Polymorphisms of the CAPN, CAST, LEP, GH, GHR, IGF-1 and MSTN *loci* of Colombian Creole hair x Pelibuey sheep sheep crossbreeds

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Abstract

The objective of this study was to characterize single nucleotide polymorphisms at the Calpain (CAPN), Calpastatin (CAST), Leptin (LEP), Growth Hormone (GH), Growth Hormone Receptor (GHR), Insulin-Like Growth Factor 1 (IGF-1) and myostatin (MSTN) *loci* in Columbian Creole hair sheep crossed with Pelibuey sheep. In 192 individuals, the CAST and MSTN *loci* were genotyped by PCR-RFLP, and the CAPN, GH, GHR, IGF-1 and LEP *loci* by PCR-SSCP and sequences. The following parameters were calculated: allelic and genotypic frequencies, observed (Ho) and expected (He) heterozygosity, fixation index and deviations from Hardy-Weinberg equilibrium (HWE). In the CAPN *locus*, higher frequencies of the T allele (70%) were found with respect to C (30%) and the frequencies of the TT (46%) and TC (48%) genotypes exceeded the CC (6%) genotype. For the CAST locus, the MM genotype (83%) was the most frequent, followed by the other genotypes (MN 16% and NN 0.5%) and thus the allelic frequencies were M: 91% and N: 9%. The allele A (66%) of the LEP *locus* was more frequent than the G allele (34%) and the AA, AG and GG genotypes had frequencies of 43, 47 and 10%, respectively. Only two genotypes (AA 64% and AG 36%) were found in the GH *locus* with allelic frequencies of 82 and 18% for A and G respectively. In the *locus* IGF-1, the genotypes AA, GG, and AG presented frequencies of 36, 49 and 15%, respectively, with a greater presence of the G allele (56%) than A (44%). In the analysed population, the GHR and MSTN *loci* were monomorphic, and only the AA and MM genotypes were found in each. The CAST, GH, and IGF-1 *loci* showed deviations from HWE. He deficit was found only in the IGF-1 *locus*. The CAPN, CAST, LEP, GH, and IGF-1 *loci* were polymorphic with high He and excess heterozygotes, in contrast, the GHR and MST *loci* were monomorphic. The data presented here could be used in animal breeding programmes.

Key words: *animal breeding; genetic diversity; zoogenetic resource*

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Introduction

The genetic resources of native breeds are irreplaceable and must be preserved, particularly given the growing demand for products of animal origin for human consumption. One of the first requirements in the process of conservation of genetic resources is the knowledge of the genetic variables (Bahrami et al., 2013). Molecular tools are used in genetic improvement programmes of animals through assisted selection by molecular markers, which improves accuracy and increases genetic progress by identifying, mapping and analysing polymorphisms of the genes involved in the main metabolic pathways related to animal growth, the distribution of nutrients in different tissues, and product quality (Sutikno et al., 2011).

Candidate genes having potential for use in animal selection programmes include: Calpain (CAPN), Calpastatin (CAST), Leptin (LEP), Growth Hormone (GH), Growth Hormone Receptor (GHR), Insulin-Like Growth Factor 1 (IGF-1) and Myostatin (MSTN).

The CAPN gene, located on chromosome 7, is involved in the degradation of myofibrillar proteins of the muscle after death, forming the biochemical basis of the softening of the meat (Georgieva et al., 2015). The calpain system is made up of the μ-calpain (CAPN1), m-calpain (CAPN2) and calpain 3 (CAPN3) genes, in which numerous SNPs correlated with gene expression and meat tenderness have been identified in different species (Knight et al., 2012). The CAST gene, located on chromosome 5, is the main inhibitor of the action of calpain, slowing the speed and the degree of post-mortem softening of the meat, which is counterproductive, since it reduces tenderness (Bagatoli et al., 2013).

In sheep, the LEP gene is located on chromosome 4. Leptin is an information transporting hormone that intervenes in the processes of food consumption, animal growth, and lipid metabolism, and it also acts on adipose tissue as a regulator of fat deposits (Bahrami et al., 2013), and on reproduction and body composition (Radhi et al., 2015). Growth hormone (GH) is produced in the pituitary gland, and is encoded by a gene located on chromosome 11 (Moradian et al., 2012). GH regulates postnatal growth and various metabolic processes such as cell division and growth, cartilage and bone formation and the increase of bone, muscle and visceral tissue (Hajihosseinlo and Negahdary, 2013). It plays a key role in the acceleration of metabolism (Jiang et al., 2014). GHR is located on chromosome 11, it binds to GH causing the dimerization of GHR initiating a signalling in the cytoplasmic domain, facilitating the transcription of other genes, regulating the effect of GH on the target cells (Bahrami et al., 2013).

The IGF-1 or Somatomedin C gene is a peptide of 70 amino acids synthesized in the liver, kidneys and cartilage. It stimulates cell growth and multiplication, and is an inhibitor of programmed cell death (Negahdary et al., 2013), increases glucose absorption, stimulates myogenesis, activates genes in the cell cycle, controls somatic growth and regulates the effects of growth hormone (Honarvar et al., 2012). Myostatin or the differentiation factor 8 (GDF8 or MSTN) gene is found on chromosome 2. It is a major regulator of myogenesis (Tellam et al., 2012), and it influences fat reduction, acting as an adipogenic regulator in muscle hypertrophy and conformation of the carcass, changing the number of muscle fibres, their composition and hypertrophy, and increasing the musculature, the number of myofibrils and fast glycolytic contraction fibres (Tellam et al., 2012).

In crossbreeds of Colombian Creole hair sheep (OPC) and Pelibuey sheep,

different genes have been studied and associated with reproductive (Pineda et al., 2018; Hernández et al., 2020) and productive characteristics (Montes et al., 2019), but not those related to growth and quality of meat. The objective of this study was to characterize single nucleotide polymorphisms (SNPs) in the CAPN, CAST, LEP, GH, GHR, IGF-1 and MSTN *loci* of genes in Columbian Creole hair sheep crossed with Pelibuey sheep.

Materials and methods

Sampling, DNA extraction and amplification conditions of the examined *loci*

A total of 192 blood samples were taken from individuals of Colombian Creole hair x (OPC) Pelibuey (F1) crossbred sheep from flocks located in the Department of Valle del Cauca, Colombia. DNA extraction was performed using the QIAamp® DNA Mini Kit from QIAGEN. The quantity and quality of the DNA were evaluated using a Colibrí Spectrometer (Gentech) and diluted to 30 ng/μL.

Amplification of the samples by PCR was performed in a final volume of 25 μL containing 30 ng DNA, 1U Taq polymerase, 1X Taq buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 2 mM of each primer, 1% BSA and 10% trehalose. The sequences of the primers used and the amplification conditions of each *loci* were carried out according to the authors as outlined in Table 1. The visualization of the size and quality of amplified fragments was verified by electrophoresis in 1.2% agarose gels, stained with GelRed (Biotium, Inc. USA).

Genotyping of the CAST and MSTN *loci* by PCR-RFLP

DNA fragments amplified by PCR from the CAST and MSTN *loci* were

Table 1. Sequences of the primers and alignment temperature at the *loci* studied

Locus	Primers	Annealing temperature	Fragment length
CAST[a,b]	F 5'-TGGGGCCCAATGACGCCATCGATG-3'	58 °C	622 pb
	R 5'-GGTGGAGCAGCACTTCTGATCACC-3'		
CAPN[b,c]	F5'-AACATTCTCAACAAAGTGGTG-3'	60 °C	190 pb
	R5-ACATCCATTACAGCCACCAT-3'		
LEP[d]	F5'-CGCAAGGTCCAGGATGACACC-3'	62.5 °C	260 pb
	R5'-GTCTGGGAGGGAGGAGAGTGA-3'		
GH[e,f]	F5'-CTGCCAGCAGGACTTGGAGC-3'	62 °C	200 pb
	R5'-GGAAGGGACCCAACAATGCCA-3'		
GHR[g,h]	F5'-GCCAAAACAATAAGACTGGGAACC-3'	60 °C	218 pb
	R5'-GGCTGTAGTGGTAAGGCTTTCTGTG-3'		
IGF-1[f]	F5'-ATTACAGCTGCCTGCCCCTT-3'	58 °C	295 pb
	R5'-CACATCTGCTAATACACCTTACCCG-3'		
MSTN[b]	F 5'-CCGGAGAGACTTTGGGCTTGA-3'	58.5 °C	337 pb
	R5'-TCATGAGCACCCACAGCGGTC-3'		

[a]Sutikno et al., 2011; [b]Azari et al., 2012; [c]Dehnavi et al., 2012; [d]Valeh et al., 2009; [e]Moradian et al., 2012; [f]Hajihosseinlo and Negahdary, 2013; [g]Bahrami et al., 2013; [h]Barzehkar et al., 2009

digested with 1U of the restriction enzymes *MspI* and *HaeIII* respectively, for 2 hours at 37 °C. The identification of genotypes was carried out as reported by Sutikno et al. (2011) and Azari et al. (2012). The digestion product was observed in vertical polyacrylamide electrophoresis at 9% (37:1) using 150 volts for 40 minutes, stained with GelRed (Biotium, Inc., USA) and analysed by Software Quality One® v4.1, in a system Image Gel 2000 (Bio-Rad Laboratories, Richmond CA).

Genotyping of the CAPN, GH, GHR, IGF-1 and LEP loci by PCR-SSCP

Briefly, 5 μL of the PCR product was mixed with 8 μL denaturing buffer (95% formamide, 0.05% Xylene-Cyanol, 0.05% bromophenol blue, 20 mM EDTA pH 8.0) and heated at 95 °C for 5 minutes, then subjected to vertical electrophoresis in 12% polyacrylamide (100:1) denaturing gels with 3.7% glycerol. The gels were run for 4 hours at 180-200 volts. Band visualization was performed by staining with GelRed (Biotium, Inc. USA) using the software Quality One® v4.1 Software, in the Gel Doc 2000 image system (Bio-Rad Laboratories, Richmond CA). The interpretation of the patterns and assignment of genotypes was performed as reported by the authors referred to in Table 1.

Statistical Analysis

The genotypic and allelic frequencies, observed (Ho) and expected (He) heterozygosity, fixation index (F) and Hardy-Weinberg equilibrium (HWE) deviations were calculated using the Arlequin program, version 3.5.2.2 (Excoffier and Lischer, 2010).

Results

Five of the seven SNPs evaluated were polymorphic. For the CAPN *locus*, a higher frequency of the T allele (70%) was found in relation to the C allele; the frequencies of the TT and TC genotypes were higher than the CC genotype (6%). In the CAST *locus*, the MM genotype was the most frequent, followed by the other two genotypes, and the M allele exceeded the N allele (9%) in frequency. In the LEP *locus*, the A allele was represented by 66%, and the G allele by 34%, where the AA, AG, and GG genotypes had frequencies of 43, 47 and 10%, respectively. The frequency of the A allele for the GH *locus* was higher than the G allele, and only the AA and AG genotypes were found, the first being the most frequent (64%). In exon 1 of the IGF-1 gene studied, the G allele was found in high frequency, and the three identified genotypes were present in frequencies of 36, 49 and 15% for AA, GG, and AG, respectively. In the analysed population, the GHR and MSTN *loci* were monomorphic; only the AA and MM genotypes were found in each. The detailed genotypic and allelic frequencies are presented in Table 2.

The average value of Ho found for all *loci* was 0.324±0.16. The highest percentage of observed heterozygotes was found in the CAPN, LEP and GH *loci*. Likewise, in the GHR and MSTN *loci*, the value of Ho was zero. The average He for all *loci* was 0.363±0.13. The IGF-1, LEP and CAPN *loci* showed the highest value of expected heterozygotes. He was higher than Ho only for the IGF-1 *locus*, presenting a high value of F and significant deviations from HWE. For the other *loci* evaluated, negative values of F were significant in the GH *loci* (Table 3).

Discussion

This is the first report of polymorphisms in the CAPN, CAST, LEP, GH, GHR, IGF-1 and MSTN *loci* in Colombian Creole hair sheep crossed with Pelibuey sheep. In the CAPN gene, variation is associated with growth characteristics (Fang et al., 2013). It is located between exons 5 and 6, and consists of a synon-

Table 2. Genotypic and allelic frequencies at the CAPN, CAST, LEP, GH, GHR, IGF-1 and MSTN *loci* in Colombian Creole hair (OPC) x Pelibuey sheep.

Locus	Frequencies				
	Genotype			Allelic	
CAPN	TT	TC	CC	T	C
	0.46	0.48	0.06	0.70±0.02	0.30±0.02
CAST	MM	MN	NN	M	N
	0.835	0.16	0.005	0.91±0.01	0.09±0.01
LEP	AA	AG	GG	A	G
	0.43	0.47	0.10	0.66±0.02	0.34±0.02
GH	AA	AG	GG	A	G
	0.64	0.36	-----	0.82±0.02	0.18±0.02
GHR	AA	AG	GG	A	G
	1.00	-----	-----	1.00±0.00	-----
IGF-1	AA	AG	GG	A	G
	0.36	0.15	0.49	0.44±0.03	0.56±0.03
MSTN	MM	MN	NN	M	N
	1.00	-----	-----	1.00±0.00	-----

Table 3. Ho, He, F values and deviations of the EHW at the *loci* studied in Colombian Creole hair (OPC) x Pelibuey sheep.

Locus	Ho	He	F	^{P}HWE
CAPN	0.479	0.422	-0.136	0.059^{ns}
CAST	0.161	0.157	-0.028	0.015^{ns}
GH	0.359	0.295	-0.219	0.002*
GHR	0.000	0.000	N/C	N/C
IGF-1	0.151	0.492	0.693	0.000**
LEP	0.469	0.448	-0.047	0.518^{ns}
MSTN	0.000	0.000	N/C	N/C

Ho: observed heterozygosity. He: expected heterozygosity. F: fixation index. ^{P}HWE: Probability of Hardy-Weinberg equilibrium (chi-square value). N/C: not calculated. *P<0.01. **P<0.001. ns: not significant.

ymous transition of T/C in nucleotide 44 of the gene (Azari et al., 2012; Dehnavi et al., 2012) (eg. AF309634.1). Similar results were reported for two biotypes of the Colombian creole sheep breed (OPC) (Montes et al., 2019). In Coloured Polish Merino sheep, three alleles were reported: the A1 allele and A1B1 genotype were predominant with frequencies of 0.467 and 0.419, respectively (Grochowska et al., 2017). In Baluchi sheep, three band patterns were reported with genotype frequencies of 0.082 (G1), 0.89 (G2) and 0.03 (G3). Contrary to the results presented here, the CC genotype and the C allele as the most frequent in three Egyptian

sheep breeds (Barki, Rahmani, and Ossimi), with the absence of the TT genotype in the Barki and Ossimi breeds (Mahrous et al., 2016). In Bandur sheep, two alleles were reported with frequencies of 0.82 (A) and 0.18 (B), and two genotypes AA (0.67) and AB (0.30) (Khan et al., 2012). In Iranian Zel sheep, similar allelic and genotype frequencies were found in Bandur sheep (Azari et al., 2012) and Kurdi sheep (Nassiry et al., 2007). The results reported here for the CAPN *locus* were higher than for the Bandur (He: 0.295; Naveen et al., 2015), Zel (He: 0.260; Azari et al., 2012), Kurdi (He: 0.07; Nassiry et al., 2007) breeds, and were similar to reports for the Colombian creole sheep breed (OPC) (Montes et al., 2019). On the other hand, in this *locus* a similar non-significant excess of heterozygotes was found in the Kurdi race (Nassiry et al., 2007), though this differed from the Bandur (Naveen et al., 2015), OPC (Montes et al., 2019) and Zel (Azari et al., 2012) breeds, where the deficit of heterozygotes in the latter was significant.

In the CAST gene (AF016006, AF016007 and AF016008.1), the fragment digested by the *MspI* enzyme (M allele) is a substitution of A for G located in the intronic region between exons 1C and 1D27. For this *locus,* the PCR-RFLP and PCR-SSCP techniques have been compared as alternatives for genotyping, and it was possible to determine that the A and C alleles obtained by SSCP could be digested with the *MspI* enzyme, that the AA, AC and CC genotypes are similar to the MM genotype determined by RFLP, that the MN genotype is similar to the AB and BC genotypes (by SSCP) and finally, that the NN genotype agrees with the BB genotype (Valeh et al., 2009). Similar frequencies were found in the OPC breed (Montes et al., 2019). In Dalagh sheep, the genotypes found were MM (36%), MN (38%) and NN (26%) with a majority of the M allele (55%) (Dehanavi et al., 2012); this latter frequency was higher in the OPC (Table 2). In Iranian sheep of the Zel and Afshari breeds, alleles A and B were reported with frequencies of 85.5 and 14.5%, respectively, and alleles M and N with frequencies of 74% and 26%, respectively. However, the frequency of the NN genotype in these reports (10%) was higher than that presented here (Dehanavi et al., 2012; Nikmard et al., 2012). As in this study, significant deviations of HWE as an excess of heterozygotes were found in the Dalagh sheep, which had a considerably higher value of He (Azari et al., 2012). The M allele of this *locus* has been associated with high birth weights (Byun et al., 2008).

The fragment amplified in the LEP *locus* is located between exon 2 and part of intron 2 (HE605296.1), presenting two SNPs that are synonymous transitions of A/G and C/T (Valeh et al., 2009). These SNPs were observed in the population examined here. Allele A has been shown to significantly affect lamb weaning weight, carcass composition (subcutaneous fat) and metabolic characteristics (citrate synthase activity) in the Longissimus dorsi muscle (Valeh et al., 2009). Found in the Shal, Zandi and Zel breeds, the transition of A/G has allele frequencies of 0.74 and 0.26 (Shal), 0.85 and 0.15 (Zandi) and 0.82 and 0.15 (Zel) respectively (Valeh et al., 2009), which is consistent with our results in which the frequency of the A allele was always higher (0.66/0.34; A/G). However, we found the GG genotype, which previous studies did not. Similar results were reported in the Dorset and Suffolk breeds, where the A allele was also the most frequent (0.87 and 0.93 for Dorset and Suffolk, respectively) (Boucher et al., 2006). Nevertheless, six alleles and six genotypes were found in Awassi sheep using the PCR-SSCP technique (Radhi et al., 2015), as compared with the two alleles and three genotypes found here. Similarly, high polymorphism (10

conformational patterns) was reported in Kermani sheep in a 275bp fragment located in exon 328. HWE was found in the breeds of Shal, Zandi, Zel, Dorset, and Suffolk (Boucher et al., 2006; Valeh et al., 2009) with an average He of 0.216, which is lower than our results.

The GH gene amplifies a fragment of approximately 200bp, located in exon 5 of chromosome 11 (eg. AF002110.1). In Nilagiri sheep native to the Nilgiris of Tamil Nadu, the frequency of GG, GA and AA genotypes were 0.48, 0.43 and 0.09, respectively (Hajihosseinlo and Negahdary, 2013), while in the present study, the GG genotype was not found. Additionally, the proportion of allele frequencies differed, with a higher proportion of the G allele than the A allele (Cauveri et al., 2016). In Kermani sheep, ten genotypes were found using PCR-SSCP, including A/A, C/C, A/B, A/C, A/B/C, A/B/E, A/B/F, A/C/F, A/B/D/E and A/B/C/F (Shojaei et al., 2010). Also, in Baluchi sheep, three band patterns with genotype frequencies of 0.082 (G1), 0.89 (G2) and 0.03 (G3) were reported by Valeh et al. (2009). At this *locus,* a significant excess of heterozygotes was found with deviations from the theoretical proportions of the HWE. On the other hand, the AA genotype was associated (P<0.05) with weight at weaning and daily gain of preweaning weight, but not with weights at ages after weaning (Cauveri et al., 2016).

The fragment amplified for the GHR *locus* is located in exon 10 (eg. AY292283.1). In the present study, this *locus* was monomorphic, as also reported in the Mehraban breed (Bahrami et al., 2013). Additionally, the animals genotyped here came from a single flock, which can imply less variation by inbreeding effects. On the other hand, in Baluchi sheep, this *locus* was polymorphic with frequencies of 31, 61 and 8% for the genotypes AA, AG and GG, respectively (Valeh et al., 2009).

In the amplified *locus* of exon 1 of the IGF-1 gene (eg. AF492765.1), three genotypes and two alleles were identified (Table 2). Similar band patterns were found in the Makooei (Negahdary et al., 2013) and Zel sheep (Kazemi et al., 2011), and similar allelic frequencies were reported in the Barki breed (Darwish et al., 2017), with slight changes in genotype frequencies. On the other hand, in Mehraban sheep, the reported frequencies differ significantly (Bahrami et al., 2013) to our results.

Other authors report more polymorphisms at this *locus*, finding three band patterns in Mehraban sheep (Behzadi et al., 2015), four in Zel sheep (Honarvar et al., 2012) and five in Makooei sheep (Hajihosseinlo et al., 2013). A heterozygous deficit was found for this *locus*, affecting HWE, and this differed from reports for the Barki (Darwish et al., 2017), Mehraban and Makui breeds (Bahrami et al., 2013; Hajihosseinlo and Negahdary, 2013; Behzadi et al., 2015). The AA genotype for this *locus* has been associated with birth weight, weaning weight, weight at six months, a daily gain of preweaning and postweaning weight, body length and thickness of fat on the rump (Bahrami et al., 2013; Negahdary et al., 2013; Chelongar et al., 2014).

Finally, the MSTN *locus* for the studied population was monomorphic (MM), as also reported in Barki, Ossimi, Najdi and Harri (Elkorshy et al., 2013) sheep and in Dalagh sheep (Azari et al., 2012). According to Mirhoseini and Zare (2012), in Karakul, Zel and Makoie Iranian sheep, a substitution in exon 3 where the G/A nucleotide change occurs is a possible marker of double musculature in sheep. The mutation of this gene has been found in different breeds such as Texel Belga, Romney, Bulgarian and Dalagh (Azari et al., 2012; Georgieva et al., 2015) and the crosses Texel x Poll Dorset and Texel x Welsh Mountain (Tellam et al., 2012).

Due to its simplicity, the PCR-SSCP procedure has been applied successfully in many laboratories, though some reports have shown differing mobility patterns. Some reasons that could explain why more than two bands can be observed in single-stranded DNA strands of the same sequence are related to the molecular stability of the chain, which can be affected by an excess of primers, slow handling after the denaturation that causes a hybridization of DNA strands (Bahrami et al., 2013), or by electrophoresis conditions, where factors such as temperature, running time, buffer concentration, gel composition, position of the base change in the analysed fragment, length fragment, etc. can influence sensitivity (Shojaei et al., 2010). In the sequencing of samples, we observed SNPs similar to those reported previously for each *locus*, supporting the results found.

Based on our results, the investigated population showed a high degree of genetic variability for the examined *loci*. This might be explained by the lack of conservation and breeding strategies in these animals. However, this may open interesting prospects for future selection programmes, especially for the use of marker-assisted selection for improving weight gain and meat quality.

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Polimorfizmi lokusa CAPN, CAST, LEP, GH, GHR, IGF-1 i MSTN u križanaca kreolske dlakave ovce iz Kolumbije s ovcama pelibuey pasmine

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Cilj ove studije bio je okarakterizirati polimorfizam pojedinačnih nukleotida kod Calpaina (CAPN), kalpastatina (CAST), leptina (LEP), hormona rasta (GH), receptora hormona rasta (GHR), inzulinu sličnog čimbenika rasta 1 (IGF-1) i miostatina (MSTN) u križanaca kreolske dlakave ovce iz Kolumbije s ovcama pelibuey pasmine. U 192 jedinke, lokusi CAST i MSTN genotipizirani su PCR-RFLP, a lokusi CAPN, GH, GHR, IGF-1 i LEP pomoću PCR-SSCP i sekvenci. Izračunati su sljedeći pokazatelji: alelna i genotipska frekvencija, uočena (Ho) i očekivana heterozigotnost (He), indeks fiksacije i odstupanja od ravnoteže Hardy-Weinberga (HWE). U lokusu CAPN nađene su veće frekvencije alela T (70 %) u odnosu na C (30 %), a frekvencije genotipa TT (46 %) i TC (48 %) su nadmašile CC (6 %). Kod CAST-a, MM genotip (83 %) je bio najčešći, a slijede ga ostali genotipovi (MN: 16 % i NN 0,5 %), tako da su alelne frekvencije bile M: 91 % i N: 9 %. Alel A (66 %) lokuse LEP bio je češći od alela G (34 %), a AA, AG i GG genotipovi imali su frekvencije 43, 47 i 10 %. U lokusu GH pronađene su samo dva genotipa (AA: 64 % i AG: 36 %) s alelnim frekvencijama 82 i 18 % za A i G. U lokusu IGF-1, genotipovi AA, GG i AG prikazali su frekvencije od 36, 49 i 15 %, s većom prisutnošću alela G (56 %) od A (44 %). U analiziranoj populaciji, lokusi GHR i MSTN bili su monomorfni, a u svakom su pronađeni samo AA i MM genotipovi. Lokusi CAST, GH i IGF-1 pokazali su odstupanja od HWE. Jedino je u IGF-1 lokusu ustanovljen manjak He. Lokusi CAPN, CAST, LEP, GH i IGF-1 bili su polimorfni s visokim He i viškom heterozigota. Nasuprot tome, lokusi GHR i MST bili su monomorfni. Rezultati prikazani u ovom istraživanju mogu se koristiti u programima uzgoja životinja.

Ključne riječi: *uzgoj životinja, genetska raznolikost, zoogenetski resurs*