Bulgarian Journal of Agricultural Science, 30 (No 3) 2024, 523–526

PCR-RFLP analysis of PGAM2 gene in two rabbit breeds

Denis Viryanski[1], Milena Bozhilova-Sakova[1*], Maya Ignatova[1], Ivona Dimitrova[2] and Mostafa Helal[3]

[1] *Agricultural Academy, Department of Genetics, Breeding and Reproduction, Institute of Animal Science, 2232 Kostinbrod, Bulgaria*
[2] *University of Forestry, Department of Plant Protection, Faculty of Agronomy, 1756, Sofia, Bulgaria*
[3] *Cairo University, Department of Animal Production, Faculty of Agriculture, Giza 12613, Egypt*
[*]*Corresponding author:* bojilova_milena@abv.bg

Abstract

Viryanski, D., Bozhilova-Sakova, M., Ignatova, M., Dimitrova, I. & Helal, M. (2024). PCR-RFLP analysis of PGAM2 gene in two rabbit breeds. *Bulg. J. Agric. Sci., 30*(3), 523–526

The present study was conducted in order to investigate the genetic variability of the PGAM2 gene associated with meat productivity and body weight in rabbits. Blood samples were collected from *v. saphena* of 60 adult breeding animals from New Zealand White and Californian rabbit breeds (50 does and 10 bucks) from the experimental flocks raised in the rabbit farm part of the Institute of Animal Science – Kostinbrod, Bulgaria. The genetic diversity was determined by means of PCR-RFLP method using a specific set of primers and restriction enzyme *Csp6I*. As a result, it can be concluded that in the tested group of New Zealand White rabbit breed, polymorphism was not detected in the selected region of the PGAM2 gene. All animals were monomorphic for the investigated region and only genotype CC was presented. In California rabbit breed, the genetic diversity was existed in this gene with the presence of two genotypes – homozygous wild genotype CC and heterozygous genotype CT with frequencies 0.70 and 0.30, respectively. The experimental work will continue with further investigation in association of the live weight and different genotypes of the PGAM2 gene in rabbits from Californian breed.

Keywords: rabbit breeding; genetic diversity; PCR-RFLP; PGAM2 gene; polymorphism

Introduction

According to the UN, by 2050 the population of the Earth will reach 9.6 billion, which inevitably raises the question of feeding this population. According to FAO, millions of people around the world cannot eat healthy and have serious deficiencies in vital nutrients. The use of every food resource is essential. Rabbits are very prolific animals with rapid and effective reproduction, high feed conversion rate and low maintenance cost. Between the third and fifth months, a rabbit could be considered a fully developed adult animal suitable for reproduction and the meat industry. The requirement and demand for high-quality rabbit meat products are gradually increasing because of their dietary and nutritional qualities (Frinza et al., 2023).

Various methods and strategies are being developed worldwide to obtain a larger amount of output with less cost and in a shorter period of time. Therefore, the implementation of marker-assisted selection in rabbit breeding, which is mainly aimed at the production of meat with good nutritional values, will facilitate the whole process. Candidate genes associated with weight development in different rabbit breeds have already been developed. One of these genes is PGAM2 (El-Sabrout & S.A Aggag, 2017).

Phosphoglycerate mutase 2 (PGAM2, ENSOCUG00000027853) is located at chromosome 10 in the rabbit genome (44,639,812–44,641,590) and it consists of 3 exons and 2 introns with alength of 1840 nucleotides. The main function of the PGAM enzyme is to regulate glycolysis and glycogenesis in the mammalian cells. There are two

known forms of PGAM, PGAM1 is the brain form (B form, also known as PGAM1), which is commonly expressed, and the muscle form (M form, also known as PGAM2), which is expressed only in skeletal and cardiac muscles of adult individuals (Helal et al., 2021).

Restriction analysis of the PGAM2 gene in rabbits using restriction enzyme Csp6I distinguishes between the two alleles (C and T). Allele C produced two fragments of 613-bp and 242 bp; allele T resulted in three fragments of 309 bp, 304 bp and 242 bp (fragments 304 and 309 bp appeared as a single band after electrophoresis). The wild genotype CC was visualized with two bands on the agarose gel with lengths of 613 bp nd 242 bp. The heterozygous genotype CT was visualized with three bands with lengths of 613 bp, 309/304 bp and 242 bp. The homozygous mutant genotype TT revealed two bands on the agarose gel with lengths of 309/304 bp and 242 bp (Nahácky et al., 2018).

The aim of the present study was to investigate the genetic variability of the PGAM2 gene associated with meat productivity and body weight in rabbits in 60 adult breeding animals from New Zealand White and Californian rabbit breeds (50 does and 10 bucks) from the experimental flocks raised in the rabbit farm part of Institute of Animal Science – Kostinbrod, Bulgaria. This is the first study of PGAM2 gene in rabbit breeds in Bulgaria.

Materials and Methods

Animals

In this study were included 60 adult breeding rabbits (50 does and 10 bucks) from New Zealand White (25 does and 5 bucks) and Californian breeds (25 does and 5 bucks). The female and male animals from the two breeds were selected for their typical phenotypic characteristics. Does from the New Zealand White breed had an average weight of 4.3 kg and they were aged 1 to 1.5 years. The bucks from this breed had an average weight of 4 kg. The does from the Californian breed had an average weight of 4 kg and they were at the age from 1 to 1.5 years. The bucks had an avarage weight of 3.8 kg. The rabbits were raised in enclosed oyne-floor building. Animals were housed separately in galvanized wire cages (40 × 60 × 50 cm) fitted with a nipple drinking system and a manual feeder. Animals were maintained under standard conditions of humidity, temperature and photoperiod. Food and water intake were available *ad libitum*. Rabbits were fed by commercial pelleted feed. All procedures involving animals were done according to protocols for animal welfare.

DNA extraction

For the purpose of the present experiment, 60 blood samples were collected from *vena saphena* in vacuum tubes containing EDTA. DNA was extracted from whole blood using llustra 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).

PCR amplification

PCR amplification was conducted by a thermal cycler (QB-9, Quanta Biotech) in a final volume of 20 µL containing 8 µL of DNA template, 0.4 µL of ddH_2O, 0.8 µL of each primer (Bioneer) and 10 µL of 2×(1.5 mmol/L $MgCl_2$) 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 a final extension step at 72°C for 10 min. Primers were chosen according to Fontanesi et al. (2013) and were with sequences:
forward primer, 5´GAA TGC TGA TTG GCA GTT GGC3´
reverse primer, 5´CCA GTT GTC TGA AAC CCC TGT G 3´

Restriction analysis

After amplification, 60 PCR products were obtained, and then digested by the restriction enzyme Csp6I (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 100 V for 40–50 min. The bands were visualized under ultraviolet transilluminator and photographed in Hi-UVTM Duo Capture (HIMEDIA).

Results

After the DNA extraction, 60 samples were purified with a DNA concentration of approximately 30–40 ng/µL. The samples were equaled to work concentration of approximately 10–11 ng/µl using TE buffer (Thermo Fisher Scientific, UK), and the quality of DNA was tested on 1% agarose gel (Figure 1).

In the present study could be announced that the PCR-RFLP approach was suitable for genotyping PGAM2 gene in both studied breeds. In NZW rabbits, only the wild allele C

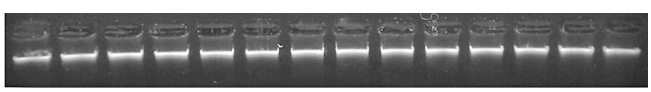

Fig. 1 The concentration of extracted DNA samples was levelled to 10 ng/µl

and the wild genotype CC were presented as shown in Figure 2. All animals were monomorphic for the tested region of PGAM2 gene. However, In the Californian rabbits,the two alleles C and T of PGAM2 gene were detected with frequencies of 0.67 and 0.33, respectively. Two genotypes were identified – homozygous wild genotype CC with a frequency of 0.70 and heterozygous genotype CT with a frequency of 0.30.According to the result the tested Californian rabbit breed consisted with Hardy-Weinberg equilibrium ($p > 0.05$) (Table 1, Figure 3)

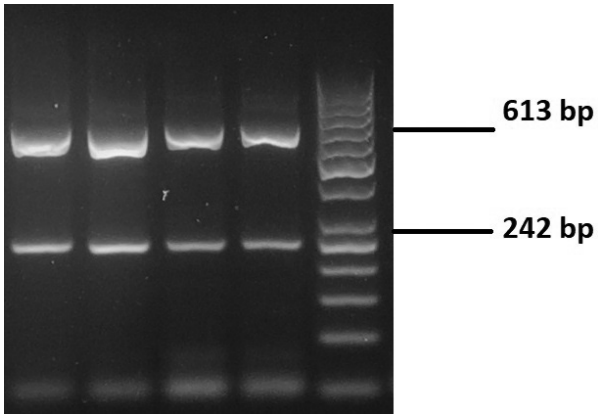

Fig. 2. Restriction fragments of PGAM2 gene in animals from NZW breed after digestion with *Csp6I* enzyme visualized on 2.5 agarose gel

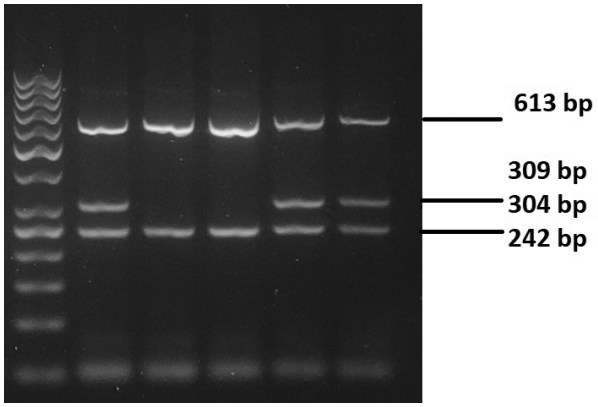

Fig. 3. Restriction fragments of PGAM2 gene in animals from CA breed after digestion with Csp6I enzyme visualized on 2.5 agarose gel

Discussion

Although the above-mentioned importance of the PGAM2 gene, it has not been sufficiently studied in rabbits. Only a few authors worldwide have studied the genetic diversity by PCR RFLP method. However, statistical significance has been reported between different genotypes and weight traits in rabbits. This sets a serious prerequisite for the more extensive study of the gene with a larger number of animals and its inclusion as a candidate gene in marker-assisted selection.

Differ to results in this study, Nahácky et al. (2018) performed PCR-RFLP analysis in 44 rabbits to investigate the PGAM2 gene (195C > T) and identified all three genotypes CC, CT and TT. Rabbits with genotype TT had higher levels of all observed parameters compared to CC and CT genotypes for live weight, body weight, carcass weight, weight of skin and weight of the thigh.

The c.195C > T was genotyped by PCR-RFLP in a total of 222 rabbits of three rabbit breeds (Tianfu black, 53 animals; Ira, 91 animals; Champagne, 78 animals). Allele T was prevalent with a mean frequency of 0.52 compared with 0.48 for allele C. The heterozygosity and effective number of alleles were 0.499 and 1.996, respectively. According to their results, the heterozygous genotype CT was associated significantly ($P < 0.05$) with higher body weight at 84 days of age (BW84) and with average daily weight gain (ADG) (Wu et al., 2015).

Conclusions

This was the first study on the genetic diversity of the PGAM2 gene conducted in Bulgaria. As a result, it can be concluded that in the tested group of New Zealand White rabbit breed polymorphism was not detected in the selected region of the PGAM2 gene. All animals were monomorphic for the investigated region. However, in the Californian rabbit breed, the genetic diversity was discovered in this gene with the presence of two genotypes – homozygous wild genotype CC and heterozygous genotype CT with observed frequencies 0.70 and 0.30, respectively. The experimental work will continue with further investigation in association of the live weight and different genotypes of the PGAM2 gene in rabbits from Californian breed.

Table 1. Allele and genotype frequencies, Ho and He, and Fis of PGAM2 gene

Breed	n	Allele frequency		Genotype frequency			Heterozygosity		Fis	X^2	p-value
		C	T	CC	CT	TT	Ho	He			
NZW	30	1.00	0.00	1.00	0.00	0.00	0.000	0.000	1.000	–	–
CA	30	0.67	0.33	0.70	0.30	0.00	0.300	0.255	-0.176	1.13	0.26*

*statistically non-significant difference ($p > 0.05$)

Acknowledgements

This research was part of the project Ж 175 "Identification of genetic markers associated with productive traits in Californian and New Zealand White rabbits ", Agricultural Academy, Sofia, Bulgaria.

References

El-Sabrout, K. & Aggag, S. A. (2017). Associations between single nucleotide polymorphisms in multiple candidate genes and body weight in rabbits. *Vet. World, 10*(1), 136-139. doi:10.14202/vetworld.2017.136-139.

Fontanesi, L., Scotti, E., Cisarova, K., Battista, P. D., Dall'Olio, S., Fornasini, D. & Frabetti, A. (2013). A missense mutation in the rabbit melanocortin 4 receptor (MC4R) gene is associated with finishing weight in a meat rabbit line. *Animal Biotechnology, 24*(4), 268-277. https://doi.org/10.1080/10495398.2013.781034.

Frinza, G., Murariu, O., Ciobanu, M., Radu-Rusu, R., Simeanu, D. & Biosteanu, P. (2023). Meat Quality in Rabbit (*Oryctolagus cuniculus*) and Hare (*Lepus europaeus* Pallas) – A Nutritional and Technological Perspective. *Agriculture, 13*(1), 126: 1-17.

Helal, M., Hany, N., Maged, M., Abdelaziz, M., Osama, N., Younan, Y., Ismail, Y., Abdelrahman, R. & Ragab, M. (2021). Candidate genes for marker-assisted selection for growth, carcass and meat quality traits in rabbits. *Animal Biotechnology, 32*(1), 1691-1710.

Nahácky, J., Židek, R., Gabor, M., Miluchová, M., Bučko, O. & Kovacik, A. (2018). MC4R and PGAM2 genes polymorphism association with production traits in rabbit (*Oryctolagus cuniculus*). *Journal of Microbiology*, *Biotechnology and Food Sciences, 7*(5), 493-495. DOI: 10.15414/jmbfs.2018.7.5.537-539.

Wu, Z., Chen, S., Jia, X. & Lai, S. (2015). Association of a synonymous mutation of the PGAM2 gene and growth traits in rabbits. *Czech Journal of Animal Science, 60*(3), 139–144.

Received: **November, 21, 2023**; *Approved*: **December, 06, 2023;** *Published:* **June, 2024**