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MC4R gene polymorphisms for classification of growth efficiency and carcass measurements in two rabbit breeds in Egypt

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Abstract

Melanocortin 4 receptor (MC4R), a protein derived from the *MC4R* gene, is involved in feed intake, metabolism control, and body weight regulation in humans. The purpose of this research was to explore *MC4R* polymorphisms alongside metabolic marker changes and their relationship with growth and carcass measurements in rabbits. Using synthetic line V (V-line) and Baladi Black rabbit breeds (60 rabbits per breed), blood samples were collected for DNA extraction and biochemical analysis. The polymerase chain reaction (PCR) product of *MC4R* (493 bp) revealed five nonsynonymous single nucleotide polymorphisms (SNPs; submitted to GenBank with accession numbers gb|MT832144|, gb|MT832145|, and gb|MT832146|). Four SNPs were characteristic of the V-line breed, and one was characteristic of the Baladi Black. For classification of the defined SNP-dependent groups within and between breeds, a discriminant analysis model correctly classified a percentage of cases with the following predictor variables: 90.8% for body weight at 5–14 weeks of age; 85% for feed consumption, daily feed intake, and feed conversion ratio; and 93.3% for carcass measurements (for which hind part weight, liver weight, and liver percentage were the best predictors in both breeds). There were significant differences between and within V-line and Baladi Black breeds in agreement with metabolic biochemical marker profiles and the defined SNPs. The identified SNPs in the *MC4R* gene and profile of the investigated metabolic biomarkers could be used as candidates and reference for the effective characterization of the two rabbit breeds. This study could therefore facilitate the introduction of marker-assisted selection for growth performance characteristics in rabbits.

Keywords: discriminant analysis, feed conversion efficiency, *Melanocortin 4 receptor*, slaughter traits

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Introduction

Wild rabbits are commonly considered to have been first domesticated in 600 A.D by French medieval monks (Doherty & Driscoll, 2017). 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*. The *Oryctolagus* genus includes the European rabbit species as well as its descendants, the world's 305 domestic rabbit breeds. European rabbits can be found in the wild on all continents, with the exception of Asia and Antarctica (DAD-IS, 2017).

Recently, domestic rabbits have been proposed as a good substitute source of dietary protein for the rising human populations in developed countries (Dalle Zotte, 2014; Ezema & Eze, 2015; Trocino, 2019). According to the Food and Agriculture Organization of the United Nations (FAO) China, North Korea, Spain, Egypt, and Italy were the five leading rabbit meat producers globally in 2017 (FAO, 2019). Rabbit meat is healthy and nutritious; it is commonly given to children and the elderly because of its nutritional benefits and efficient digestibility (Dalle Zotte & Szendro, 2011; Dalle Zotte, 2014). Rabbit meat is characterized by a high

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protein content (~22%), a large proportion of essential amino acids, a loin lipid content of ~1.8g/100g meat (Dalle Zotte & Szendro, 2011), and high vitamin B content and it is low in fat, cholesterol, and sodium (Ramirez *et al*., 2006; Ezema & Eze, 2015). Moreover, rabbit meat qualifies as one of the most valuable meat sources due to its effectiveness in dietary manipulation, as well as a promising improvement in oxidative stability and "functional" properties (Pla *et al*., 2004; Dalle Zotte & Szendro, 2011; Dalle Zotte *et al*., 2016; Martins *et al*., 2018). Indeed, as a tender and nearly cholesterol-free white meat, rabbit meat could potentially replace chicken meat (Dalle Zotte, 2014). Overall, the rabbit has high value as a meat product.

In 1981, the V-line (or line V) was developed as a synthetic line in Spain (Polytechnic University of Valencia) by crossing the progeny of four specific maternal lines chosen to improve litter size at weaning (Estany *et al*., 1989). A maternal synthetic line is created by improving doe-related traits such as litter weights and sizes, as well as milk yield, which are employed as selection criteria in the development of maternal rabbit lines (Estany *et al*., 1989; Gómez *et al*., 1996; Rochambeau *et al*., 1998; Baselga, 2004).

A variety of characteristics related to climate adaptation and litter size at weaning dictated the decision to select line V in this study. Valencia seems to have a long selection history (García & Baselga, 2002), and its climate is akin to that of Egypt's Nile delta. The V line breed has also been examined in hot climates such as Adana in Turkey and Zagazig in Egypt, where it surpassed other exotic breeds (Yamani, 1994) according to the first international hot climate rabbit development conference in Cairo (Yamani,1994) and the sixth world rabbit congress in Toulouse (Testik, 1996). New reports in Saudi Arabia for the V-line have reaffirmed the line's superior heat stress tolerance (Khalil *et al*., 2002). This line is also notable for having a higher litter size at weaning.

According to the Egyptian Ministry of Agriculture's Poultry Breeding Section, the Baladi rabbit breed originated from Egypt as a consequence of crossbreeding between native rabbits and the exotic Flemish Giant breed for many generations at research stations to develop a heat-resistant, meat-producing rabbit that could endure Egypt's harsh climate (Badawy, 1975; Galal & Khalil, 1994). This selection approach for the adapted lines for the Egyptian climate resulted in the establishment of three native Baladi strains: Baladi Red, Baladi White, and Baladi Black (Khalil, 2011).

Growth performance and carcass traits are of high importance in the overall breeding objective of rabbit selection programs. However, the effects of a large number of known gene polymorphisms associated with these traits require assessment to improve breeding operations (Migdal *et al*., 2019). SNP genetic markers have revolutionized previous achievements in conservation decisions, biodiversity assessment, and genetic characterization of breeds (Groeneveld *et al*., 2010). Few studies using genotypes dependent on single nucleotide polymorphisms (SNPs) of candidate genes have been undertaken to enhance the genetic potential of rabbits for human food purposes (El-Sabrout, 2017). The diversity of rabbit breeds coupled with tailored breeding strategies could potentially enhance the efficiency of commercial meat production (Piles *et al*., 2004).

Molecular genetic markers (e.g., restriction fragment length polymorphisms, RFLP; SNP) can be used in rabbit management to improve selection and mating systems by favouring the preferable genotype; and thereby reducing kindling intervals among generations (Hirose *et al*., 2014). Using these markers could also improve characteristics such as reproductive performance, growth and development, quality of meat, and milk production (Dekkers, 2004). Molecular markers may also be used to increase the precision of selection and, therefore, the genetic progress of major economic traits. Several previous investigations have demonstrated correlations between candidate gene polymorphisms and meat characteristics in rabbits. Many polymorphisms have been correlated with rabbit body weight (BW), e.g., myostatin SNPs (Sternstein *et al*., 2014), growth hormone (*GH*) (Fontanesi *et al*., 2012a), GH receptor (Zhang *et al*., 2012), and insulin-like growth factor 2 (*IGF-2*) (Fontanesi *et al*., 2012b). Moreover, Zhang *et al.* (2013) reported that SNPs within the fat mass and obesity-associated (*FTO*) gene are linked to the longissimus lumborum muscle having higher intramuscular fat content.

Melanocortin 4 receptor (*MC4R)*, a member of the G-protein-coupled receptor family, is expressed in the human hypothalamus. It is known to be involved in food intake as well as the regulation of metabolism and body weight (Li & Li, 2006). Polymorphisms in the *MC4R* gene have been associated with growth performance in domestic animals such as pigs (Houston *et al*., 2004; Meidtner *et al*., 2006), cattle (Zhang *et al*., 2009), sheep (Song *et al*., 2012), and chickens (Zhou *et al*., 2012). It was established that the *MC4R* gene could be a potential candidate for production traits; however, the possible association of *MC4R* gene polymorphisms in rabbits has rarely been reported. This study therefore aims to explore the association between *MC4R* gene polymorphisms and growth traits, carcass traits, feed intake, and feed conversion ratio in V-line and Baladi Black rabbit breeds using a PCR–DNA sequencing approach, to subsequently identify whether there are associations between the SNPs identified in the *MC4R* region and serum levels of various metabolic biochemical markers including growth hormone (GH), leptin (Lep), and thyroid stimulating hormone (TSH) in these breeds.

Materials and Methods

This study was conducted from November, 2017 to March, 2019 on rabbits raised at the El-Serw Experimental Station, which belongs to the Animal Production Research Institute (Agricultural Research Center, Ministry of Agriculture, Egypt) from birth to weaning at 5 weeks of age. Thereafter, growth and feed performance records were collected from 120, five-week-old rabbits from the V-line (n = 60) and Baladi Black (n = 60) breeds until 14 weeks of age whereafter the animals were slaughtered and carcass traits were recorded. The collection of samples and care of rabbits used in this study followed the guidelines of Mansoura University and the protocol of the study was approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (code R/21).

The rabbits were raised in an open-sided hutch. Breeding animals were housed separately in galvanized wire cages (40 × 60 × 50 cm^3) fitted with a nipple drinking system and a manual feeder.

Diazinon (20%) was routinely used to wash and purify the hutches before each kindling. Cages and nest boxes were regularly cleansed to get rid of mange. Affected animals were handled with injectable Ivomac (Ivermectin injection 2%) biweekly for non-pregnant does and young, and Benzylbenzoate cream for pregnant does. Urine and faecal pellets on the floor of the cages were cleaned each morning.

The environmental temperature was preserved as far as possible between 16 °C and 24 °C. Efficient ventilation and fresh air were provided by fans to minimize the accumulation of ammonia in the building. Photoperiod was set at 16 light vs 8 dark in the rabbitry.

A tubular-shaped, pelletized (4 mm diameter, 9 mm length) commercial ration was utilized. The ration formulated according to Zaghloul *et al.* (2019), contained 24% soybean meal, 23% barley, 21% berseem hay, 19% wheat bran, 18.01% crude protein 13% yellow corn, 11.5% crude fiber, 2.5% fat, 1% limestone, 0.5% table salt, 14 kg di-calcium phosphate/ton, 1 kg anti-toxicity/ton, 1 kg anti-coccidial/ton, and 1 kg of a mineral mixture/ton, as per the National Research Council (NRC,1977).

The rabbits were supplied with safe, fresh water at all times. Prophylactic antibiotics and anti-coccidial medications were applied to the water for 3–5 days until weaning. Kits were injected every two weeks with multi-vitamins to mask any shortages and operate against declining immunity arising from weaning (Lebas, 2000). In addition, injectable vitamin E and selenium were also used for fertility enhancement. Kits were vaccinated at three and six weeks of age, respectively, against pasteurellosis and infectious rabbit haemorrhagic disease as well as a booster dose at ten weeks of age, according to the history of the El-Serw Animal Production Research Station vaccination program and area circumstances.

Mating was done at random, with the exception of parent–offspring, full-sibling, and half-sibling mating. Each doe was mated with the appropriate buck of the same breed in a ratio of 1 buck to 3 does from each breed. Ten days after mating, each doe was palpated to confirm pregnancy, and those found to be non-pregnant were reintroduced to the same buck. Attached to the doe's cage was a metal nest box (40 × 40 × 40 cm^3) for kindling and nursing kits. The nest boxes were supplied with a thick layer of rice straw on the 27th day of pregnancy, which was placed in the bottom of the nest box to assist the doe in creating a warm and comfortable nest for her offspring. At weaning (five weeks), litters were ear-tagged, and separated into cages, each cage containing five litters to start the fattening period until marketing (14 weeks).

During the fattening period, male and female rabbits were weighed weekly to determine their body weight (BW). Weight gain (WG) was expressed as the difference between the current and previous weight relative to the subsequent period of time. Average daily gain (ADG) was defined as the weight gain over the computed number of days.

Rabbits were supplied with a precisely weighed amount of feed per day to calculate feed intake (FI). At the end of the day, the residual feed was excluded and the intake amount was determined by the difference according to the method of Wagner *et al.* (1983). The feed conversion ratio (FCR) was determined by calculating FI per kg and BW gain per kg according to the method of Iyayi & Odueso (2003).

Rabbits were fasted for 12 h at the end of the fattening period and weighed before being slaughtered. They were subsequently euthanized via IP injection of a substantial dose (20 to 60 mg/kg) of sodium thiopental for organ and body part collection (Archimedes, France) according to Hellebrekers *et al.* (1990). The skin of the slaughtered animals was removed and dressed, and the hot carcasses were separately weighed and examined, along with the head and giblets.

The dressing percentage (dressing %) was calculated as follows:

dressing percentage = 100 × hot carcass (giblets weight + head weight + carcass weight) / live BW,

where giblets weight = kidney weight + heart weight + liver weight.

The ratio of body organs (e.g., kidneys, heart, abdominal fat, mid parts, foreparts, hind parts, liver, and head) to carcass weight was estimated according to the method of Szendrő *et al.* (2010).

Blood samples were collected aseptically before slaughter from 120 rabbits (60 of each breed). After washing and disinfection, approximately 2 ml of blood was obtained from a rabbit's ear vein using a piece of cotton filled with xylol in tubes containing EDTA for DNA extraction. Tubes without anticoagulants were used to obtain serum by centrifugation. The serum samples were subsequently placed in a cold gel icebox, which was sealed and stored at 4 °C prior to a hormonal assay.

Genomic DNA was extracted from whole blood using a Gene JET Whole Blood Genomic DNA Extraction Kit following the manufacturer's instructions (Thermo Scientific, Lithuania). The quality, purity, and concentration of DNA were assessed using a Nanodrop before further analysis.

Polymerase chain reaction (PCR) was used to amplify fragments of the *MC4R* gene, which spanned exons I and II, with an expected amplicon size of 493 bp (Fontanesi *et al*., 2013). The primers used were: forward: 5′-CCATTGCAGTGGACAGGTATT-3′; reverse: 5′-TCCGGAGTGCATAAATGAGA-3′. The PCR mixture (50 µl) contained DNA (3 µl), H_2O (double-distilled water, 21 µl), PCR master mix (25 µl; Jena Bioscience, Germany), and forward and reverse primers (0.5 µl of each). The final reaction mixture was placed in a thermal cycler, and the PCR program was conducted with an initial denaturation at 95 °C for 3 min, followed by 30 cycles of 95 °C for 1 min for DNA denaturation, annealing at 55 °C for 1 min, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The samples were then maintained at 4 °C; representative PCR results were confirmed by agarose gel electrophoresis, and then fragment patterns were visualized under ultraviolet light using a gel documentation system.

PCR products with target bands were used for DNA sequencing. These products were sequenced in forward and reverse directions with an ABI 3730XL DNA sequencer (Applied Biosystems, USA) according to the enzymatic chain terminator technique developed by Sanger *et al*. (1977). DNA sequencing data were analysed using Chromas 1.45 and BLAST 2.0 (Altschul *et al*., 1990). Differences were classified as SNPs on the basis of a comparison between the PCR products of the *MC4R* gene and the reference sequence available in GenBank. On the basis of an alignment of sequences, variation in the amino acid sequences of *MC4R* genes among the breeds was identified using MEGA4 (Tamura *et al*., 2007).

Using an ELISA analyser, growth hormone (GH) was assessed by adding 100 µl of weak dilution antibody to each well of a plate, which was then stored at room temperature (20–25 °C) for 1 h, after which 200 µl of blocking solution was added for 30 min, and the plate was washed multiple times. Subsequently, 100 steps were applied to each well, which was then maintained for 1 h at room temperature. Another 100 µl of dilution antibody was then added to the wells for 1 h at room temperature. The wells were washed, containing 100 µl of the well's TMP substrate solvent, and were positioned in a dark place at room temperature for 15 min, including 100 µl of the well suspension solution. Color absorption was then calculated on a plate reader at approximately 450 nm (Medgyesi *et al*., 1975).

Leptin hormone (Lep) was evaluated using IMMULITE and an IMMULITE 1000 Analyzer for the quantitative estimation of leptin in serum, according to the method of Tian (2005). A solid-stage, chemical (enzyme)-labelled chemiluminescent competitive immunoassay was used, with the bead (solid stage) coated in anti-leptin polyclonal rabbit antibody. The fluid phase was composed of leptin-conjugated alkaline phosphatase (bovine calf intestine).

Thyroid stimulating hormone (TSH) concentration was determined according to the method of Baloch *et al*. (2003). Kits from Siemens Health Diagnostic (USA) were used with Speedy TSH IMMULITE/IMMULITE 2000, a powerful immunochemical assay.

Data were arranged, compiled, and analysed using the statistical package, SPSS version 23 (USA). The sample size was calculated using the following equation based on the population size:

$$n = \frac{S^2 Z^2_{1-\alpha/2}}{d^2}$$

where: S^2 = population variance; Z_1^2 - α/2 = critical value for significance level which equals 1.96 for 95% confidence level; d = level of error.

A chi-squared test was used to evaluate the frequencies of *MC4R* SNPs across various classes of the V-line (GV1, GV2, GV3, and GV4) and Baladi Black (GB1 and GB2) breeds. A linear discriminant analysis (LDA) (SPSS, 2015) was then used to evaluate the significance of various determinants and classify gene group SNPs as the dependent variables, with BW, WG, FI, FCR, and carcass traits as the independent attributes. The discriminating statistical model was:

$$DF = V1X1 + V2X2 + V3X3 + \cdots + VIXI,$$

where DF= the discriminating grouping function (score) for grouping variables; V = the standardized discriminant or loading coefficient for predictors; X = the respondent's score for the predictors; and I is the number of predictor variables.

The coefficients of discriminant equation, V, or the idealized type, beta, represent the partial contribution of each predictor to the mechanism of discrimination. Finally, a one-way ANOVA was used to compare the means of BW, WG, FI, FCR, and carcass traits among *MC4R* groups, dependent on SNP classes. All data were represented as means ± SE; P <0.05 was considered statistically significant.

Results and Discussion

In this study, DNA sequencing of the *MC4R* gene (493 bp) revealed five SNPs (submitted to GenBank with accession numbers gb|MT832144|, gb|MT832145|, and gb|MT832146|; Table 1). Four non-synonymous SNPs were characteristic for the V-line breed; rabbits that harboured the A47C SNP, the A50C SNP, the A43G SNP, the A44T SNP, and those that did not harbour the identified SNPs, are hereafter respectively referred to as GV1MC4R, GV2MC4R, GV3MC4R, and GV4MC4R. For Baladi Black rabbits, one identified synonymous SNP (G333A) was identified and this group is hereafter referred to as GB1MC4R; whereas Baladi Black rabbits that did not exhibit the identified SNP are hereafter referred to as GB2MC4R.

Chi-square tests revealed a difference in the frequency of *MC4R* gene SNPs between the two breeds (Table 1). Nucleotide sequence variation for the *MC4R* gene between the two breeds, as well as between the breeds and reference sequences available in GenBank (HF970577.1), confirmed all five identified SNPs. The identification of some SNPs specific to each breed is probably related to the founder effect associated with origin, history, evolution, and genetic constituents of each breed (Carneiro *et al*., 2011; Ateya *et al*., 2021).

The *MC4R* gene is known to play an active role in food intake, metabolic control, and body weight in humans (Li & Li, 2006). *MC4R* is a descendant of the G-protein paired receptor family, which plays a key role in managing rodent feeding behaviour (Wardlaw, 2001). Indeed, variations of *MC4R* were found to be consistent with genetic human obesity (Farooqi & O'Rahilly, 2006). Previous studies have focused on the association between the *MC4R* gene and control of eating behavior in humans, mice, and pigs (El-Sabrout, 2017). Furthermore, *MC4R* gene mutations in many animal species are correlated with carcass quality and growth, e.g., in chickens (Wang *et al*., 2009) and cattle (Zhang *et al*., 2009). According to our results, the identified SNPs in the *MC4R* gene were associated with growth traits in the two breeds enabling for marker assisted selection within and between breeds.

The association between *MC4R* gene polymorphisms and productive traits in rabbits has not previously been studied in detail. However, El-Sabrout & Soliman (2017) reported an association between SNPs of the *MC4R* gene and FI in V-line rabbits and El-Sabrout & Aggag (2018) reported an association between *MC4R* gene polymorphisms and carcass quality in rabbits. According to the results, the authors reported that identified SNPs were associated with feed intake. Fontanesi *et al.* (2013) also found that a missense mutation in the rabbit *MC4R* gene was associated with finishing weight in a meat rabbit line. Additionally, Osaiyuwu *et al.* (2020) reported that a *MC4R* gene polymorphism was associated with body weight in some breeds of rabbit. Previous studies have investigated the association of *MC4R* gene polymorphisms and their association with growth performance in rabbit breeds using other genetic markers (e.g., RFLP and single-strand conformational polymorphism, SSCP) (Jiang *et al*., 2008; Nahácky *et al*., 2018).

Due to their abundance and distribution across the genome, high automation, and multiplex ability, SNP markers have revolutionized previous achievements in conservation decisions, biodiversity assessment, and genetic characterization of breeds. Although these markers are applied intensively across other production animal species, the same has not been true for rabbits. Studies like this one might assist in improving SNP-based applications for rabbits (Groeneveld *et al*., 2010). Jiang *et al.* (2008), for example, reported SNP markers in the coding sequence of the *MC4R* gene detected by the PCR-SSCP and DNA sequencing methods. By means of a general linear model analysis for the effect of genotypes on performance traits, these authors demonstrated that an A/G genotype 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 *MC4R* gene polymorphism that was associated with production traits in rabbit.

Table 1 Distribution of single nucleotide polymorphisms (SNPs) identified within the *MC4R* gene in V-line and Baladi Black rabbits

SNPs	V-line	BB	Total	Chi Square	P-Value
A47C	18	0	18/120	18.89	<0.0001**
A50C	22	0	22/120	24.54	<0.0001**
A43G	14	0	14/120	13.67	<0.0002**
A44T	14	0	14/120	13.67	<0.0002**
G333A	0	49	49/120	79.47	<0.0001**
Total	68	49	117/600	3.44	<0.0636^{NS}

P <0.05 is considered statistically different, NS: non-significant, BB: Baladi Black

The hormonal assay revealed that GH and TSH varied greatly among the V-line and Baladi Black breeds with various *MC4R* SNPs. GB2*MCR4* showed the lowest GH levels (1.26 ng/ml) and the highest TSH levels (1.21 µl/ml). The relationship between the SNP genotypes and the corresponding metabolic profiles holds potential for explaining the phenotypic variation in the growth performance between the two breeds. On the other hand, there was no substantial difference in leptin hormone among breeds (Table 2). A significant effect of breed on T3 and T4 has been recorded in livestock; for instance, Ghanem *et al.* (2016) concluded that the choice of broiler breed may have a significant impact on serum T3 and T4 levels, and these findings may be reflected in growth and body composition. Leptin hormone levels in Baladi Black rabbits were found to be insignificantly higher than those in NZW rabbits for both does and bucks (El-Werdany *et al.*, 2016).

Table 2 Hormonal assay of V-line and Baladi Black rabbits harbouring *MC4R* single nucleotide polymorphism (SNP) genotypes

Hormone	Mean ± SE							ANOVA	
	GV1*MC4R*	GV2 *MC4R*	GV3*MC4R*	GV4*MC4R*	GB1*MC4R*	GB2*MC4R*	Total	F	Sig
GH	2.34^{a} ±0.20	2.18^{a} ±0.09	2.28^{a} ±0.14	1.85^{b} ±0.16	2.22^{a} ±0.07	1.26^{c} ±0.07	2.14±0.06	6.53	0.00
Lep	1.63^{a} ±0.18	1.98^{a} ±0.08	1.75^{a} ±0.06	1.83^{a} ±0.16	1.75^{a} ±0.08	1.83^{a} ±0.19	1.79±0.05	1.05	0.39
TSH	0.99^{b} ±0.033	0.86^{c} ±0.05	0.80^{c} ±0.026	0.82^{c} ±0.11	0.98^{b} ±0.03	1.21^{a} ±0.09	0.95±0.02	6.63	0.00

GH: Growth hormone, Lep: Leptin hormone, TSH: Thyroid stimulating hormone, N: Number (17, 23, 13, 7, 49, 11, 120 for *GV1MC4R,* GV2*MC4R,* GV3*MC4R,* GV4*MC4R,* GB1*MC4R,* GB2*MC4R* & Total, respectively), GV1*MC4R*: Group SNP 1 for *MC4R* in V-line breed, GV2*MC4R*: Group SNP 2 for *MC4R* in V-line breed, GV3*MC4R*: Group SNP 3 for *MC4R* in V-line breed, GV4*MC4R*: Group SNP 4 for *MC4R* in V-line breed, GB1*MC4R*: Group SNP 1 for *MC4R* in Baladi Black breed, GB2*MC4R*: Group SNP 2 for *MC4R* in Baladi Black breed, means of various hormone levels with different superscripts are different (*P* <0.05)

Studied traits were entered into a DF model using a stepwise method. All variables except DFI, WG, and DWG were selected for use in the equation as predictors for the allocated groups based on identified SNPs in the *MC4R* gene. For BW at 5–14 weeks of age, there was a genetic variation between the V-line and Black Baladi breeds, with GV1*MC4R,* GV2*MC4R,* GV3*MC4R,* and GV4*MC4R* SNP-allocated groups showing a higher BW compared to the Baladi Black (GB1*MC4R*, GB2*MC4R*) breed. In the same respect, there was a genetic variation within the V-line and Black Baladi breeds in BW, where GV1*MC4R* and GV2*MC4R* SNP-allocated groups had a higher BW than the others. The GB1*MC4R* group showed a higher BW than GB2*MC4R* in the Black Baladi breed (Table 3). The observed results were consistent with those of El-Sabrout & Soliman (2018), where authors analysed the relationship between *MC4R* gene polymorphisms and rabbit feed intake. Results revealed that identified SNPs were correlated with feed intake, which facilitate marker assisted selection (MAS). Their findings can also be used to select rabbits for high body weight at a marketing age. Additionally, it was established that *MC4R* gene polymorphisms were associated with finishing weight (Fontanesi *et al.*, 2013). *MCR4* was associated through sequencing with high BW at market for Alexandria and V-line breeds by El-Sabrout & Aggag (2017). Conversely, Shan *et al.* (2020) found no significant association between the 19 detected SNPs of the *MC4R* gene and body weight traits of Hu sheep; however, they noticed two SNPs (g.706 CA and g.732 CG) that were significantly associated with adult female body height traits.

Table 3 Mean (± SE) of body weight of different *MC4R* single nucleotide polymorphism-dependent on allocated groups from week 5 to week 14

Trait	Mean ± SE						
	GV1*MC4R*	GV2 *MC4R*	GV3*MC4R*	GV4*MC4R*	GB1*MC4R*	GB2*MC4R*	Total
BW5	721.11^{A}±6.65	695.83^{B}±6.97	663.00^{C} ±11.52	632.14^{D}±27.81	492.71^{E}±4.63	450.00^{F}±7.60	588.92±10.10
BW6	936.56^{A}±9.33	876.78^{B}±9.32	831.08^{C}±15.51	779.29^{D}±40.51	618.80^{E}±5.59	526.00^{F}±17.59	740.53±13.75
BW7	1173.8^{A}±9.94	1134.0^{A}±12.22	1085.2^{B}±14.62	1053.7^{B}±53.79	806.24^{C}±8.80	668.50^{D}±19.75	957.38±17.29
BW8	1431.3^{A}±11.12	1356.7^{B}±15.09	1292.0^{C}±22.10	1276.0^{C}±34.04	1099.2^{D}±7.48	982.50^{E}±10.25	1219.8±14.54
BW9	1620.0^{A}±12.57	1597.4^{A}±9.39	1485.2^{B}±17.71	1427.0^{C}±24.17	1247.4^{D}±9.46	1032.0^{E}±32.48	1388.7±18.37
BW10	1821.7^{A}±12.61	1810.0^{A}±12.13	1686.2^{B}±17.49	1629.3^{C}±31.63	1462.1^{D}±11.17	1201.0^{E}±33.78	1595.0±18.96
BW11	2053.6^{A}±13.76	1972.9^{B}±9.96	1896.8^{C}±16.94	1801.1^{D}±20.66	1650.3^{E}±12.62	1428.0^{F}±11.53	1789.6±18.70
BW12	2182.9^{A}±13.63	2177.9^{A}±14.79	2034.3^{B}±23.87	1944.4^{C}±31.31	1869.6^{D}±14.35	1565.0^{E}±33.27	1972.53±18.49
BW13	2321.4^{A}±16.10	2323.4^{A}±16.19	2176.2^{B}±16.85	2044.3^{C}±36.88	2020.3^{D}±14.43	1730.0^{E}±35.31	2117.6±18.13
BW14	2555.2^{A}±27.50	2403.7^{B}±10.62	2306.2^{C}±6.17	2258.7^{D}±0.68	2090.3^{E}±13.19	1825.5^{F}±17.10	2231.2±20.50

BW5 - BW14: Weekly body weight measurements, N: Number (17, 23, 13, 7, 49, 11, 120 for *GV1MC4R,* GV2*MC4R,* GV3*MC4R,* GV4*MC4R,* GB1*MC4R,* GB2*MC4R, &* Total, respectively)*,* GV1*MC4R*: Group SNP 1 for *MC4R* in V-line breed, GV2*MC4R*: Group SNP 2 for *MC4R* in V-line breed, GV3*MC4R*: Group SNP 3 for *MC4R* in V-line breed, GV4*MC4R*: Group SNP 4 for *MC4R* in V-line breed, GB1*MC4R*: Group SNP 1 for *MC4R* in Baladi Black breed, GB2*MC4R*: Group SNP 2 for *MC4R* in Baladi Black breed; means of various body weights with different superscripts are different (P <0.05)

Weekly body weight during the fattening period (5 to14 weeks of age) was used as a predictor in the LDA for classification of the SNP groups of *MC4R*. Correctly classified cases are the number of cases that have been correctly classified to the appropriate category in terms of the determinant (BW, 5–14 weeks). Overall, 90.8% of cases were correctly classified by the model, with 100% (17/17) for GV1*MC4R*, 78.26% (18/23) for GV2*MC4R*, 84.62% (11/13) for GV3*MC4R*, 71.43% (5/7) for GV4*MC4R*, 97.96% (48/49) for GB1*MC4R*, and 90.09% (10/11) for GB2*MC4R*. It was also shown that BW10 was the best predictor for function 1, BW6 was the best predictor for function 2, BW8 was the best predictor for function 3, BW13 was the best predictor for function 4, and BW11 was the best predictor for function 5 (estimates of 0.369, 0.904, 0.719, −1.960, and −1.313, respectively) (Table 4). On the basis of these results, rabbits with weight problems can be eliminated early in the fattening period, e.g. up to the sixth week after birth, as in the second formula.

Table 4 Standardized coefficients of the classification process of *MC4R* single nucleotide polymorphism-dependent allocated groups using weight from week 5 to week 14 as predictors for classification

Trait	Function				
	1	2	3	4	5
BW5	-0.003-	-0.389-	0.025	-0.177-	0.423
BW6	0.022	0.904	0.617	-0.388-	-0.356-
BW7	0.102	-0.340-	-0.382-	0.772	-0.694-
BW8	0.034	0.183	0.719	0.125	1.089
BW9	-0.361-	-0.378-	-0.254-	- 0.237-	0.976
BW10	0.369	0.830	-0.317-	0.417	-0.014-
BW11	-0.130-	0.129	0.020	-0.758-	-1.313-
BW12	0.088	- 0.782-	-0.665-	1.793	-0.321-
BW13	-0.367-	0.541	-0.117-	-1.960-	0.342
BW14	-0.204-	0.647	0.440	0.471	0.043

BW5–BW14: Weekly body weight measurements

The SNP-dependent allocated groups of the two studied breeds were strongly associated with carcass traits; there were genetic differences between and within SNP-allocated groups from both the V-line and Baladi Black rabbit breeds (Table 5). The variation/diversity in these genetic resources may be beneficial for future breed management and improvement.

Table 5 Mean (± SE) of carcass traits of different *MC4R* single nucleotide polymorphism -dependent allocated groups

Trait	Mean ± SE						
	GV1*MC4R*	GV2 *MC4R*	GV3*MC4R*	GV4*MC4R*	GB1*MC4R*	GB2*MC4R*	Total
Live wt	2567.7^{A}±12.98	2473.1^{B}±4.88	2443.2^{B}±0.57	2437.6^{B}±0.78	2161.0^{C}±19.46	1853.4^{D}±9.72	2302.9±20.97
Hot carcass wt	1540.3^{A}±22.22	1369.0^{B}±24.33	1347.2^{BC}±33.03	1352.7^{B}±60.47	1283.6^{C}±13.67	1084.6^{D}±15.90	1332.8±13.96
Dressing %	59.95^{AB}±0.61	57.07^{D}±0.73	57.98^{CD}±1.02	61.21^{A}±0.79	59.40^{ABC}±0.35	58.52^{BCD}±0.78	58.91±0.27
Head wt	127.83^{A}±1.76	122.74^{AB}±1.73	127.69^{A}±2.10	119.29^{B}±3.43	112.45^{C}±1.60	93.90^{D}±1.35	117.23±1.20
Head %	8.33^{C}±0.17	9.03^{AB}±0.20	9.53^{A}±0.22	8.90^{BC}±0.37	8.79^{BC}±0.13	8.67^{BC}±0.13	8.84±0.08
Liver wt	78.00^{A}±2.01	69.78^{B}±1.86	63.48^{B}±0.90	62.53^{B}±2.01	83.38^{A}±1.97	63.64^{B} ±2.96	74.95±1.23
Liver %	5.08^{C}±0.18	5.11^{C}±0.12	4.73^{C}±0.08	4.65^{C}±0.16	6.51^{A}±0.14	5.87^{B}±0.26	5.68±0.10
Heart wt	6.50^{A}±0.17	6.43^{A}±0.21	6.05^{AB}±0.21	5.84^{BC}±0.40	5.36^{C}±0.11	5.33^{C}±0.12	5.83±0.09
Heart %	0.42^{C}±0.01	0.47^{AB}±0.02	0.45^{ABC}±0.01	0.43^{BC}±0.03	0.42^{C}±0.01	0.49^{A}±0.01	0.44±0.01
Kidney wt	14.00^{A}±0.42	13.54^{A}±0.50	14.64^{A}±0.47	13.06^{A}±0.63	13.63^{A}±0.22	13.29^{A}±1.02	13.72±0.18
Kidney %	0.91^{D}±0.03	0.99^{BCD}±0.03	1.09^{B}±0.03	0.97^{CD}±0.04	1.07^{BC}±0.02	1.23^{A}±0.10	1.04±0.02
Abdom fat wt	20.14^{AB}±3.50	13.37^{B}±1.40	17.80^{AB}±1.82	18.64^{AB}±4.63	23.90^{A}±1.55	17.89^{AB}±2.74	19.85±1.00
Abdom fat %	1.29^{BC}±0.22	0.99^{C}±0.11	1.34^{ABC}±0.16	1.46^{ABC}±0.41	1.89^{A}±0.13	1.64^{AB}±0.24	1.52±0.08
Hind part wt	504.94^{A}±6.44	447.48^{B}±8.90	459.54^{B}±13.04	435.29^{B}±27.57	402.61^{C}±5.70	334.30^{D}±5.45	428.94±5.54
Hind part %	32.82^{B}±0.31	32.68^{B}±0.31	34.21^{A}±0.97	32.03^{BC}±0.82	31.33^{CD}±0.19	30.82^{D}±0.15	32.12±0.18
Fore part wt	416.50^{A}±7.18	383.04^{B}±8.37	376.46^{B}±5.68	389.29^{B}±19.31	328.92^{C}±5.14	280.90^{D}±6.20	357.10±4.77
Fore part %	27.03^{B}±0.20	27.98^{A}±0.30	28.07^{A}±0.54	28.79^{A}±0.64	25.60^{C}±0.23	25.88^{C}±0.18	26.74±0.17
Mid part wt	360.61^{A}±8.84	307.04^{BC}±8.08	324.69^{B}±6.96	308.00^{BC}±14.84	311.14^{BC}±4.71	297.10^{C}±5.70	317.89±3.47
Mid part %	23.37^{BC}±0.31	22.39^{C}±0.33	24.19^{B}±0.54	22.77^{C}±0.39	24.24^{B}±0.24	27.45^{A}±0.70	23.93±0.19

N: Number (17, 23, 13, 7, 49, 11, 120 for *GV1MC4R,* GV2*MC4R,* GV3*MC4R,* GV4*MC4R,* GB1*MC4R,* GB2*MC4R, &* Total, respectively)*,* GV1*MC4R*: Group SNP 1 for *MC4R* in V-line breed, GV2*MC4R*: Group SNP 2 for *MC4R* in V-line breed, GV3*MC4R*: Group SNP 3 for *MC4R* in V-line breed, GV4*MC4R*: Group SNP 4 for *MC4R* in V-line breed, GB1*MC4R*: Group SNP 1 for *MC4R* in Baladi Black breed, GB2*MC4R*: Group SNP 2 for *MC4R* in Baladi Black breed; means of various slaughter traits with different superscript are different (P <0.05)

All carcass traits except forepart weight, foreparts %, and middle parts % were used as predictors in LDA for the classification of SNP groups. In terms of determinant (carcass traits), correctly classified cases corresponded to the number of cases that had been correctly classified into the correct category. Overall, the model correctly classified 93.3% of cases, with 100% (17/17) for GV1*MC4R*, 91.3% (21/23) for GV2*MC4R*, 69.23% (9/13) for GV3*MC4R*, 85.71% (6/7) for GV4 *MC4R*, 100% (49/49) for GB1 *MC4R*, and 81.82% (9/11) for GB2*MC4R*. It was also shown that liver weight was the best predictor for function 1, hind part weight was the best predictor for function 2, liver % was the best predictor for function 3, and hind part weight was the best predictor for functions 4 and 5 (estimates of −3.315, −1.559, 7.033, 8.130, and −11.260, respectively) (Table 6). These results demonstrate the efficiency of determining the breeding method and mating system within rabbit farms by selecting rabbits with desirable traits to be the parents of future generations. Xuemei *et al.* (2006) discovered an association between various genotypes of the chicken *MC4R* gene and body weight, growth, and carcass traits. They revealed that the body weight, carcass weight, and leg muscle weight of the BB were dramatically higher than those of the AA and AB genotypes (P <0.05), but the difference among the three genotypes did not attain the significance level (P >0.05). The afore-mentioned authors also concluded that the *MC4R* gene is the most likely candidate gene for chicken growth and carcass traits.

In terms of feed intake, DFI, and FCR there was substantial variation between the V-line and Black Baladi class SNPs, with the Black Baladi SNPs outperforming the V-line SNPs at most intervals for these traits (Table 7). Jiang *et al.* (2008) reported that allele A of the *MC4R* gene was the predominant allele in five studied rabbit breeds; therefore, the AA genotype level was greater than the AG genotype. GLM analysis for the influence of genotypes on productive traits demonstrated that the genotype of AG was truly associated with BW, eviscerated weight, and feed conversion efficiency. G/A 1426 *MC4R* mutations were genotyped using real-time PCR approach by Piórkowska *et al.* (2010) in 1191 gilts of five breeds to determine the impact of missense mutations on meat quality, carcass composition, and growth traits. They noticed that the A allele was linked to higher daily feed intake (AA – 2.51 kg; GG – 2.31 kg in the Puławska breed, P <0.05), daily gain, and backfat thickness (AA –1.67 cm, GG –1.52 cm in PL, P <0.01). Reduced lean meat content was exhibited by the AA genotype.

Table 6 Standardized coefficient of classification process of *MC4R* single nucleotide polymorphism-dependent allocated groups using carcass traits as predictors for classification

Slaughter Traits	Function				
	1	2	3	4	5
Hot carcass wt	1.624	0.019	-0.765-	-7.238-	7.620
Dressing %	-0.026-	0.283	0.835	1.049	-0.810-
Head wt	-0.302-	0.315	-4.219-	1.590	1.781
Head %	0.588	-0.379-	5.682	-1.600-	-1.856-
Liver wt	-3.315-	-0.522-	-6.139-	-1.598-	-1.439-
Liver %	3.250	0.401	7.033	1.954	1.495
Heart wt	2.885	0.539	3.837	0.996	1.813
Heart %	-2.805-	-0.631-	-4.583-	-0.880-	-1.774-
Kidney wt	0.869	0.492	4.241	-3.033-	1.911
Kidney %	-1.091-	-0.400-	-5.100-	2.935	-2.016-
Abdom fat wt	1.846	-1.207-	3.886	4.474	-1.055-
Abdom fat %	-1.860-	1.052	-3.976-	-3.966-	1.318
Hind part wt	-1.935-	-1.559-	3.601	8.130	-11.260-
Hind part %	1.313	0.610	-1.559-	-3.038-	6.373
Mid part wt	-0.252-	0.121	-0.845-	0.430	0.066

The effect of *MC4R* on rabbit FI indicates that it may be a significant genetic marker for the growth-related features of rabbits. Moreover, nucleotide sequence assessment of whole amplified fragments and evaluation of the standard errors and means of individual rabbits' BW at 9 weeks of age have confirmed that significant correlations exist between *MC4R* SNPs and high rabbit BW at marketing age (El-Sabrout & Aggag, 2017; El-Sabrout, 2017). Interestingly, our study elicited novel SNPs in the *MC4R* gene associated with growth traits in rabbits. El-Sabrout & Soliman (2018) explained the relationship between two parts of the *melanocortin* gene (*MC4R-1* and *MC4R-2*) and FI for V-line rabbits (low and high FI); they observed that the alignment of sequence data from each group revealed that there is a variation detected in *MC4R*-1 at nucleotide 35 (T–G) (sense mutation) and another variation was detected in *MC4R-2* gene at nucleotide 19 (T–C) (sense mutation) for high feed intake rabbits. The *MC4R* gene's function is substantially altered as a result of these sense mutations, which modify certain amino acids. The average daily feed intake findings showed that the high feed intake group had a substantially higher feed intake than the V-line rabbits' high feed intake group. The indicated mutations, as well as the assessment of daily feed intake means, demonstrated a substantial link between the *MC4R* polymorphism and rabbit feed intake. The same association with BW, obesity, and development was reported by Nahácky *et al*. (2018). On the other hand, Dvoáková *et al*. (2011), found no effect of the p.Asp298Asn mutation of the *MC4R* gene on feed intake, feed conversion, and growth rate in a Czech pig population.

Table 7 Mean (± SE) of feed intake, daily feed intake, and feed conversion ratio of different *MC4R* single nucleotide polymorphism-dependent allocated groups from week 5 to week 14

Trait	Mean ± SE						
	GV1*MC4R*	GV2 *MC4R*	GV3*MC4R*	GV4*MC4R*	GB1*MC4R*	GB2*MC4R*	Total
FI 5-8 wk	1.61^{A}±0.03	1.52^{A}±0.04	1.52^{A} ±0.06	1.50^{A}±0.14	1.51^{A}±0.02	1.55^{A}±0.02	1.53±0.02
FI 8-11 wk	2.15^{AB}±0.08	2.06^{C}±0.06	2.25^{A}±0.09	1.96^{B} ±0.16	2.24^{AB}±0.04	2.03^{B}±0.05	2.164±0.03
FI 11-14 wk	2.38^{B}±0.08	2.33^{B}±0.07	2.40^{B}±0.09	1.98^{C} ±0.23	2.68^{A} ±0.04	2.56^{AB}±0.11	2.48±0.04
FI 5-14 wk	6.14^{AB}±0.17	5.91^{B}±0.15	6.17^{AB}±0.23	4.97^{C}±0.37	6.42^{A}±0.10	6.15^{AB}±0.18	6.15±0.07
DFI 5-8 wk	76.88^{A}±1.34	72.58^{A}±1.70	72.45^{A}±2.84	71.31^{A}±6.53	71.67^{A}±0.100	73.96^{A}±0.98	72.88±0.74
DFI 8-11 wk	102.17^{AB}±3.58	98.07^{AB}±2.84	106.99^{A}±4.17	93.39^{B}±7.61	106.54^{A}±1.88	96.83^{B}±2.43	102.73±1.31
DFI 11-14 wk	113.24^{BC}±3.64	110.82^{C}±3.24	114.22^{BC}±4.13	94.46^{D}±11.00	127.42^{A}±2.09	122.06^{AB}±5.43	118.31±1.68
DFI 5-14 wk	97.51^{AB}±2.68	93.83^{B}±2.36	97.88^{AB}±3.61	78.86^{C}±5.87	101.88^{A}±1.56	97.62^{AB}±2.88	97.55±1.14
FCR 5-8 wk	2.60^{A}±0.07	2.45^{B}±0.02	2.27^{C} ±0.02	2.18^{C} ±0.21	2.72^{A} ±0.03	2.46^{B}±0.02	2.55±0.03
FCR 8-11 wk	4.35^{B}±0.15	3.86^{C}±0.06	3.85^{C} ±0.02	4.04^{C}±0.17	4.68^{A}±0.05	3.91^{C}±0.14	4.28±0.05
FCR 11-14 wk	7.00^{A}±0.41	6.23^{B}±0.06	5.35^{C} ±0.19	5.62^{C}±0.23	6.47^{B}±0.03	6.35^{B} ±0.05	6.32±0.08
FCR 5-14 wk	4.15^{B}±0.05	3.86^{C}±0.01	3.61^{D}±0.03	3.29^{E} ±0.28	4.43^{A}±0.04	3.94^{C}±0.02	4.08±0.04

FI: Feed intake at 5–8, 8–11, 11–14, and 5–14 weeks, DFI: Daily feed intake at 5–8, 8–11, 11–14, and 5–14 weeks, FCR: Feed conversion ratio at 5–8, 8–11, 11–14, and 5–14 weeks, N: Number (17, 23, 13, 7, 49, 11, 120 for *GV1MC4R,* GV2*MC4R,* GV3*MC4R,* GV4*MC4R,* GB1*MC4R,* GB2*MC4R, &* Total, respectively), GV1*MC4R*: Group SNP 1 for *MC4R* in V-line breed, GV2*MC4R*: Group SNP 2 for *MC4R* in V-line breed, GV3*MC4R*: Group SNP 3 for *MC4R* in V-line breed, GV4*MC4R*: Group SNP 4 for *MC4R* in V-line breed, GB1*MC4R*: Group SNP 1 for *MC4R* in Baladi Black breed, GB2*MC4R*: Group SNP 2 for *MC4R* in Baladi Black breed; means of various feed conversion ratios, feed, and daily feed intake with different superscripts are different (P <0.05)

Feed intake, DFI, and FCR were entered in the DF model using a stepwise method; FI and FCR were selected for use in the equation as predictors. FCR and FI were also used as predictors in LDA for classification of the SNP groups of *MRC4*. Correctly classified cases are the number of cases that have been correctly classified to the appropriate category in terms of the determinants (FCR & FI). Overall, the model correctly classified 85% of cases, with 64.71% (11/17) for GV1*MC4R*, 95.65% (22/23) for GV2*MC4R*, 92.31% (12/13) for GV3*MC4R*, 5.6% (2/7) for GV4*MC4R*, 89.79% (44/49) for GB1*MC4R*, and 90.91% (10/11) for GB2*MC4R*. It was also shown that breed was the best predictor for function 1; FI 5–14 was the best predictor for functions 2, 3, 4, and 5 for both FI and FCR; followed by FI 8–11 for functions 2, 3, and 5; and then FI 5–8 for function 4; followed by FCR 8–11 for functions 2 and 5; and then FCR 5–8 for functions 3 and 4 (Table 8).

Table 8 Standardized coefficient of classification process of *MC4R* single nucleotide polymorphism-dependent allocated groups using feed intake and feed conversion ratio as predictors for classification

Trait	Function				
	1	2	3	4	5
breed	1.244	-0.175-	-0.012-	-0.026-	0.066
FI 5-8 wk	-0.299-	1.133	-0.992-	-1.896-	-3.158-
FI 8-11 wk	-0.437-	2.366	-2.664-	-0.428-	-3.494-
FI 11-14 wk	-0.128-	0.474	-0.424-	-1.350-	-2.522-
FI 5-14 wk	0.818	-4.085-	4.182	3.561	9.188
FCR 5-8 wk	0.194	-0.799-	0.519	0.998	2.837
FCR 8-11 wk	0.421	-0.995-	0.019	0.801	2.906
FCR 11-14 wk	0.136	-0.380-	0.409	-0.047-	2.091
FCR 5-14 wk	-1.141-	2.971	-0.986-	-1.422-	-5.396-

FI: Feed intake at 5–8, 8–11, 11–14, and 5–14 weeks, FCR: Feed conversion ratio at 5–8, 8–11, 11–14, and 5–14 weeks

MC4R SNP-allocated groups in both breeds had an effect on weight gain and daily weight gain; there was genetic variation between and within the group *MC4R* SNPs for both V-line and Baladi Black rabbit breeds (Table 9). Animal breeders can use this difference as a preliminary step to improve subsequent generations of rabbits.

Table 9 Mean (± SE) of weight gain and daily weight gain of different *MC4R* single nucleotide polymorphism-dependent allocated groups from week 5 to week 14

Trait	Mean ± SE						
	GV1*MC4R*	GV2 *MC4R*	GV3*MC4R*	GV4*MC4R*	GB1*MC4R*	GB2*MC4R*	Total
WG 5-8	710.17^{A}±7.92	660.83^{B}±13.60	629.00^{BC}±18.10	643.86^{B}±17.74	606.51^{C}±6.30	532.50^{D}±6.64	630.92±6.15
WG 8-11	622.33^{A}±7.47	616.22^{A}±10.24	604.77^{A}±25.31	525.14^{B}±16.53	551.08^{B}±6.47	445.50^{C}±9.79	569.76±6.42
WG 11-14	501.56^{A}±37.57	430.78^{BC}±7.92	409.38^{BC}±16.82	457.57^{AB}±20.10	439.96^{BC}±5.92	397.50^{C}±8.54	441.62±7.08
WG 5-14	1834.1^{A}±30.82	1707.8^{B}±9.17	1643.2^{C}±15.57	1626.6^{C}±27.27	1597.6^{C}±12.47	1375.5^{D}±18.66	1642.3±12.89
DWG 5-8	33.82^{A} ±0.38	31.47^{B}±0.64	29.95^{BC}±0.90	30.66^{B}±0.84	28.88^{C}±0.30	25.36^{D}±0.32	30.04±0.29
DWG 8-11	29.63^{A} ±0.36	29.34^{A}±0.49	28.80^{A}±1.21	25.01^{B}±0.79	26.24^{B}±0.31	21.21^{C}±0.47	27.13±0.31
DWG 11-14	23.88^{A} ±1.79	20.51^{BC}±0.38	19.49^{BC}±0.80	21.79^{AB} ±0.96	20.95^{BC}±0.28	18.93^{C}±0.41	21.03±0.34
DWG 5-14	29.11^{A} ±0.49	27.11^{B}±0.15	26.08^{C}±0.25	25.82^{C}±0.43	25.36^{C}±0.20	21.83^{D}±0.30	26.07±0.20

WG: Weight gain at 5–8, 8–11, 11–14, and 5–14 weeks, DWG: Daily weight gain at 5–8, 8–11, 11–14, and 5–14 weeks, N: Number (17, 23, 13, 7, 49, 11, 120 for *GV1MC4R,* GV2*MC4R,* GV3*MC4R,* GV4*MC4R,* GB1*MC4R,* GB2*MC4R, &* Total, respectively), GV1*MC4R*: Group SNP 1 for *MC4R* in V-line breed, GV2*MC4R*: Group SNP 2 for *MC4R* in V-line breed, GV3*MC4R*: Group SNP 3 for *MC4R* in V-line breed, GV4*MC4R*: Group SNP 4 for *MC4R* in V-line breed, GB1*MC4R*: Group SNP 1 for *MC4R* in Baladi Black breed, GB2*MC4R*: Group SNP 2 for *MC4R* in Baladi Black breed; means of various weight gains and daily weight gains with different superscript are different (P <0.05)

Conclusions

The findings of the study revealed a significant association between *MC4R* gene polymorphisms and growth, carcass, and feed intake traits in rabbits. The results presented in this study suggest that SNPs in the *MC4R* gene could be beneficial in selecting the highest performing rabbits for these traits, which may be useful in rabbit breeding strategies. The correlation of *MCR4* SNPs with various economic traits in pure rabbit breeds should be investigated in further research and extended to also include crossbred rabbits. Metabolic profiles of GH, TSH, and Lep could also be proxy biomarkers for discrimination between these breeds.

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Authors' contributions

HAR designed, performed the experiment, and collected data; AIA collected blood samples, performed PCR–DNA Sequencing; EAA analysed the data and interpreted the results; SAS performed the experiment, and collected data; MMF, RAD, and AEE wrote the initial manuscript. The manuscript was revised by all authors, and the final version was accepted before release.

Conflict of interest declaration

The authors declare that they have no conflict of interest.

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