traits with low heritability or for which measurement of phenotype is difficult, expensive, only

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possible late in life, or not possible on selection candidates) (Ruane and Colleau, 1996; Baselga, 2004; Dekkers, 2004).

Californian rabbit is one of the most commonly used breeds for rabbit meat production. The first Californian rabbits in Bulgaria were imported in 1970 from Italy. The next larger import of rabbits of the Californian breed took place in 2002, again from Italy (Marinov *et al.*, 2009), the offspring of which are the experimental animals in this study. At present, a total of 842 Californian rabbits (705 does and 137 bucks in 10 herds) are under the selection control of the Executive Agency for Selection and Reproduction in Animal Breeding in Bulgaria and rabbit breeding activities are carried out by different breeding associations (Grigorov, 2005; Dimitrova *et al.*, 2008; EASRAB, 2017).

Several candidate genes have been already successfully applied to identify molecular markers associated with growth efficiency in different livestock species. In particular, for rabbits, growth is an important commercial trait. Growth hormone gene (GH), one of the most investigated genes that possess an important endocrine function has already been cloned and sequenced in the rabbit (Wallis and Wallis, 1995). Southern blotting analysis revealed that the rabbit's GH gene has a single-copy without GH-like genes and contains five exons separated by non-coding regions. Consistently, Fontanesi *et al.* (2008) re-sequenced the GH gene in four different rabbit breeds (Belgian Hare, Burgundy Fawn, Checkered Giant, and Giant Grey), but mutation was not detected in the sequenced regions encompassing exons 2, 3 and 4 and introns 1, 2, 3 and 4. Moreover, Fontanesi *et al.* (2012) re-sequenced all five exons and two single nucleotide polymorphisms (SNPs) –one (c.C>T) at position 78 and another (the rare c.A>G) at position 33 of the starting codon of exon I– have been identified within a total of 1337 bp of the GH in 14 rabbits of different breeds. In addition, c.78C>T SNP has been significantly associated with market weight (finishing weight) in a commercial rabbit population. The c.78C>T polymorphism in the rabbit's GH gene has also been reported. Recently, based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, a sufficient number of heterozygous individuals in terms of c.78C>T SNP was established by Hussein *et al.* (2015) for APRI rabbits and by Hristova *et al.* (2018) in two rabbit populations (purebred and crossbred NZW lines) under Bulgarian conditions. Mutation C>T SNP at –78 bp has been previously related with changes in body weight gain in a previous study with other type of rabbits (Ramadan *et al.*, 2020).

It is well known that the somatotropin or GH gene regulates the metabolic processes of body growth and development in animal biology. During the physiology function of the GH gene, the initial step is binding to its receptor —growth hormone receptor (GHR)— which results in receptor dimerisation and signal-transducing at the cytoplasmic domain of the GHR (VanderKuur *et al.*, 1994; Frank, 2001; Herrington and Carter-Su, 2001). There is strong evidence that the variability of the GHR gene could affect GH-GHR interaction (Bai *et al.*, 2011). Therefore, not only the function pathway of the GHR gene needs to be established, but also the genetic variability within its sequence.

The rabbit GHR gene was initially sequenced by Leung *et al.* (1987) and as a result, ten exons encoding a total of 638 amino acids have been established. The genetic variability in exon 10 of the rabbit GHR gene was reported by Polasik *et al.* (2005) in the Chinchilla breed and by Deng *et al.*, (2008), who identified two polymorphisms (c.705C>T and c.810C>T) associated with carcass traits and feed efficiency in Chinese developed lines and others cosmopolitan rabbit breeds (Belgian Hare, Tianfu Black, Great line of Zika, Harbin White and Californian). Furthermore, a missense mutation (c.106C>G) located in exon 3 that changes the amino acid valine to leucine at position 36 (p.L36V) of the GHR protein was identified by Zhang *et al.*, (2012) in three rabbit breeds (Tianfu Black, Ira and Champagne), by Fontanesi *et al.*, (2016) in a commercial meat rabbit line, and also in a total of 100 rabbits from New Zealand White and Californian breeds by Gencheva *et al.* (2017), studying the genetic variation in GHR gene through the PCR-RFLP assay. The same exon in the rabbit GHR gene was investigated in a recent study by Helal (2019) and highly significant associations were found between CC genotype and body weight at 6, 10 and 12 wk in both Baladi Red and New Zealand White rabbit breeds in Egypt. The 1st exon of the GHR gene was sequenced by Sahwan *et al.* (2014) in three rabbit breeds (Alexandria, New Zealand White and V-line) reared under Egyptian conditions, and a total of sixteen new SNPs have been reported. Associations between SNPs in multiple candidate genes and body weight in rabbits were also examined in Egypt by El-Sabrout and Aggag (2017).

The present research was carried out to identify genetic variation within two main growth genes (GH and GHR) of the rabbit somatotropic axis and to assess the molecular association with post-weaning individual body weight of a Californian rabbit population reared under Bulgarian conditions.

MATERIAL AND METHODS

Animals and phenotypic traits

The experimental animals originated from the rabbit farm of the Institute of Animal Science (Kostinbrod, Sofia, Bulgaria). A total of 107 post-weaning rabbits (50 male and 57 female) were studied. They were born from 24 does mated with 10 bucks. The information about sick and dead animals from litters was removed from the experiment. The weaning was done between the age of 40 and 45 d when forming a batch. All rabbits were ear-tagged and raised in groups, in single-floor cage complexes (cage size 80×60×35 cm), with an area of at least 0.14-0.22 m^2 per rabbit, according to age and body weight. The rabbits were fed with commercial pellets (crude protein=17%, crude fibre=12%, ether extract=2.92%) *ad libitum*, except for a period of 2 wk post-weaning. During this period, the rabbits were fed with a gradually increasing diet of pellets from 40-50 g per rabbit to feeding *ad libitum* on the farm husbandry technology as prevention of digestive disorders. They had constant access to water through nipple drinkers. During the growing period, individual body weight was recorded at 35, 70 and 90 d of age in all tested animals.

Blood samples and DNA extraction

Blood samples were collected from the rabbits *v. auricularis* into sterile 3 mL of potassium salt of ethylene diamine tetra acetic acid (K_2EDTA) containing tubes (Biosigma, Italy). Genomic DNA was extracted from the whole rabbit blood using a commercial purification kit (Illustra Blood GenomicPrep DNA, GE Healthcare, UK), following the manufacturer's instructions. The concentration and purity of the obtained genomic DNAs were measured via NanoVue Plus Spectrophotometer (GE Healthcare) at 260/280 nm and verified by agarose gel electrophoresis. The samples were frozen and stored at –18°C until the PCR amplification was performed.

PCR-RFLP and genotyping

Two polymorphic sites in rabbit genes GH and GHR, respectively, were screened for SNPs by PCR-RFLP approach. The exact gene regions, their prime sequences and obtained length of the amplicons, annealing temperatures for PCR, specific enzymes for digestion and restriction profiles corresponding to examined SNPs are presented in Table 1.

The amplifications were carried out using a Doppio thermal cycler (2×48 well) (VWR®, Germany) in a final reaction volume of 20 µL that included: 2×Red Taq DNA Polymerase Master mix (VWR, Belgium), 80 ng DNA template, 20 pM of each primer and nuclease-free water (ddH_2O). The reactions were performed under the following cycling conditions: a preliminary denaturation at 94°C/5 min, followed by 30 cycles at 94°C/30 s, primer annealing for 45 s. at the appropriate temperature, extension at 72°C/1 min, final extension at 72°C/10 min, and stored at 4°C/∞. The restrictions of the PCR products were carried out in a total volume of 25 µL, containing 10 µL PCR product, 10 U/µL

Table 1: Gene regions, primer sequences, amplicons length (bp), annealing temperature (°C), restriction enzyme and restriction fragment length polymorphism (RFLP) patterns for genotyping of the investigated single nucleotide polymorphism (SNPs) of growth hormone (GH) and growth hormone receptor GHR genes.

	GH	GHR
Gene region	Part of the 5'-flanking region, 5'-untranslated (UTR) region, exon 1 (CDS) and part of intron 1	Part of intron 2, exon 3, part of intron 3
Forward and reverse primers (5'-3')	GTATAGTGGGATGGGGTTGG* TTAGCGTCCCATTCAGAAGC	AGGTGAAGCGTGCTCTCATT** TTTGGCCTAGCTTAGCCTTT
Amplicons length (bp)	231	479
Annealing T (°C)	60	56.4
Restriction enzyme	Bsh1236 I	Hinf I
RFLP patterns	Allele C=169+62 bp, Allele T=231 bp	Allele C=210+162+107 bp, Allele G=317+162 bp

*primers suggested by Fontanesi *et al.* (2012); **primers suggested by Fontanesi *et al.* (2016).

restriction enzyme (Bioneer, South Korea) and ddH_2O, and incubated at 37°C/overnight. The obtained PCR products and restriction fragments were stained with GelRed® fluorescent nucleic acid dye (Biotium, USA) and separated on 2.5% agarose gel. Visualisation of the generated banding patterns was performed using Electrophoresis Gel Imaging Analysis System (Bio-Imaging Systems, Israel).

Statistical analysis

To estimate the population genetic parameters, PopGene v.1.31 software was used (Yeh *et al.*, 1999; Labate, 2000). The following parameters were calculated for each SNP in the studied rabbit population: effective number of alleles (Ne), allele and genotype frequencies, observed (Ho) and expected (He), heterozygosity calculated as per Nei (1973), coefficient of inbreeding (Fis), and chi-square test (χ^2) of Hardy-Weinberg equilibrium (HWE).

The influence of the particular genotype of two SNPs – c.78C>T in GH and c.106C>G in GHR gene on individual body weight in California rabbit population during the growing period (35, 70 and 90 d of age) were examined by univariate data analysis using the following model:

$$Y=\overline{x}+G+e$$

where Y are the measurements of the IBW on 35th, 70th or 90th growing day of weaned rabbits, $\overline{x}$ are the overall mean values, G are the fixed factors (the different genotypes at GHR c.106C>G SNP or at GH c.78C>T SNP; the haplotype combinations or the diplotype combinations of two SNPs), and e are the residuals of the model.

Significant differences between the different least square means (LSM) of the genotypes were calculated by post hoc multiple comparisons with Dunnett or Tukey test (depending on Levene's test of equality of error variances) at $P<0.05$. The data analysis was performed using the General Linear Model (GLM) of SPSS Statistics v17.0 package (SPSS Statistics, 2007).

Additionally, the genetic effects for dominance (D) and additivity (A) for each SNP in the Californian rabbit population were estimated, according to the equations suggested by Russo *et al.* (2008): D=pq–½(pp+qq) and A=½(pp–qq), where pp and qq are homozygous groups. Estimates of dominance and additive effects were tested for deviation from zero through the Student's t-test at $P<0.05$. The D/A ratio was considered to indicate actual gene effects, as follows: D/A<0.2 – additive; 0.2<D/A<0.8 – partial dominance; 0.8< D/A<1.2 – dominance; D/A>1.2 – overdominance, according to Stuber *et al.* (1987).

RESULTS AND DISCUSSION

Population genetic structure in Californian rabbits

In the present research, we applied a candidate gene approach to identify SNPs (c.78C>T and c.106C>G) within two polymorphic regions of the GH and GHR genes in the Californian rabbit population. On the basis of the PCR-RFLP assay, we identified restriction fragments that revealed all three possible genotypes in c.78C>T SNP at polymorphic

Table 2: Effective number of alleles (Ne), allele and genotypic frequencies, expected (Ho) and observed (He) heterozygosity, coefficient of inbreeding (Fis) and chi-square test (χ^2) of HWE et degree of freedom df=1 for growth hormone and growth hormone receptor genes in Californian rabbits.

						Heterozygosity			
		Allele				Observed	Expected		
SNPs	Ne	frequencies		Genotypic frequencies		Ho	He	Fis	χ^2
c.78C>T	1.992	C	0.533	CC (n=17)	0.159	0.747	0.498	–0.508	26.439
		T	0.467	CT (n=80)	0.748				
				TT (n=10)	0.093				
c.106C>G	1.986	C	0.542	CC (n=30)	0.281	0.523	0.497	–0.054	0.262
		G	0.458	CG (n=56)	0.523				
				GG (n=21)	0.196				

GH locus —two homozygous CC and TT, and heterozygous CT— observed in 15.9, 9.3 and 74.8% of the Californian rabbit population, respectively. Regarding the c.106C>G SNP at GHR locus, the genotype distribution in the examined rabbit population was 28.1% for homozygous (CC) genotype, 52.3% for heterozygous (CG) genotype and 19.6% for another homozygous (GG) genotype, respectively (Table 2).

The calculated values of the allele frequencies, expected (Ho) and observed (He) heterozygosity and coefficient of inbreeding (Fis) in the studied rabbit population for GH and GHR genes are summarised in Table 2. The results presented for the allele frequencies show a preponderance of the C allele (0.533) over the T allele (0.467) in c.78C>T SNP at the GH gene. The observed prevalence of allele C compared to allele T (0.625 *vs.* 0.375) was also reported by Fontanesi *et al.* (2012) in Californian and also in New Zealand White (NZW) rabbits by Hristova *et al.* (2018) (0.613 for allele C *vs.* 0.387 for allele T). On the contrary, Hussein *et al* (2015) established a prevalence of the T allele (0.540) over the C allele (0.460) in the APRI rabbit line.

Regarding the c.106C>G SNP in GHR locus, a similar trend for the allele distribution was observed in the rabbit population examined. Thus, the frequency of allele C (0.542) was higher than that of allele G (0.458) and the obtained results agreed with those reported by Gencheva *et al.* (2017) for Californian rabbits (0.541 *vs.* 0.459 for C and G alleles, respectively).

In both SNP sites, the value of observed heterozygosity (Ho = 0.747 for c.78C>T and Ho = 0.523 for c.106C>G) was higher compared to the expected ones (He=0.498 and 0.497, respectively), resulting in a negative coefficient of inbreeding (Fis=–0.508 for c.78C>T and Fis=–0.054 for c.106C>G). These results indicated a sufficient number of heterozygous forms and a moderate level of genetic diversity in the Californian rabbit population in terms of the SNP loci examined in GH and GHR genes.

Association analysis based on single-gene approach

An association analysis based on the single-gene approach for SNP loci in targeted genes was performed in the Californian rabbit population. The presence of significant differences between the different genotypes (CC, CT, and TT) of the c.78C>T SNP at the GH gene for the studied trait (IBW) was determined by post hoc multiple comparisons for the observed means, performing the Dunnett test.

The calculated mean values of individual body weight and significant differences among the GH gene genotypes presented in Table 3 are the indication of possible association and reveal that c.78C>T polymorphism in the GH gene had a significant influence on the weight at 70 and 90 d of age in the Californian rabbit population.

Significant differences ($P<0.05$) were observed between individuals with two homozygous genotypes CC and TT, and between those with the CT and TT genotypes, respectively. Thus, the highest IBW (2530.4±66.63 g) was observed in rabbits carrying the c.78C>T TT genotype, and detected individuals were significantly affected by the dominance effect (D=–72.65, A=–89.55, ratio D/A=0.81). No significant differences ($P>0.05$) were observed for c.78C>T genotypes at 35 d of age for the studied rabbit population. The coefficients of determination (R^2) obtained for the IBW in the CAL rabbit population revealed that the highest percentage of influence in the particular genotype was established in IBW at 70 d of age (R^2=7.9%). Association analysis between c.78C>T SNP and the market weight at 70 d performed by Fontanesi *et al.* (2012) indicated a significant influence (P=0.013) on the recorded trait of the

Table 3: Least square means±standard deviations for the investigated individual body weights (IBW) at three growing periods for the Californian rabbits corresponding to different genotypes at growth hormone c.78C>T single nucleotid polymorphism.

		IBW (g)		
Trait	R^2 (%)	CC (n=17)	CT (n=80)	TT (n=10)
IBW-35d	0.3	787.65^{a}±62.52	804.70^{a}±129.55	804^{a}±60.39
IBW-70d	7.9	1861.29^{a}±162.74	1805.52^{a}±221.03	1990.8^{b}±67.58
IBW-90d	5.8	2351.29^{a}±161.24	2368.20^{a}±214.37	2530.4^{a}±66.63

^{a,b}Different superscripts within the same row represent significant differences at the level of significance $P<0.05$; R^2 – coefficients of determination based on observed means through Dunnett test; IBW-35d: is the individual body weight at 35 d of ages. IBW-70d: is the individual body weight at 70 d of ages. IBW-90d: is the individual body weight at 90 d of ages.

Table 4: Least square means±standard deviations for the investigated individual body weights (IBW) at three growing periods for the Californian rabbits corresponding to different genotypes at growth hormone receptor c.106C>G single nucleotid polymorphism.

		IBW (g)		
Growing period	R^2 (%)	CC (n=30)	CG (n=56)	GG (n=21)
IBW-35d	10.6	857.21^a±125.29	769.46^b±108.15	819.14±100.80
IBW-70d	7.9	1895.93^a±186.68	1767.54^b±213.90	1849.81±160.46
IBW-90d	9.4	2462.0^a±198.30	2323.57^b±201.10	2419.62±172.03

^{a,b} Different superscripts within the same row represent significant differences at the level of significance $P<0.05$; R^2 – coefficients of determination based on observed means through Tukey test; IBW-35d: is the individual body weight at 35 d of ages; IBW-70d: is the individual body weight at 70 d of ages; IBW-90d: is the individual body weight at 90 d of ages.

rabbits from a commercial line. Fontanesi *et al.* (2012) reported that heterozygous rabbits with CT genotype reached a higher mean value (2778.83) of body weight at 70 d compared to both homozygous CC and TT ones (2720.04 and 2693.94, respectively), supporting overdominance.

Regarding the c.106C>G SNP at the GHR gene, post hoc multiple comparisons for observed means were performed by Tukey test to analyse the possible association between the GHR gene genotypes (CC, CG, and GG) and the IBW. The obtained mean values of the rabbits' IBW and significant differences between the genotypes of c.106C>G SNP at the GHR gene are presented in Table 4. Significant differences ($P<0.05$) were observed between individuals with homozygous CC genotype and those with heterozygous CG genotype. The highest IBW (2462.0±198.30 g) was observed in rabbits carrying the c.106C>G CC genotype, and detected individuals were significantly affected by the additive effect (D=–117.24, A= 21.19, ratio D/A=–5.53).

The results for additive (A) and dominance (D) effects indicated the part of the genetic variance of body weight that may be affected by allele C more than allele G. The highest coefficients of determination (R^2) for the IBW was obtained at 35 d of age (R^2=10.6%).

Zhang *et al.* (2012) reported that the less frequent CC genotype was the most favourable in terms of body weight at 84 d and carcass traits in three different rabbit breeds (Tianfu Black, Ira, and Champagne). In contrast, Fontanesi *et al.* (2016) stated that the most frequent genotype GG was associated with higher weight at 70 d ($P<0.05$) in a commercial meat rabbit line.

Association analysis based on haplotype and diplotype groups

Haplotype analysis of both c.78C>T and c.106C>G SNPs revealed four haplotype combinations (H1 (CC), H2 (CT), H3 (CG), and H4 (TG)), with frequencies of 0.710, 0.654, 0.664 and 0.561, respectively (Table 5).

The significant differences between the haplotype combinations of two SNPs ($P<0.05$) were calculated by post hoc multiple comparisons for the observed means, performing the Tukey test. However, there were no significant differences ($P>0.05$) among the haplotype combinations for body weight in the Californian rabbit population. Moreover, the value

Table 5: Association between four haplotype combinations of growth hormone and growth hormone receptor single nucleotide polymorphism and individual body weight (IBW) at three growing periods in Californian rabbit population.

		IBW (g)		
Haplotype combinations	n	IBW-35d	IBW-70d	IBW-90d
H1 (CC)	76	796.89±125.50	1788.68±214.49	2350.21±212.27
H2 (CT)	70	806.00±128.23	1813.89±227.20	2383.83±221.98
H3 (CG)	71	784.11±111.36	1772.23±200.36	2335.04±198.30
H4 (TG)	60	784.10±118.20	1791.17±208.89	2372.67±196.51

R^2 – coefficients of determination based on observed means through Tukey test; n – number of the individuals; R^2=0.006 for haplotype combinations at 35 d; R^2=0.005 for haplotype combinations at 70 d; R^2=0.008 for haplotype combinations at 90 d. IBW-35d: is the individual body weight at 35 d of ages; IBW-70d: is the individual body weight at 70 d of ages; IBW-90d: is the individual body weight at 90 d of ages.

Table 6: Association between four diplotype combinations of growth hormone and growth hormone receptor single nucleotide polymorphism and individual body weight (IBW) at three growing periods in Californian rabbit population.

		IBW (g)		
Genotype combinations	n	IBW-35d	IBW-70d	IBW-90d
CT/CC	17	853.18±135.25	1867.76±191.37	2447.29[a]±213.84
CC/CG	10	774.0±68.67	1756.0±118.76	2232.8[b]±73.55
CT/CG	40	770.75±123.38	1735.3±225.92	2316.2±217.39
CT/GG	18	817.56±113.09	1813.78±143.76	2392.89[a]±171.81

[a,b] Different superscripts within the same column represent significant differences at the level of significance $P<0.05$; R^2 – coefficients of determination based on observed means through Dunnett test; n – number of the individuals; $R^2=0.076$ for diplotype combinations at 35 d; $R^2=0.072$ for diplotype combinations at 70 d; $R^2=0.108$ for diplotype combinations at 90 d. IBW-35d: is the individual body weight at 35 d of ages; IBW-70d: is the individual body weight at 70 d of ages; IBW-90d: is the individual body weight at 90 days of ages.

of the coefficient of determination ($R^2=0.6\%$; 0.5%; 0.8% at 35, 70, 90 d, respectively) showed a very weak relation between different haplotype groups and rabbits' IBW.

For the combination of c.78C>T and c.106C>G SNPs, a total of nine combined genotypes were found in the study, of which only four major groups (CT/CC, CC/CG, CT/CG, and CT/GG) were concerned in the diplotype analysis (Table 6).

Significant differences were observed at IBW-90d between individuals with CT/CC and CC/CG diplotypes, and between those with the CC/CG and CT/GG genotypes, respectively. The highest IBW-90d (2447.29±213.84 g) was associated with rabbits carrying the CT/CC genotype combinations. No significant differences were observed among the individuals with various diplotypes at IBW-35d and IBW-70d ($P>0.05$). The highest coefficient of determination ($R^2=10.8\%$) found for at IBW-90d demonstrates the high effect of genotype combinations at studied SNP loci on the rabbit IBW at 90 d of age. However, further studies are needed to validate the associations detected between GH and GHR genes and body weight at different ages in the investigated population of Californian rabbits, and to check the possibility of using these genes in marker-assisted selection.

CONCLUSIONS

In conclusion, the association analysis in the present study revealed that c.78C>T and c.106C>G polymorphisms at GH and GHR, respectively, had a significant influence ($P<0.05$) on the individual body weight at 90 d of age in the Californian rabbit population. The individuals with the c.78C>T TT and c.106C>G CC genotypes reached a higher level of performance on the recorded trait. The result obtained based on genotype combinations revealed that the highest body weight at 90 d of age was associated with rabbits carrying the CT/CC diplotype ($P<0.05$). Therefore, results obtained in the study suggested that c.78C>T and c.106C>G SNPs could be useful candidate gene markers for rabbit growth performance with potential application in marker-assisted selection. However, before the practical application of the obtained results they should be evaluated more precisely by means of validation in a larger rabbit population.

REFERENCES

Bai W.L., Zhou C.Y., Ren Y., Yin R.H., Jiang W.Q., Zhao S.J., Zhang S.C., Zhang B.L., Luo G.B., Zhao Z.H. 2011. Characterization of the GHR gene genetic variation in Chinese indigenous goat breeds. *Mol Biol Rep., 38: 471-479. https://doi.org/10.1007/s11033-010-0130-2*

Baselga M. 2004. Genetic improvement of meat rabbits. Programmes and diffusion. *In Proc.: 8th World Rabbit Congress, September 7-10, 2004, Pueblo, Mexico, 1-13.*

Dekkers J.C. 2004. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons, *J. Anim. Sci., 82, 13: 313-328.*

Deng X.S., Wan J., Chen S.Y., Wang Y., Lai S.J., Jiang M.S., Xu M. 2008. The correlations between polymorphism of growth hormone receptor gene and butcher traits in rabbit. Yi chuan. *Hereditas, 30: 1427-1432. https://doi.org/10.3724/SP.J.1005.2008.01427*

Dimitrova I., Dimitrov T., Teneva A., Tzvetkova H. 2008. Rabbit production in Bulgaria. *Biotech. Anim. Husbandry, 24: 1-2: 149-154. https://doi.org/10.2298/BAH0802149D*

EASRAB. Livestock Breeds in the Republic of Bulgaria Executive Agency for Selection and Reproduction in Animal Breeding, Catalogue, 5th ed. *In: Nikolov V. (editor), Sofia, Bulgaria; 2017. pp. 80-81.*

El-Sabrout K., Aggag S.A. 2017. Associations between single nucleotide polymorphisms in multiple candidate genes and body weight in rabbits. *Vet. World, 10: 136-139. https://doi.org/10.14202/vetworld.2017.136-139*

Fontanesi L., Tazzoli M., Scotti E., Russo V. 2008. Analysis of candidate genes for meat production traits in domestic rabbit breeds. *In Proc.: 9th World Rabbit Congress, June 10-13, 2008, Verona, Italy, 79-84.*

Fontanesi L., Dall'Olio S., Spaccapaniccia E., Scotti E., Fornasini D., Frabetti A., Russo V. 2012. Asingle nucleotide polymorphism in the rabbit growth hormone (GH1) gene is associated with market weight in a commercial rabbit population. *Livest. Sci., 147: 84-88. https://doi.org/10.1016/j.livsci.2012.04.006*

Fontanesi L., Sparacino G., Utzeria V.J., Scottia E., Fornasini D., Dall'Olioa S., Frabetti A. 2016. Identification of Polymorphisms in the Rabbit Growth Hormone Receptor (GHR) Gene and Association with Finishing Weight in a Commercial Meat Rabbit Line. *Anim. Biotechnol., 27: 77-83. https://doi.org/10.1080/10495398.2015.1101697*

Frank S.J. 2001. Growth hormone signalling and its regulation: preventing too much of a good thing. *Growth Horm IGF. Res., 11: 201-212. https://doi.org/10.1054/ghir.2001.0237*

Gencheva D., Georgieva S., Velikov K., Koynarski T., Tanchev S. 2017. Single nucleotide polymorphism of the Growth Hormone Receptor (GHR) encoding gene in *Oryctolagus cuniculus*. *J. BioSci. Biotechnol., 6: 197-201.*

Grigorov I. 2005. How to grow rabbits. *Zemizdat, Sofia.*

Helal M.M. 2019. Association between growth hormone receptor gene polymorphism and body weight in growing rabbits. *Adv. Anim. Vet. Sci., 7: 994-998. https://doi.org/10.17582/journal.aavs/2019/7.11.994.998*

Herrington J., Carter-Su C. 2001. Signaling pathways activated by the growth hormone receptor. *Trends Endocrinol Metab., 1: 252-257. https://doi.org/10.1016/S1043-2760(01)00423-4*

Hristova D., Tanchev S., Velikov K., Gonchev P., Georgieva S. 2017. Rabbit Growth Hormone and Myostatin Gene Polymorphisms. *J. Agri. Res., 2: 1-6. https://doi.org/10.23880/OAJAR-16000133*

Hristova D.G., Tanchev S.G., Velikov K.P., Gonchev P.G., Georgieva S.J. 2018. Single nucleotide polymorphism of the growth hormone (GH) encoding gene in inbred and outbred domestic rabbits. *World Rabbit Sci., 26, 1: 49-55. https://doi.org/10.4995/wrs.2018.7211*

Hussein B., Abdel-Kafy E.M., Abdel-Ghany S.M., Gamal A.Y., Badawi Y.M. 2015. Single nucleotide polymorphism in growth hormone gene are associated with some performance traits in rabbit. *Int. J. Biol. Pharm. Allied Sci., 4: 490-504.*

Labate J. 2000. Software for population genetic analyses of molecular marker data. *Crop Sci., 40: 1521-1528. https://doi.org/10.2135/cropsci2000.4061521x*

Leung D.W. Spencer S.A., Cachianes G., Hammonds R.G., Collins C., Henzel W.J., Barnard, R., Waters M.J., Wood W.I. 1987. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature, 330: 537-543. https://doi.org/10.1038/330537a0*

Marinov B., Grigorov I., Gurov B., Peshev R. 2009. Raising rabbits for meat. *Sofia, pp. 1-334.*

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U.S.A. 70: 3321-3323. https://doi.org/10.1073/pnas.70.12.3321*

Polasik D., Kmiec M., Liefers S.,Terman A. 2005. Single nucleotide polymorphisms in exon 10 of the chinchilla growth hormone receptor (GHR) gene. *J. Appl Genet. 46: 403-406.*

Ramadan S., Manaa E., El-Attrony M., EL Nagar A. 2020. Association of growth hormone (GH), insulin-like growth factor 2 (IGF2) and progesterone receptor (PGR) genes with some productive traits in Gabali rabbits. *World Rabbit Sci., 28: 135-144. https://doi.org/10.4995/wrs.2020.12610*

Ruane J., Colleau J. J. 1996. Marker-assisted selection for a sex-limited character in a nucleus breeding population. *J. Dairy Sci., 79: 1666-1678. https://doi.org/10.3168/jds.S0022-0302(96)76531-1*

Russo V., Fontanesi L., Scotti E., Beretti F., Davoli R., Nanni Costa L., Buttazzoni L. 2008. Single nucleotide polymorphisms in several porcine cathepsin genes are associated with growth, carcass, and production traits in Italian Large White pigs. *J. Anim. Sci., 86: 3300-3314. https://doi.org/10.2527/jas.2008-0920*

Sahwan F.M., El-Sheik A.I., Sharaf M.M., El-Nahas A.F. 2014. Genetic Polymorphism in Growth hormone receptor Gene (GHR) and its Relationship with Growth Trait in Pure and Hybrid Rabbit Breeds. *Alex. J. Vet. Sci., 43: 1. https://doi.org/10.5455/ajvs.165197*

SPSS Statistics 17.0.0 WinWrap Basic, Copyright 1993-2007 *Polar Engineering and Consulting, https://www.ibm.com/products/spss-statistics.*

Stuber CW, Edwards MD, Wendel JF. 1987. Molecular-marker facilitated investigations of quantitative trait loci in maize. II Factors influencing yield and its component traits. *Crop Sci., 27: 639-648. https://doi.org/10.2135/cropsci1987.0011183X0027000040006x*

VanderKuur J.A., Wang X., Zhang L., Campbell G.S., Allevato G., Billestrup N., Carter-Su C. 1994. Domains of the growth hormone receptor required for association and activation of JAK2 tyrosine kinase. *J. Biol. Chem., 269: 21709-21717. https://doi.org/10.1016/S0021-9258(17)31863-X*

Wallis O. C., Wallis M. 1995. Cloning and characterisation of the rabbit growth hormone-encoding gene. *Gene, 163: 253-256. https://doi.org/10.1016/0378-1119(95)00429-A*

Yeh F.C., Yang R.C., Boyle T. 1999. POPGENE. Microsoft Windows-Based Freeware for Population Genetic Analysis. *Release 1.31. University of Alberta, Edmonton.*

Zhang W. X., Zhang G. W., Peng J., Lai, S. J. 2012. The polymorphism of GHR gene associated with the growth and carcass traits in three rabbit breeds. *In Proc.: 10th World Rabbit Congress, Egypt, September, 3-6, pp 75-78.*