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Polymorphism of MC4R gene in New Zealand White and Californian rabbit breeds

Milena Bozhilova-Sakova[1*], Denis Viryanski[1], Maya Ignatova[1], Ivona Dimitrova[2]

[1] *Department of Genetics, Breeding and Reproduction, Institute of Animal Science, Agricultural Academy, Kostinbrod, Bulgaria*
[2] *Agronomy Faculty, Department of Plant Protection, University of Forestry, Sofia, Bulgaria*
[*]*Corresponding author:* bojilova_milena@abv.bg

Abstract

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The aim of present work was to study the genetic variation of MC4R gene, associated with meat production and body weight in New Zealand White (NZW) and Californian (CA) rabbit breeds. Blood samples were collected from *v. saphena* of 60 animals (50 does and 10 bucks) from the experimental flocks raised in rabbit farm part of Institute of Animal Science – Kostinbrod, Bulgaria. Blood samples were stored in vacuum tubes at -20°C until DNA purification. The genetic variation was determined by means of PCR-RFLP method, using specific set of primers and restriction enzyme *BcuI*.In both breeds were identified all three possible genotypes (GG, AG and AA) of MC4R gene, and in both studied breeds the frequency of heterozygous genotype AG was higher than the other genotypes – 0.68 for NZW and 0.53 for CA. The mutant genotype AA was with lowest frequency in both breeds – 0.12 for NZW and 0.07 for CA. This is the first study in Bulgaria of MC4R gene. Further tests are pending to establish the influence of each genotype on the growth development of several rabbit generations from both breeds.

Keywords: Rabbit breeding; PCR-RFLP; MC4R gene; Polymorphism; Body weight

Introduction

In recent years, with the development and implementation of modern genetic approaches in animal breeding, the possibilities for achieving selection that is more effective are increasing. The use of candidate genes and marker-assisted selection could improve traits, which are difficult to manage through conventional selection (Gencheva et al., 2022).

In Bulgaria, rabbits are raised mainly for meat production. Despite the fact that the sector is not well developed, rabbit meat production is in demand on the market due to its dietary properties, such as low fat and high protein content (Wang et al., 2022).

The growth characteristics are the most important economic factor in rabbit breeding. They depend on non-genetic factors, such as breed, sex and feeding, but also could be genetically determined.

Genetic markers are widely used for the identification of polymorphisms at the DNA level, genotyping and genetic mapping. Genes, whose allelic variants are associated with the phenotypic expression of economically important traits are considered as DNA markers. That is why the knowledge of the genetic variants, which are related to productive characteristics in rabbits, is extremely important, as it could help to predict the results of selection very accurately (Yadav et al., 2017).

According to NCBI database rabbit genome consists 44 chromosomes, 2737.46 Mb, comprising 29098 genes and more than 19000 described proteins. Finding of suitable genes associated with productive traits is in great interest

in many countries worldwide (Miller et al., 2014). Several genes affect weight gain in rabbits. One of this gene considered as genetic marker in rabbits is MC4R (Song et al., 2022)

The MC4R receptor gene encodes the protein known as melanocortin. A G protein-coupled receptor binds to Alpha-melanocyte stimulating hormone. In mice part of its function is responsible for the feeding behavior, regulation of metabolism, sexual behavior and male erectile function. It is also linked with the central melanocortinergic pathway, which plays an important role in the control of mammalian energy homeostasis (Radwan et al., 2022).

Melanocortin 4 Receptor (MC4R) gene is linked with rabbit body weight gain. The melanocortin 4 receptor gene (MC4R, ENSOCU G00000025457) is located on chromosome 9 (100,687,330-100,688,331) in rabbits and has a single exon transcript. In addition, the MC4R gene controls food intake, body weight, and fat deposition. Mutations in the MC4R gene are associated with different types of obesity. The MC4R gene also controls glucose homeostasis and insulin sensitivity (Helal et al., 2022). Polymorphisms in the melanocortin 4 receptor (MC4R) gene have been already associated with growth performance in different species. MC4R is mainly expressed in the hypothalamus, which plays a key role in controlling energy homeostasis and food intake with effects on body weight and fat storage (Fontanesi et al., 2013; Osaiyuwu et al., 2020; Radwan et al., 2022).

There are three known genotypes of rabbit MC4R gene for locus – GG, AG and AA. It was found that the mutant genotype AA have been associated to higher live weight in rabbits (Nahácky et al., 2018; Osaiyuwu et al., 2020).

Studies in different species showed similar patterns and results associated to the MC4R gene. For example, in pigs a missense mutation of the swine melanorcortin-4 receptor gene (MC4R) has been related to growth and fatness. In the F2 population of 111 animals, Chen et al. (2004) examined the association between the MC4R polymorphism and growth and obesity-related: age at 100 kg weight (Days), ham circle (HC), body length (BL), rear quarters weight (RW), backfat (probed, BF), average carcass backfat (ABF) and etc. They studied TaqI recognition site. The polymorphism revealed a misssence mutation that replaces aspartic acid (GAU) with asparagine (AAU) at the position identical to amino acid 298 of pig MC4R protein. The research team discovered that mutant genotype had tendency for higher weight performances.

MC4R was also studied in cattle. Maharani et al. (2018) investigated different traits, such as birth weight (BW), weaning weight (WW), birth body length (BBL), birth chest circumference (BCC), birth shoulder height (BSH), weaning body length (WBL), weaning chest circumference (WCC), weaning shoulder height (WSH) and average daily gain (ADG). High birth body length was associated with the GG genotype and the MC4R gene SNP at 1133 C>G with the frequency of (0.59) for the G allele relative to the C allele (0.41). As a conclusion, the SNP at g. 1133 C>G may be a relevant marker for calf selection based on birth body length.

In Bulgaria several genetic studies have been carried out involving rabbits. They included PCR-RFLP and sequence analysis of myostatin gene (MSTN), growth hormone receptor gene (GHR) and growth hormone gene (GH) (Hristova et al., 2017; Hristova et al., 2018; Gencheva et al., 2021; Gencheva et al., 2022).

The aim of present study was to identify the genotypic variants of the melanocortin-4 receptor gene (MC4R) associated with body weight in 60 animals from Californian (CA) (30 bucks and does) and New Zealand White (NZW) (30 bucks and does) broiler rabbit breeds. The investigation was conducted on experimental flocks from Institute of Animal Science in Kostinbrod, Bulgaria. This was the first study on rabbit MC4R gene in Bulgaria.

Materials and Methods

Animals

In this study were used a total of 60 adult rabbits (50 does and 10 bucks) from New Zealand White (25 does and 5 buck) Californian breeds (25 does and 5 bucks) (Figure 1 and 2). The female and male animals from the two breeds were selected for their typical phenotypic characteristics. The does from New Zealand White breed were with mean weight of 4,3 kg and at age from 1 to 1,5 years. The bucks from this breed were with average weight of 4 kg. The does from Californian breed were with mean weight of 4kg and at age from 1 to 1,5 years. The bucks were with average weight of 3,8 kg.

Fig. 1. Animal from experimental herd of New Zealand White rabbit breed from Institute of Animal Science – Kostinbrod

Fig. 2. Animal from experimental herd of Californian rabbit breed from Institute of Animal Science – Kostinbrod

DNA extraction

Blood samples were collected from *vena saphena* in vacuum tubes containing EDTA. Rabbits were fed *ad libitum* by commercial pelleted feed and had free access to water. Animals were maintained under standard conditions of humidity, temperature and photoperiod. The rabbits were raised in enclosed one-floor building. Adult animals were housed separately in galvanized wire cages (40 × 60 × 50 cm3), fitted with a nipple drinking system and a manual feeder. During the experiment, all institutional and national guidelines for the care and use of animals were followed. All tested animals were healthy during the experiment. All activities have been carried out in accordance with the recommendations contained in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

PCR amplification

Genomic DNA was extracted from whole blood using Illustra Blood Genomic Prep DNA Purification Kit of GE Healthcare (UK), according to the manufacturer's instructions. The DNA concentration of each sample was determined using a Biodrop spectrophotometer. The quantity of the obtained DNA was about 10–50 ng and it was tested using gel monitoring on 1% agarose gel (Healthcare) prepared with Tris-acetate-EDTA (TAE) buffer (Jena Bioscience). Primers were chosen according to Fontanesi et al. (2013) and were with sequences:

forward primer, 5′ CAT GAA CTC CAC CCA CCA C 3′

reverse primer, 5′ CTC ATA GCA CCC TCC ATC AGA CTA G 3′

DNA amplification was carried out by thermal cycler (QB-9, Quanta Biotech) in a final volume of 20μL containing 8 μL of DNA template, 0.4 μL of ddH2O, 0.8 μL of each primer (Bioneer) and 10 μL of 2× (1.5 mmol/L MgCl2) Red Taq DNA Polymerase Mastermix (Bioline). The specific PCR conditions were: primary denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, elongation at 72°C for 1 min. and the process was completed by final extension at 72°C for 10 min.

Restriction analysis

PCR products were digested by restriction enzyme*BcuI* (Thermo Fisher Scientific, UK). The restriction fragments were subjected to electrophoresis in 2.5% agarose gel stained by Red Gel Nucleic Acid Stain (Biotium), 1× TBE buffer at 90 V for 30-40 min. The bands were visualized under ultraviolet transilluminatior and photographed in Hi-UVTM Duo Capture (HIMEDIA).

Results

As a result, in this study was detected genetic polymorphism of MC4R (195C>T) gene in the two rabbit breeds. After DNA extraction were purified 60 samples with DNA concentration approximately 30-40 ng/μL and the quality of DNA was tested on 1% agarose gel (Figure 3).

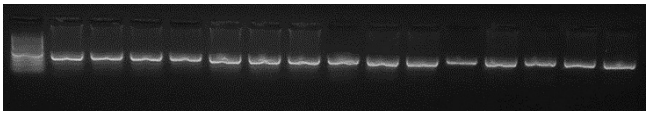

Fig. 3. Electrophoretic testing of DNA samples on 1% agarose gel

All samples were amplified and resulted in PCR products with the expected length of 127 bp (Figure 4).

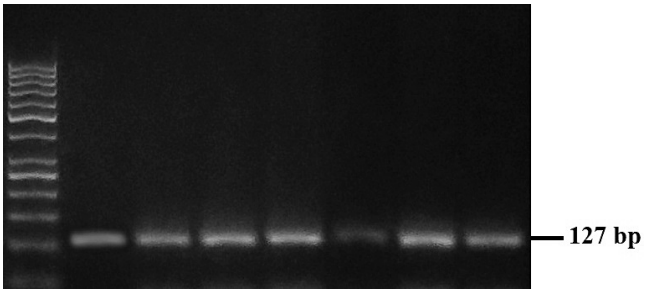

Fig. 4. PCR products of rabbit MC4R gene visualized on agarose gel

RFLP analysis of PCR product using *BcuI* restriction endonuclease produced two fragments of 100-bp and 27-bp for mutant allele A and one uncut fragment of 127 bp for wild allele G. The wild genotype GG was visualized with

only one band on the agarose gel with length of 127 bp. The heterozygous genotype AG was visualized with three bands with lengths of 127 bp, 100 bp and 27 bp. The homozygous mutant genotype AA revealed two bands on the agarose gel with lengths of 100 bp and 27 bp (Figure 5).

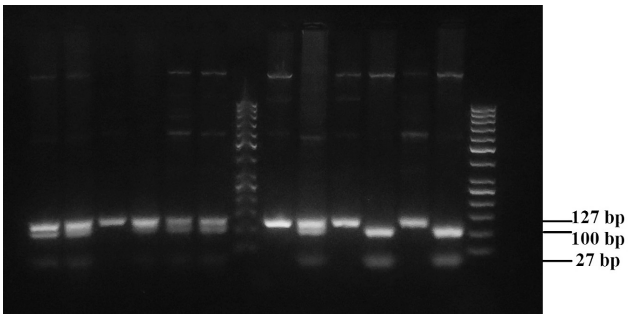

Fig. 5. Restriction fragments of MC4R gene after digestion with *BcuI* enzyme visualized on 2,5 agarose gel

As a result, it can be reported that in both studied breeds presented the two alleles (G and A) of MC4R gene and all three possible genotypes (GG, AG and AA) were determined with different distribution. However, the heterozygous genotype AG was predominant in both breeds with frequency of 0.63 for NZW and 0.53 for CA breed. The homozygous mutant genotype AA was with higher frequency of 0.12 in NZW compared to 0.07 for CA breed. The homozygous wild genotype GG was more frequent in CA (0.40) in comparison of NZW (0.20). In both breeds the coefficient of inbreeding for MC4R gene was negative – -0.357 for NZW and -0.206 for CA. According to the result both breeds consisted with Hardy-Weinberg equilibrium (p>0.05) (Table 1, Figure 6).

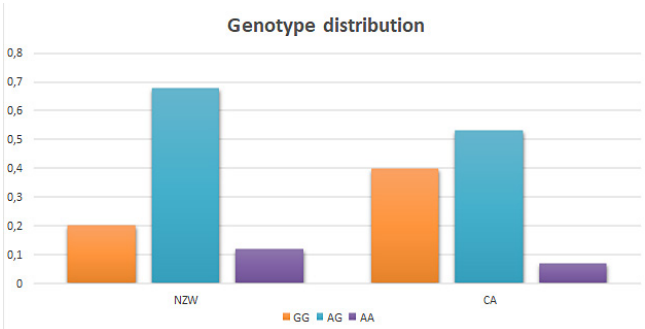

Figure 6. Genotype distribution of the three studied genotypes between the two rabbit breeds

Discussion

Osaiyuwu et al. (2020), using 6 different rabbit breeds (20 Fauve de Bourgogne, 26 Chinchilla, 10 New Zealand white, 11 Dutch, 4 English Spot, and 3 Californian), studying the polymorphism of MC4R gene and detected SNP at c.101 > A, which produced three genotypes (AA, AG and GG), linked with body weight in rabbits. The highest allele frequency was observed in the AG genotype (0.69), which is similar to the results in the current experiment. According to their study, the genotype AA may have the potential to be associated with higher body weight compared to genotypes AG and GG.

Similar to the results in the present study, Nahácky et al. (2018) also detected the presence of two alleles (A and G) and three genotypes (AA, AG and GG) in their investigation on 44 breed lines 84 post-weaned rabbits (meat line P91 – 18 males, meat line M91 – 26 females). The research team reported that the heterozygous genotype AG was with highest frequency, which is in agreement with results in the present study.

Obtained genotype frequencies in this study of SNP c.101G>A of MC4R were comparable with Fontanesi et al. (2013). They resequenced 1729 bp of the rabbit melanocortin 4 receptor (MC4R) gene in 31 rabbits from different breeds/lines and identified ten polymorphisms: one was an indel and 9 were single nucleotide polymorphisms (SNPs). The indel and 5 SNPs were in the 5'-flanking region, 3 were synonymous SNPs and one was a missense mutation (c.101G>A; p. G34D), located in a conserved position of the extracellular tail of the MC4R protein, which was similar to the present study. The missense mutation was analyzed in a panel of 74 rabbits of different breeds. Association analysis indicated that rabbits with the genotype DD had a lower weight at 70 postnatal days than animals with genotype GD ($P < 0.10$) and animals with genotype GG ($P < 0.05$).

Conclusions

This was the first genetic study on MC4R gene conducted in Bulgaria. According to the results, the MC4R gene was

Table 1. Allele and genotype frequencies, Ho and He, and Fis of SNP c.101G>A of MC4R

Breed	n	Allele frequency		Genotype frequency			Heterozygosity		Fis	X^2	p-value
		G	A	GG	AG	AA	Ho	He			
NZW	30	0.54	0.46	0.20	0.68	0.12	0.677	0.499	-0.357	3.33	0.07*
CA	30	0.67	0.33	0.40	0.53	0.07	0.533	0.442	-0.206	1.77	0.18*

* p > 0.05 – statistically non-significant difference

found to be polymorphic with presence of two alleles and three genotypes in both tested rabbit breeds. This was a preliminary study on the genetic diversity of does and bucks. The experimental work continues with their offspring and investigation of association between different genotypes and body weight of rabbits is pending.

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