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Effect of the MC4R gene polymorphism on the body weight in New Zealand White rabbits

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Abstract

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The present study is a follow-up to a previous study of the genetic diversity in does and bucks of the New Zealand White (NZW) rabbit breed. Does and bucks with the required genotype were bred and after birth, blood was taken from the offspring for DNA testing. The experiment included 50 kits of NZW breed. The live weight of the rabbits was monitored from birth to day 90. After carrying out PCR-RFLP analysis to establish the genotypes and estimate the genetic diversity of the studied groups of animals, a statistical analysis was conducted to demonstrate the genotype's effect on the animals' body weight. The results of the present study identified all three possible genotypes (GG, AG and AA) of MC4R gene. The frequency of heterozygous genotype AG (0.52) was higher than the other genotypes. The mutant genotype AA was with lowest frequency of 0.18. The student's T-test was used to estimate the effect of the genotype on the body weight of the rabbits. According to the results, the animals with the mutant genotype AA had significantly higher body weight. A statistically significant difference was observed in individual birth weight in New Zealand White breed, at 70 days of age between the average values of genotype GG (1.703 kg) and AA (1.858 kg), where p=0.006 and between genotypes AG (1.722 kg) and AA (1.858 kg) where p v=0.02. At 90 days of age, a statistically significant difference was observed between genotypes GG (2,207) and AA (2,447) where p=0,003.

Keywords: rabbit breeding; polymorphism; MC4R gene; PCR-RFLP; body weight

Introduction

The domestication of Wild rabbits probably had started around 600 A.D by French medieval monks (Doherty and Driscoll, 2017). Today, more than 300 domestic rabbit breeds. The domestic rabbit (*Oryctolagus cuniculus domesticus*) is a subspecies of the European rabbit (*Oryctolagus cuniculus*), which belongs to the *Leporidae* family, of the order *Lagomorpha* (Radwan et al., 2022).

According to FAO, some of the largest producers of rabbit meat in the world are Egypt, China, and Spain. Nowadays, rabbits are important agricultural animals that are qualifiers to fill the gap between production and consumption of animal protein (Siddiqui et al., 2023). Rabbit meat is healthy and possesses good nutritional qualities. It is recommended for children, the elderly, and people with health issues because of its benefits and efficient digestibility. Rabbit meat is low in fat, cholesterol, and sodium, and at the same time, it has a high vitamin B content, a high protein content (~22%), a large amount of essential amino acids, a low lipid content of ~1.8 g/100 g meat (Cullere and Dalle Zotte, 2018). These factors led to the characterization of rabbit meat as high-quality meat, and currently, consumers are willing to pay more for high-quality meat, leading to an increase in the

demand for rabbit meat (Szendrő et al., 2020). This, in turn, has induced the shift in rabbit production from small-scale to intensive production (Trocino et al., 2019). Moreover, growth and meat production traits are among the highest traits of interest for breeders when setting breeding goals (Blasco et al., 2018).

Rabbit breeds are not raised only for their ability to produce animal protein, but they also play a crucial role in achieving sustainability goals, and therefore, the genetic improvement of those breeds is essential for maintaining sustainable production. Since the introduction of molecular markers, their reliability and accuracy have made them dominant in animal breeding approaches. Molecular markers have greatly accelerated the rate of genetic changes in animals by facilitating various applications. A prominent application is marker-assisted selection (MAS), which is an effective and advanced approach used in animal breeding (El-Sabry et al., 2021). MAS utilizes molecular markers to assist in the selection of animals with desirable traits. It involves identifying genetic markers associated with specific traits of interest, and using this information to make informed breeding decisions. Therefore, a key step in MAS is identifying different molecular markers associated with different traits. In rabbits, there is still a gap between the number of required markers and the existing ones (Khalil, 2020; Safaa et al., 2023).

The Melanocortin 4 Receptor (MC4R, ENSOCUG00000025457) gene is located on chromosome 9 in rabbits. This gene is found and expressed in multiple regions of the central nervous system and plays a crucial role in facilitating the effects of melanocortin on food intake and energy homeostasis (Helal et al., 2022). The gene was genotyped in rabbits, and different variants were detected and associated with several growth traits, including body weight (Osaiyuwu et al., 2020; Radwan et al., 2022), feed intake, average daily gain (El-Sabrout, F. Soliman, 2018). Moreover, the MC4R gene was also associated with growth traits in cattle (Prihandini et al., 2019), sheep (Al-Thuwaini et al., 2020), and goats (Belgania et al., 2023), and therefore, the MC4R gene is a suggested to be a candidate gene for growth in livestock.

The present study aimed to establish the effect of the genotype of MC4R gene on the individual body weight in rabbits from New Zealand white rabbit breed until 90 days of age.

Material and Methods

Animals

This study was a follow-up to a previous study of the genetic diversity in does and bucks of the New Zealand White (NZW) rabbit breed. Does and bucks with the required genotype were bred and post-birth, blood was taken from the offspring for DNA testing. The experiment included 50 kits of NZW breed. The live weight of the rabbits was monitored from birth to day 90. Individual body weights were used for the present experiment at 34, 70, and 90 days of age. The adult female and male breeding rabbits were selected for their typical phenotypic characteristics. The does have a mean weight of 4.3 kg and are aged 1 to 1.5 years. The bucks had a mean weight of 4 kg. The experimental work spanned from April, 2022, to March, 2023.

All experimental work involving animals has been carried out following the recommendations, contained in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes, or the National Research Council's Guide for the Care and Use of Laboratory Animals.

Housing and feeding management

From birth until day 34 (± 2-3 days), the kits were kept together with their mothers. After weaning, the experimental rabbits were fed *ad libitum* commercial pelleted feed and had free access to water. Rabbits were maintained under standard conditions of humidity, temperature, and photoperiod. The rabbits were raised in an enclosed one-floor building. They were housed separately in galvanized wire cages (40 × 60 × 50 cm^3), 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 animals tested remained healthy throughout the experiment. All activities have been carried out following the recommendations contained in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

DNA extraction

Blood samples were collected from vena saphena in vacuum tubes containing EDTA. Genomic 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

DNA amplification was carried out by thermal cycler (QB-9, Quanta Biotech) in a total 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 presented in Table 1.

Table 1. Summary of rabbit's MC4R gene fragment, primer sequences, restriction enzyme used for the current study

MC4R rabbit gene	
Region	Chromosome 9
Primer set	F: 5´CAT GAA CTC CAC CCA CCA C 3´ R: 5´CTC ATA GCA CCC TCC ATC AGA CTA G 3´
Amplicon length (bp)	127 bp
Annealing Temperature	59 ^{0}C
Restriction enzyme	BcuI
Restriction fragment length (bp)	G allele – 127 bp A allele- 100 bp + 27 bp

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

In this study, the PCR analysis and the amplification of the MC4R gene revealed PCR products with an expected length of 127 bp (Figure 1). The restriction analysis revealed two alleles – wild G and mutant A, and three genotypes – homozygous wild GG (one fragment of 127 bp), heterozygous AG (three fragments with lengths of 127 bp, 100 bp, and 27 bp), and mutant homozygous AA (two fragments of length of 100 bp and 27 bp) (Figure 2).

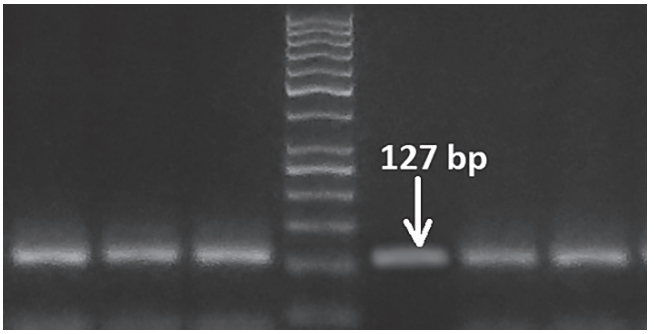

Fig. 1. PCR products of tested region in rabbit MC4R gene

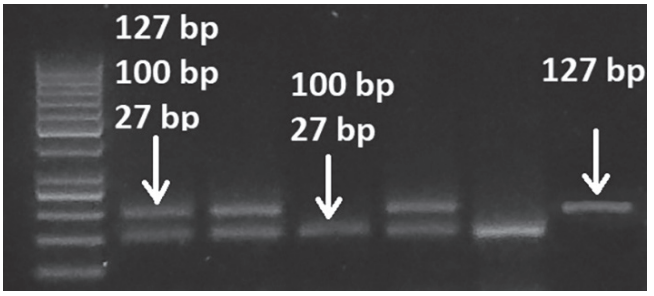

Fig. 2. Restriction profile of investigate region of MC4R rabbit gene

Mutant allele G had a higher frequency of 0.56. The frequency of heterozygous genotype AG (0.52) was higher than the other genotypes. The mutant genotype AA had the lowest frequency of 0.18. Chi-square tests revealed a non-significant difference between Ho and He (p-value > 0.05). The coefficient of inbreeding (Fis) was -0.055 (Table 2).

According to data from individual birth weights of the tested animals at 35 days of age (Table 3), the mean weights of the three genotypes were close to equal. Despite this fact, there was a tendency of excellence in the mutant genotype AA, with a mean weight of 0.768. According to the Student's t-test, statistically significant differences were observed in individual body weights at 70 days of age between the mean values of genotype GG (1.703 kg) and AA (1.858 kg), where p-value was 0.006 and between genotypes AG (1.722 kg) and AA (1.858 kg), where p-value was 0.02At 90 days of age, there were significant differences between genotypes GG and AA, with a p-value of 0.003.

Table 2. Allele and genotype frequencies, Ho and He, and Fis of SNP c.101G>A of MC4R

Breed	n	Allele frequency		Genotype frequency			Heterozygosity		Ne	PIC	Fis	X^2	p-value
		G	A	GG	AG	AA	Ho	He					
NZW	50	0.56	0.44	0.30	0.52	0.18	0.520	0.493	1.972	0.371	-0.055	0.33	0.57*

*p > 0.05 – statistically non-significant difference

Table 3. Results of individual body weights (IBW) at different ages of the tested New Zealand white rabbits

Age (Days)	Genotype GG – 15		Genotype AG – 26		Genotype AA – 9	
	Mean±SD	Min/Max	Mean±SD	Min/Max	Mean±SD	Min/Max
IBW-35	0.728±0.078	0.590/0.940	0.752±0.095	0.605/0.945	0.770±0.136	0.610/0.950
IBW-70	1.703±0.122	1.495/1.950	1.722±0.126	1,505/1,995	1.858±0.125	1.620/1.995
IBW-90	2.207±0.270	1.250/2.350	2.345±0.260	1.650/2.700	2.447±0.059	2.350/2.495

Although, there was no statistically significant difference between genotypes GG and AG, it was evident that the mean weight in heterozygous genotype is higher than the homozygous wild genotype.

Discussion

Polymorphisms in the melanocortin 4 receptor (MC4R) gene were associated with growth performance in different species. MC4R is mainly expressed in the hypothalamus, which plays a key role in controlling energy homeostasis and feed intake with effects on body weight and fat deposition (Fontanesi et al., 2013).

Osaiyuwu et al. (2020) used 6 different rabbit breeds (20 Fauve de Bourgogne, 26 Chinchil- la, 10 New Zealand white, 11 Dutch, 4 English Spot, and 3 Californian), to study the polymorphism of MC4R gene in those breeds, detected a SNP at c.101G > 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). According to their study, the genotype AA may have the potential to be associated with higher body weight values than genotypes AG and GG.

El-Sabrout and Aggag (2017) studied the association of several candidate genes with high BW in rabbits. They identified SNPs at nucleotide 23 (A-C) and nucleotide 35 (T-G) in MC4R gene (sense mutation) in Alexandria and V-line rabbits, which were associated with higher BW. Furthermore, they detected another SNP variation between the two lines, which was identified at nucleotide 27 of MC4R gene (sense mutation). The results of individual BW at 63 days of age indicated that Alexandria rabbits had significantly higher BW compared with V-line rabbits. MC4R polymorphism showed a significant association with high BW in rabbits.

Jiang et al. (2008) identified SNP markers in the coding region of the MC4R gene by the PCR-SSCP and DNA sequencing analysis. By means of a general linear model for the effect of genotypes on performance traits, the authors reported that genotype AG was associated with body weight, eviscerated weight, and feed conversion efficiency ($P < 0.05$), but not associated with cooking loss ($P > 0.05$). Nahácky et al. (2018) also reported a polymorphism of MC4R rabbit gene, that was associated with production traits.

The results of the current study were in agreement with the abovementioned studies, where the mutant allele A had a positive effect on body weight at 70 and 90 days of age. As the MC4R gene controlling the appetite and feed consumption in rabbits, mutations that affect MC4R function may result in decreased, or increase in appetite and energy expenditure. The impact of MC4R gene on body weight highlights its significance in the complex interplay of genetic factors influencing metabolism, which needs further studies.

Conclusions

In conclusion, the association analysis in the present study revealed that c.101G > A polymorphisms at the MC4R gene, had a significant influence ($P < 0.05$) on the individual body weight at 70 d of age, and 90 d of age in the New Zealand rabbit population. The individuals with the homozygous mutant genotype AA reached a higher score of the recorded trait. Therefore, results in this study suggested that the investigated region of the MC4R rabbit gene could be a promising candidate gene for marker-assisted selection in rabbits.

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