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Original scientific article

EFFICIENCY OF *LEPTIN* GENE POLYMORPHISMS IN THE EVALUATION OF THE GROWTH PERFORMANCE AND CARCASS MEASUREMENTS OF V-LINE AND BALADI BLACK RABBITS

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This study investigated the association between both polymorphisms and metabolic marker changes of the *leptin* gene, and body weight, weight gain, carcass traits, feed intake, and feed conversion ratio. Blood samples were collected from 60 V-line and 60 Baladi Black rabbits for DNA extraction and biochemical analysis. DNA sequencing of

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leptin (202-bp) revealed four non-synonymous single nucleotide polymorphism (SNPs) that characterized a number of V-line rabbits. Statistical analysis revealed that the identified SNPs were associated with rabbit growth and carcass measurements ($p < 0.05$). A discriminant analysis model showed a high classification percentage for the identified SNPs within and between breeds using body weight at 5–14 weeks of age (91.7%), slaughter traits (91.6%), feed intake, daily feed intake, and feed conversion ratio (76.7%). This study reveals that *leptin* gene could be a candidate for growth traits in rabbits enabling the development of marker-assisted selection (MAS) in different rabbit breeds.

Keywords: Carcass measurements, discriminant analysis, growth performance, *leptin* gene, rabbit, SNP.

INTRODUCTION

Rabbits are valuable and economic meat sources because of their rapid growth, prolificacy, and high fecundity (CARTUCHE *et al*. 2014). They require a small area for living and have low feed consumption, a short generation interval, a quick growth rate, high-efficiency feed conversion, high profiteering, and potential for genetic progress; these characteristics make rabbits an ideal species for meat production **(**HERBERT, 2011**)**. Rabbit meat is safe, high quality, and ready for human consumption. It is highly nutritional and healthy, with palatable properties including highly digestible, tender, white meat with low fat, cholesterol, and sodium contents, plus high value protein. In short, rabbit meat is overwhelmingly used as an alternative to chicken meat and is advisable to be eaten periodically by children and pregnant women as well as the elderly (EZEMA and EZE, 2015; TROCINO *et al*., 2018). In 2017, the United Nations Food and Agriculture Organization (FAO) ranked Egypt as the fourth largest rabbit meat producer worldwide after China, North Korea, and Spain (FAO, 2019).

The Valencia line (V line) was created as a synthetic line in 1981 by crossing the progeny of four specialized maternal lines that were selected to improve litter size at weaning (ESTANY *et al*., 1989). A maternal synthetic line means obtaining a distinct breed by improving traits related to doe productivity, such as litter weights and sizes, and milk yield which are considered as selection criteria in creating maternal rabbit lines (ESTANY *et al*., 1989; BASELGA, 2004). The intention of using line V rests on many characteristics. One is Valencia's long tradition of selection (GARCÍA and BASELGA, 2002), where the climate does not vary widely from the conditions of the Nile delta in Egypt. Another intriguing characteristic is that line V has been tested in hot climates such as Adana in Turkey or Zagazig in Egypt, and this line has outperformed other exotic breeds in terms of performance (YAMANI, 1994; TESTIK, 1996). Recent studies in Saudi Arabia for the V line have ascertained that line V's heat stress tolerance is outstanding (KHALIL *et al*., 2002).

Regarding Baladi Black breed, breeding of such breed is mainly to establish a heat-resistant meat rabbit that could sustain the Egyptian climate and be used principally for meat production. A breeding approach resulted in the implementation of three native strains: Baladi Red, Baladi White, and Baladi Black (KHALIL, 2011). According to BADAWY (1975) and GALAL and KHALIL (1994), the Baladi developed in Egypt as a consequence of crossbreeding between native rabbits and Flemish Giants at stations of the Ministry of Agriculture's Poultry Breeding Section for several generations.

Rabbit growth and carcass measurements are relevant for breeding procedures. Growth hormone serves to improve lean body mass, promoting muscle, bone, and cartilage cell growth and metabolism while reducing body fat EL-GHANY (2015). The thyroid hormone regulates the speed at which the body burns fat, creates proteins, and is responsible for the body's response to other hormones; the thyroid contributes to these processes through the production of triiodothyronine and thyroxine. These hormones control the growth process (JAFF and SALIH, 2009). A major regulator for food intake and energy homeostasis is leptin. Leptin deficiency can lead to severe problems in humans, such as obesity, diabetes, and infertility, as mentioned by ZHANG *et al*. (2005).

Currently, a large number of edited polymorphisms require that their effect be evaluated before their inclusion in breeding methods MIGDAL *et al*. (2019). Selection based on growth characteristics is of considerable significance to the rabbit industry due to the high feed expenses associated with rabbit production and customer demand for lean meat. The mechanism that regulates feed intake and its conservation of body weight is complex. Genes related to meat quality and body weight has been characterized using single-nucleotide polymorphisms (SNPs) identification of the various candidate genes (ZHANG *et al*., 2013; WU *et al*., 2015).

Single-nucleotide polymorphisms (SNPs) are an efficient approach to identify nucleotide sequence mutations in amplified DNA AMIE MARINI *et al*. (2012). Additionally, the ability to evaluate a specific desirable SNP is a new and prolonged procedure for identifying molecular DNA markers to assess the impact of a specific gene on traits of interest. Therefore, enabled exploration of genome-wide selection signatures can be carried out via an assessment of variation in marker allele frequencies between populations (HOLSINGER and WEIR, 2009). Recently, many experiments have been undertaken to establish the relationship between leptin polymorphisms and carcass characteristics in animals; as a result of these studies, a series of SNPs have been identified in the *leptin* gene in cattle (NKRUMAH *et al*., 2005). In pigs, JIANG and GIBSON (1999) two polymorphic sites within leptin were identified. A link between polymorphisms in the ovine *leptin* gene and meat quality characteristics was noted by BOUCHER *et al*. (2006).

Likewise, when KÖK and VAPUR (2021) evaluated the effects of thyroglobulin and *leptin* gene polymorphisms on beef quality in Holstein breed bulls in Turkey, they found that both play a major role in the regulation of energy balance and body weight control. The thyroglobulin and *leptin* genes are being explored as candidates for quantitative trait locus-based selection strategies to increase beef quality in cattle breeds (KÖK *et al*., 2015, KORKMAZ *et al*., 2015, GUIMARÃES *et al*., 2016, ARDICLI *et al*., 2019).

Further research has been conducted to improve the genetic potential of rabbits raised for meat by adopting genotypes based on candidate gene SNPs (EL-SABROUT and SOLIMAN, 2018). Nucleotide substitutions in the *leptin* gene have been associated with performance merits, making it a candidate target gene for optimizing livestock productivity (HAN *et al*., 2012). However, studies on the effects of *leptin* polymorphisms on the productive traits of rabbits are scarce (MIGDAL *et al*., 2018).

This study was conducted to detect the association between *leptin* SNPs and the growth and carcass measurements of V line and Baladi Black rabbits. A further aim of the study was to link these genetic polymorphisms with the serum levels of various metabolic biochemical markers, including growth, leptin, and thyroid-stimulating hormone.

MATERIALS AND METHODS

Animals

This experiment was performed 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 5 weeks old, which is weaning. Then begin recording growth and feed performance of 120 five-week-old V-line and Baladi Black breed rabbits until 14 weeks, when the animals are slaughtered and carcass traits are recorded. Every breed comprised 60 rabbits, with 15 males accounting for 25% of the overall group and 45 females accounting for the remaining 75%. The collection of samples and care of rabbits used in this study followed guidelines of Mansoura University and the protocol of the study was approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Farm management

Breeding rabbits were housed individually in galvanized wire hutches (40 × 60 × 50 cm) with a manual feeder and a watering nipple system. For kindling and nursing kits, a metal nest box (40 × 40 × 40 cm) was connected to the doe's cage. The nest boxes were supplied with a thick layer of rice straw on the 25th day of pregnancy, which was placed in the bottom of the nest box to assist the doe in creating a warm, comfy nest for her bunnies. Cages were periodically washed. Urine and faces dropped from the confines on the floor were swept every day in the morning. During the experiment, the environmental temperature was kept around 27 °C and fans were used to promote ventilation and fresh air, and the light period corresponded to 16L – 8D per day in the rabbit.

A tubular-shaped pelletized (4 mm diameter, 9 mm length) commercial ration was utilized. The ration was formulated according to ZAGHLOUL *et al*. (2019), The ration was comprised of 24% soybean meal, 23% barley, 21% berseem hay, 19% wheat bran, 18.01% crude protein, 13.7% crude fiber, 13% yellow corn, 2.5% fat, 1% limestone, 0.5% table salt, 14 kg di-calcium phosphate/ton, 1 kg anti-toxicity/ton, 1 kg anti-coccidian/ton, and 1 kg minerals mixture/ton. Pellets were formulated in the farm to meet the basic requirements for rabbits, according to the National Research Council (NRC, 1977). The rabbits had access to fresh water at all times.

Growth performance traits

Starting at 5 weeks of age, the rabbits were weighed weekly to calculate live body weight (BW)/week. Body weight gain (WG) was expressed as the difference between the final and initial weights, according to the relevant period. The average daily gain (DWG) was also calculated as the weight gain as a function of the measured number of days.

The rabbits were given a weighed quantity of feed daily to determine total feed intake (FI) and daily feed intake (DFI). At the end of the day, the residual feed was extracted from the total provided to determine the quantity consumed, as described by WAGNER *et al*. (1983). According to IYAYI and ODUESO (2003) the feed conversion ratio (FCR) was computed by dividing feed consumption/kg by WG/kg. WG, DWG, FI, DFI, and FCR were calculated across the following four-time intervals: 5–8, 8–11, 11–14, and 5–14 weeks of age.

Carcass measurements

At the end of the fattening period, the rabbits were fasted for 12 h and weighed prior to slaughter. After being stripped and dressed out, the hot carcass was weighed and recorded.
The dressing% was determined as follows: 100 × hot carcass (giblet weight + carcass weight + head weight)/live BW, where giblet weight = kidney + heart + liver weight. According to SZENDRŐ *et al*. (2010) the ratio of body parts and organs to carcass weight and the percentage of the fore, mid, and hind parts were calculated.

Experimental samples

At 14 weeks of age, approximately 10 ml of blood was collected from the ear vein after cleaning, disinfecting, and by means of a piece of cotton packed with alcohol placed in tubes containing EDTA and other tubes containing no anticoagulants. EDTA blood was used for DNA extraction, whereas blood without coagulant was used for serum processing via centrifugation. The EDTA blood was collected from sixty rabbits of the two breeds; while the females were selected for collection of the serum samples to avoid the effect of sex. The serum samples were subsequently stored in a cold gel sealed icebox at 4℃ prior to the hormone measurements.

Leptin gene polymorphisms

DNA extraction

The extraction of genomic DNA from whole blood was conducted using a Gene JET whole blood genomic DNA extraction kit, following the manufacturer's procedure (Thermo Scientific, Lithuania). The quality, purity, and concentration of DNA were determined by Nanodrop for further analysis (ATEYA *et al*., 2021).

Polymerase chain reaction (PCR)

PCR was performed to amplify the fragment of the *leptin* gene spanning exons I and II, with an expected amplicon size of 202bp .
Forward: 5′-GTGGTTCCTTCTGCCTTCAG-3′
Reverse: 5′-GCCTCTGTACCGTGTGTGAG-3′

The PCR mixture was as follows: 3 µl DNA, 21 µl H_2O (d.d water), 25 µl PCR master mix (Jena Bioscience, Germany), 0.5 µl forward primer, and 0.5 µl reverse primer (ATEYA *et al*., 2021).

The final reaction mixture was placed in a thermal cycler and the PCR program was carried out as follows: initial denaturation at 95℃ for 3 min, followed by 30 cycles at 95℃ for 1 min for DNA denaturation, annealing at 62℃ for 1 min, extension at 72℃ for the 30s, and final extension at 72℃ for 5 min. The samples were stored at 4℃. Representative results from PCR analysis were detected by agarose gel electrophoresis, and fragment patterns were visualized under ultraviolet light using a gel visualization system.

DNA sequencing

PCR products with target bands were used for DNA sequencing in the forward and reverse directions using an ABI 3730XL DNA sequencer (Applied Biosystem, USA) according to the enzymatic chain terminator technique developed by SANGER *et al*. (1977). Analysis of

DNA sequencing data was carried out using Chromas 1.45 and BLAST 2.0 software ALTSCHUL *et al*. (1990). Differences in the PCR products of the *leptin* gene and reference sequences available in GenBank were classified as SNPs. Based on data alignment from DNA sequencing, variation in the amino acid sequence of the *leptin* gene across the breeds was assessed using the MEGA4 software package (TAMURA *et al*., 2007).

Metabolic biochemical markers

Thyroid-stimulating hormone (TSH)

The estimated TSH concentration was measured according to the procedures described by BALOCH *et al*. (2003) using kits from Siemens US Health Diagnostics. The kits required the use of quick TSH IMMULITE/IMMULITE 2000, a strong chemical phase (enzyme) that employs chemiluminescent immunometric technology.

Growth hormone (GH)

Growth hormone was quantified via ELISA, and controlled by including 100 µl of a weak dilution of the antibody in each well of a plate, which was then kept at room temperature (20℃–25℃) for 1 h, after which 200 µl of blocking solution was added for maintenance for 30 min, followed by washing the plate several times. Afterward, 100 measures were applied to each well and kept at room temperature for 1 h, followed by the addition of 100 µl of dilution solution, which was then kept at room temperature for 1 h. The wells were washed with 100 µl of TMP substrate solution, then placed in an opaque container at room temperature for 15 min, followed by the addition of 100 µl of suspension solution. Color absorption was then assessed using a plate reader at 450 nm, as described by MEDGYESI *et al*. (1975).

Leptin hormone (Lep)

Serum leptin hormone was quantitatively assessed using the IMMULITE and IMMULITE 1000 Analyzer according to TIAN (2005). IMMULITE and IMMULITE 1000 are solid-stage, chemical (enzyme)-labeled chemiluminescent competitive immunoassays for leptin. The bead (solid) is coated with an anti-leptin polyclonal rabbit antibody, whereas the fluid consists of leptin-conjugated alkaline phosphatase (bovine calf intestine).

Statistical analysis

Data were organized, summarized, and analyzed using SPSS version 23 (USA). Chi-square tests ($\chi 2$) were used to compare the frequencies of the identified SNPs in *leptin* across the V line and Baladi Black breeds. A linear discriminant analysis (LDA) model was built to evaluate the significance of the different determinants in classifying the identified SNPs as dependent variables, using live BW, BWG, feed consumption, FCR, and carcass traits as independent variables. The discriminant statistical model used for this analysis was:

$DF = V_1X_1 + V_2X_2 + V_3X_3 + \ldots + V_IX_I$,

Where DF = discriminate function (score) of grouping variables, V = the standardized discriminant coefficient or loadings for the predictors, X = respondent score for the predictors, I = number of predictor variables. The discriminant function coefficients V or standardized form beta indicates the partial contribution of each predictor to the discrimination process.

A one-way analysis of variance was used to compare mean live BW, BWG, feed consumption, FCR, and carcass traits among SNPs in *leptin*. Data are presented as means ± SEM. Results were considered significant at $P < 0.05$.

RESULTS

DNA sequencing of *leptin* (202-bp) revealed four non-synonymous SNPs (submitted to GenBank with the accession numbers gb|MT832142|, and gb|MT832143|) characterizing V line rabbits (Table 1). Three SNPs (C62A, C70G, and C71A) seemed to be specific for a number of V line rabbits which were represented as GV1, while one SNP (G68C) was specific for V line rabbits which were represented as GV2. V line rabbits that did not exhibit the identified four SNPs were represented as GV3. None of the Baladi Black rabbits exhibited any of the four identified SNPs which were represented as GB1. Chi-square tests revealed a difference in the frequencies of *leptin* SNPs between the two breeds. Nucleotide sequence variation in *leptin* (202bp) between the two breeds as well as between these breeds and reference sequences available in GenBank (JX868865.1) confirmed all four identified SNPs (Figure 1).

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VL          GTGGTTCCTTCTGCCTTCAGGCCCGAGAAACACATCCTGGGAAGGAAAATGCGGTGTGGA   60
JX868865.1  GTGGTTCCTTCTGCCTTCAGGCCCGAGAAACACATCCTGGGAAGGAAAATGCGGTGTGGA   60
BB          GTGGTTCCTTCTGCCTTCAGGCCCGAGAAACACATCCTGGGAAGGAAAATGCGGTGTGGA   60
            ************************************************************

VL          CACCTCTCCGAACTCCTGTGGCTGTGGCCCTGTCTGTCCTGTGTTCCAGCTGTGCCCATG   120
JX868865.1  CCCCTCTGCCAACTCCTGTGGCTGTGGCCCTGTCTGTCCTGTGTTCCAGCTGTGCCCATG   120
BB          CCCCTCTGCCGACTCCTGTGGCTGTGGCCCTGTCTGTCCTGTGTTCCAGCTGTGCCCATG   120
            * ***** *  **************************************************

VL          CGGAAAGTCCAGGATGACACCAAGACCCTCATCAAAACCATTGTCACCAGGATCAGTGAC   180
JX868865.1  CGGAAAGTCCAGGATGACACCAAGACCCTCATCAAAACCATTGTCACCAGGATCAGTGAC   180
BB          CGGAAAGTCCAGGATGACACCAAGACCCTCATCAAAACCATTGTCACCAGGATCAGTGAC   180
            ************************************************************

VL             ATCTCACACACGGTACAGAGGC 202
JX868865.1     ATCTCACACACGGTACAGAGGC 202
BB             ATCTCACACACGGTACAGAGGC 202
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Figure 1. Representative DNA sequence alignment of *leptin* gene (202-bp) between V line, and Baladi Black rabbit and reference sequence available in GenBank gb| JX868865.1|. Asterisks represent similarity. VL is V line and BB is Baladi Black.

Table 1. Distribution of SNPs identified within the leptin gene in V-line and Baladi Black rabbits.

SNP_S	V line	BB	Total	Chi-Square	Amino acid type and number	P-Value
C62A	22	0	22/120	24.54	21, P to H	<0.0001**
C70G	22	0	22/120	24.54	24, Q to E	<0.0001**
C71A	22	0	22/120	24.54	24, Q to R	<0.0001**
G68C	24	0	24/120	27.55	23, C to S	<0.0001**

BB: Baladi Black, P: Proline, H: Histidine, Q: Glutamine, E: Glutamic acid, R: Arginine, C: Cysteine, S: Serine, P <0.05 is considered statistically different, *Mean significant difference, **Mean highly significant difference, **: P <0.01.

Table 2. Hormonal assay of V-line and Baladi Black rabbits harboring leptin single nucleotide polymorphisms (SNPs) genotypes

Hormone	Mean ± SE					ANOVA	
	GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	Total	F	Sig
TSH	0.97^{a} ±0.03	0.86^{b} ±0.04	0.81^{b} ±0.04	1.02^{a} ± 0.03	0.95±0.02	5.965	0.001
GH	2.31^{a} ±0.18	2.19^{a} ±0.08	2.14^{a} ±0.14	2.06^{a} ±0.08	2.14±0.06	0.929	0.429
Lep	1.62^{a} ±0.16	1.98^{a} ±0.08	1.78^{a} ±0.07	1.76^{a} ±0.07	1.79±0.05	1.823	0.147

TSH: Thyroid stimulating hormone, GH: Growth hormone, Lep: Leptin hormone, GV1-Lep: Group SNP 1 for leptin in V line breed, GV2-Lep: Group SNP 2 for leptin in V line breed, GV3-Lep: Group SNP 3 for leptin in V line breed, GB1-Lep: Group SNP 1 for leptin in Baladi Black breed. According to LSD's test, means of different levels having different superscripts are significantly different at P<0.05.

Table 2 displays the outcomes of the hormonal testing for the *leptin* SNP groups. A difference was detected in TSH across the V line and Baladi Black SNP-based groups, where GB1-leptin and GV1-Lep had the highest TSH (1.02 and 0.97 IU/ml, respectively). Conversely, there was no noticeable difference between the levels of GH and leptin across the studied groups.

All of the attributes evaluated, including BW, slaughter traits, FI, DFI, FCR, WG, and DWG were then tested in a LDA model using a stepwise technique; all of these variables, except DFI, WG, and DWG, were included in the equation as predictors for *leptin* SNP groups.

Concerning BW at 5–14 weeks of age, there were differences between the V line and Black Baladi rabbits, with GV1, GV2, GV3 having higher BWs than GB1. The BW also differed within the V line breed across the SNP groups; GV1 rabbits had higher body weights than those from all other SNP groups (Table 3). As such, the correlation between *leptin* SNPs and body weight in rabbit breeds should be investigated in future research.

Body weights from 5–14 weeks of age were used as predictors in the linear discriminant analysis for the classification of the SNP groups; overall, it correctly classified 91.7% of all cases, and 90% (18/20) for GV1, 83.33% (20/24) for GV2, 81.25% (13/16) for GV3, and 98.33% (59/60) for GB1 (Table 4). Results in Table 5 demonstrates that BW 9 was the best predictor for function 1 and BW 11 was the best predictor for functions 2 and 3, with estimates of −2.507, 3.721, and −1.964, respectively.

Table 3. Mean (±SE) of body weight of different leptin SNPs dependent allocated groups from week 5 to week 14

Trait	Mean ± SE					P-value
	GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	Total	
BW5	722.50^{a}±6.14	686.42^{b}±7.00	663.06^{c}±11.17	485.63^{d}±4.47	588.92±10.10	0.0410*
BW6	936.65^{a}±8.81	864.42^{b}±8.98	827.44^{c}±14.38	602.43^{d}±7.02	740.53±13.75	0.0345*
BW7	1174.0^{a}±9.77	1117.8^{b}±13.68	1098.9^{b}±15.83	783.28^{c}±10.29	957.38±17.29	0.018*
BW8	1428.2^{a}±10.30	1336.2^{b}±19.01	1307.9^{b}±12.84	1080.4^{c}±8.53	1219.8±14.54	0.032*
BW9	1622.0^{a}±11.46	1581.9^{a}±10.70	1467.9^{b}±15.48	1212.5^{c}±14.05	138.87±18.37	0.022*
BW10	1825.6^{a}±11.64	1792.3^{a}±13.73	1672.2^{b}±14.80	1418.6^{c}±16.51	1595.0±18.96	0.040*
BW11	2049.8^{a}±12.68	1962.7^{b}±10.16	1861.2^{c}±17.29	1614.6^{d}±15.13	1789.6±18.70	0.014*
BW12	2185.6^{a}±12.39	2160.0^{a}±16.56	1993.9^{b}±24.99	1820.7^{c}±19.76	1972.5±18.49	0.029*
BW13	2324.3^{a}±15.38	2308.0^{a}±17.28	2113.8^{b}±24.73	1973.6^{c}±19.35	2117.6±18.13	0.036*
BW14	2547.6^{a}±25.21	2388.2^{b}±10.03	2283.8^{c}±6.18	2048.9^{d}±17.32	2231.2±20.50	0.016*

BW5 - BW14: Weekly body weight measurements, GV1-Lep: Group SNP 1 for leptin in V line breed, GV2-Lep: Group SNP 2 for leptin in V line breed, GV3-Lep: Group SNP 3 for leptin in V line breed, GB1-Lep: Group SNP 1 for leptin in Baladi Black breed. According to LSD's test, means of different levels having different superscripts are significantly different at $P<0.05$.

Table 4. Classification of leptin gene SNPs dependent allocated groups using body weight from week 4 to week 15 as predictors for discriminant function.

SNPs			Predicted Group Membership				Total
			GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	
Original	Count	GV1-Lep	18	2	0	0	20
		GV2-Lep	2	20	2	0	24
		GV3-Lep	0	3	13	0	16
		GB1-Lep	0	0	1	59	60
	%	GV1-Lep	90	10	0	0	100
		GV2-Lep	8.3	83.33	8.3	0	100
		GV3-Lep	0	18.8	81.25	0	100
		GB1-Lep	0	0	1.7	98.33	100

91.7% of original grouped cases were correctly classified. GV1-Lep: Group SNP 1 for *leptin* in V line breed, GV2-Lep: Group SNP 2 for *leptin* in V line breed, GV3-Lep: Group SNP 3 for *leptin* in V line breed, GB1-Lep: Group SNP 1 for *leptin* in Baladi Black breed.

Table 5. Standardized coefficient of classification process of leptin gene SNPs dependent allocated groups using body weight from week 5 to week 14 as predictors for classification.

Trait	Function		
	1	2	3
BW5	-0.375-	-0.075-	-0.112-
BW6	-0.206-	-0.423-	-0.057-
BW7	-0.751-	-0.156-	0.031
BW8	1.701	2.727	-0.254-
BW9	-2.507-	-2.007-	-0.039-
BW10	-0.514-	-1.635-	1.442
BW11	1.114	3.721	-1.964-
BW12	0.449	-1.970-	1.865
BW13	-0.317-	0.451	-0.499-
BW14	-1.685-	0.432	-1.048-

BW5 - BW14: Weekly body weight measurements.

Table 6. Mean (±SE) of carcass traits of different leptin gene SNPs dependent allocated groups

Trait	Mean ± SE					
	GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	Total	P-value
Hot carcass wt	1528.4^{a}±21.69	1360.4^{b}±25.57	1344.8^{b}±31.83	1253.4^{c}±15.35	1332.8±13.9	0.015*
Dressing %	59.62^{a}±0.60	57.38^{b}±0.71	59.02^{a}±0.99	59.26^{a}±0.31	58.91±0.27	0.042*
Head wt	126.45^{a}±1.84	123.92^{c}±1.63	124.75^{b}±2.41	109.48^{d}±1.61	117.23±1.20	0.013*
Head %	8.30^{c}±0.15	9.17^{ab}±0.19	9.33^{a}±0.22	8.76^{b}±0.11	8.84±0.08	0.028*
Liver wt	78.96^{a}±2.04	67.28^{b}±1.36	62.76^{b}±0.76	79.93^{a}±1.93	74.95±1.23	0.041*
Liver %	5.19^{b}±0.19	4.96^{bc}±0.08	4.69^{c}±0.08	6.38^{a}±0.13	5.68±0.10	0.023*
Heart wt	6.44^{a}±0.16	6.46^{a}±0.21	5.84^{b}±0.21	5.38^{c}±0.10	5.83±0.09	0.024*
Heart %	0.42^{b}±0.01	0.48^{a}±0.02	0.43^{b}±0.01	0.43^{b}±0.01	0.44±0.01	0.039*
Kidney wt	14.01^{a}±0.37	13.49^{a}±0.50	14.19^{a}±0.46	13.59^{a}±0.24	13.72±0.18	0.066
Kidney %	0.92^{c}±0.03	0.99^{bc}±0.03	1.06^{ab}±0.03	1.09^{a}±0.02	1.04±0.02	0.044*
Abdom fat wt	19.65^{a}±3.18	13.53^{b}±1.34	19.15^{a}±2.29	22.63^{a}±1.39	19.85±1.00	0.033*
Abdom fat %	1.26^{bc}±0.20	1.01^{c} ±0.11	1.47^{ab}±0.20	1.83^{a}±0.11	1.52±0.08	0.030*
Hind part wt	501.15^{a}±6.37	445.21^{b}±9.55	449.94^{b}±14.12	392.77^{c}±6.03	428.94±5.54	0.041*
Hind part %	32.82^{a}±0.28	32.71^{a}±0.30	33.50^{a}±0.90	31.292^{b}±0.16	32.12±0.183	0.029*
Fore part wt	412.00^{a}±7.28	381.75^{b}±8.42	380.69^{b}±7.68	322.65^{c}±5.24	357.10±4.77	0.034*
Fore part %	26.94^{b}±0.20	28.06^{a}±0.27	28.41^{a}±0.50	25.71^{c}±0.20	26.75±0.17	0.019*
Mid part wt	358.05^{a}±8.19	305.96^{b}±7.88	319.81^{b}±7.92	308.77^{b}±4.00	317.89±3.47	0.044*
Mid part %	23.39^{bc}±0.28	22.47^{c}±0.33	23.83^{ab}±0.48	24.72^{a}±0.28	23.93±0.19	0.0145*

N: Number (20, 24, 16, 60, 120 for GV1-Lep, GV2-Lep, GV3-Lep, GB1-Lep, Total, respectively), GV1-Lep: Group SNP 1 for *leptin* in V line breed, GV2-Lep: Group SNP 2 for *leptin* in V line breed, GV3-Lep: Group SNP 3 for *leptin* in V line breed, GB1-Lep: Group SNP 1 for *leptin* in Baladi Black breed. According to LSD's test, means of different levels having different superscripts are significantly different at P<0.05.

Table 7. Classification of leptin gene SNPS dependent allocated groups using carcass traits as predictors for discriminant function.

SNPs			Predicted Group Membership				Total
			GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	
Original	Count	GV1-Lep	18	2	0	0	20
		GV2-Lep	0	18	6	0	24
		GV3-Lep	2	1	13	0	16
		GB1-Lep	0	0	0	60	60
	%	GV1-Lep	90	10	0	0	100
		GV2-Lep	0	75	25.0	0	100
		GV3-Lep	12.5	6.2	81.25	0	100
		GB1-Lep	0	0	0	100	100

91.6% of original grouped cases were correctly classified. GV1-Lep: Group SNP 1 for *leptin* in V line breed, GV2-Lep: Group SNP 2 for *leptin* in V line breed, GV3-Lep: Group SNP 3 for *leptin* in V line breed, GB1-Lep: Group SNP 1 for *leptin* in Baladi Black breed.

Table 8. Standardized coefficient of slaughter trait except for hind and fore part % for classification and discrimination of leptin SNPs.

Carcass Traits	Function		
	1	2	3
Hot (Carcass) wt	3.014	13.488	3.073
Dressing %	-0.210-	0.846	-1.264-
Head wt	-3.279-	-3.506-	-1.068-
Head %	3.035	3.269	0.809
Liver wt	6.783	-2.435-	2.239
Liver %	-5.740-	2.797	-2.133-
Heart wt	-1.205-	-2.632-	-5.398-
Heart %	1.097	2.453	5.733
Kidney wt	0.454	1.978	-2.804-
Kidney %	-0.445-	-3.100-	3.366
Abdom fat wt	0.207	-0.100-	-1.805-
Abdom fat %	-0.279-	0.260	1.564
Hind part wt	1.341	1.721	-2.011-
Fore part wt	-0.230-	-2.250-	0.629
Mid part wt	-2.988-	-10.294-	4.908
Mid part %	1.596	7.527	-4.235-

GV1, GV2, and GV3 had higher hot carcass weights, head weights, heart weights, fore part weights, mid part weights, hind part weights, fore part weight percentages, and hind part weight percentages than GB1. The kidney weight did not differ across the different SNP groups (Table 6).

Slaughter traits other than the hind and fore part percentages were used as predictors in LDA for the classification of the identified SNPs. Overall, they correctly classified 91.6% of all cases, and 90% (18/20) for GV1, 75% (18/24) for GV2, 81.25% (13/16) for GV3, and 100% (60/60) for GB1 (Table 7). Furthermore, liver weight, carcass weight, and heart percentages were the best predictors for functions 1, 2, and 3, respectively, with high estimates of 6.783, 13.488, and 5.733 (Table 8).

There were no differences between SNP groups for FI and DFI over the periods of 5–8 and 8–11 weeks of age, but there were significant differences at 11–14 and 5–14 weeks of age; GB1 had higher estimates than those of the V line SNP groups. There were significant differences in FCR across the different SNP groups of the two breeds as well as variation within the V line breed, leading to differences across GV1, GV2, and GV3 (Table 9).

Table 9. Mean (±SE) of FI, DFI, and FCR of different leptin gene SNPs dependent allocated groups from week 5 to week 14.

Trait	Mean ± SE					
	GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	Total	P-value
FI 5-8 wk	1.61^{a}±0.03	1.53^{a}±0.03	1.48^{a}±0.07	1.52^{a}±0.02	1.53±0.016	0.083
FI 8-11 wk	2.17^{a}±0.07	2.06^{a}±0.06	2.1^{a}±0.10	2.20^{a}±0.03	2.16±0.03	0.548
FI 11-14 wk	2.40^{b}±0.07	2.33^{bc}±0.07	2.16^{c}±0.12	2.66^{a}±0.04	2.48±0.035	0.021^{*}
FI 5-14 wk	6.19^{ab}±0.15	5.92^{b}±0.14	5.55^{c} ±0.26	6.38^{a}±0.09	6.15±0.07	0.020^{*}
DFI 5-8 wk	76.70^{a}±1.20	72.92^{a}±1.66	70.67^{a}±3.47	72.18^{a}±0.84	72.88±0.74	0.074
DFI 8-11 wk	103.48^{a}±3.33	98.07^{a}±2.76	100.30^{a}±4.73	105.00^{a}±1.65	102.73±1.31	0.069
DFI 11-14	114.33^{b}±3.35	110.92^{bc}±3.11	103.07^{c}±5.71	126.65^{a}±1.94	118.31±1.68	0.046^{*}
DFI 5-14 wk	98.24^{ab}±2.45	93.99^{bc}±2.27	88.05^{c}±4.20	101.28^{a}±1.37	97.55±1.14	0.0245^{*}
FCR 5-8 wk	2.58^{a}±0.06	2.44^{b}±0.03	2.18^{c}±0.08	2.68^{d}±0.03	2.55±0.03	0.008^{**}
FCR 8-11 wk	4.33^{b}±0.14	3.83^{c}±0.05	3.86^{c}±0.01	4.56^{a}±0.06	4.28±0.05	0.030^{*}
FCR 11-14	6.93^{a}±0.37	6.18^{b}±0.07	5.29^{c}±0.15	6.45^{b}±0.03	6.32±0.08	0.001^{**}
FCR 5-14 wk	4.13^{b}±0.04	3.83^{c}±0.01	3.38^{d}±0.09	4.35^{a}±0.04	4.08±0.04	0.010^{*}

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 week, GV1-Lep: Group SNP 1 for *leptin* in V line breed, GV2-Lep: Group SNP 2 for *leptin* in V line breed, GV3-Lep: Group SNP 3 for *leptin* in V line breed, GB1-Lep: Group SNP 1 for *leptin* in Baladi Black breed. According to LSD's test, means of different levels having different superscripts are significantly different at P<0.05.

There were no differences between SNP groups for FI and DFI over the periods of 5–8 and 8–11 weeks of age, but there were significant differences at 11–14 and 5–14 weeks of age; GB1 had higher estimates than those of the V line SNP groups. There were significant differences in FCR across the different SNP groups of the two breeds as well as variation within the V line breed, leading to differences across GV1, GV2, and GV3 (Table 9).

FI, DFI, and FCR were then entered into the DFA model. FI and FCR were selected to be included as predictors in the LDA to classify the SNP groups; they correctly classified 76.7% of all cases, and 70% (14/20) for GV1, 100% (24/24) for GV2, 87.5% (14/16) for GV3, and 66.67% (40/60) for GB1 (Table 10). FI at 5–14 weeks of age was the best predictor for functions 1 and 3, and FI at 11–14 weeks of age was the best predictor for function 2, with estimates of −4.340, 4.959, and −1.697, respectively (Table 11). The identified SNPs had a significant impact on weight gain and daily weight gain, as there was a genetic difference both between and within the SNP groups in both the V line and Baladi Black rabbit breeds (Table 12).

Table 10. Classification of leptin gene SNPs dependent allocated groups using FI, DFI, and FCR as predictors for discriminant function.

SNPs			Predicted Group Membership				Total
			GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	
Original	Count	GV1-Lep	14	5	0	1	20
		GV2-Lep	0	24	0	0	24
		GV3-Lep	0	2	14	0	16
		GB1-Lep	12	8	0	40	60
	%	GV1-Lep	70	25	0	5	100
		GV2-Lep	0	100	0	0	100
		GV3-Lep	0	12.5	87.5	0	100
		GB1-Lep	20	13.3	0	66.67	100

76.7% of original grouped cases were correctly classified. GV1-Lep: Group SNP 1 for *leptin* in V line breed, GV2-Lep: Group SNP 2 for *leptin* in V line breed, GV3-Lep: Group SNP 3 for *leptin* in V line breed, GB1-Lep: Group SNP 1 for *leptin* in Baladi Black breed.

Table 11. Standardized coefficient of classification process of leptin gene SNPs dependent allocated groups using FI and feed conversion ratio as predictors for classification.

Trait	Function		
	1	2	3
FI 5-8 wk	1.512	0.099	-2.134-
FI 8-11 wk	0.893	0.352	-1.407-
FI 11-14 wk	1.999	-1.697-	-1.377-
FI 5-14 wk	-4.340-	1.351	4.959
FCR 5-8 wk	-1.277-	0.359	1.884
FCR 8-11 wk	-1.182-	-0.274-	2.587
FCR 11-14 wk	-0.617-	0.820	1.286
FCR 5-14 wk	3.154	-0.125-	-3.628-

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.

Table 12. Mean (±SE) of weight gain of different leptin gene SNPs dependent allocated groups from week 5 to week 14.

Trait	Mean ± SE					
	GV1-Lep	GV2-Lep	GV3-Lep	GB1-Lep	Total	P-value
WG 5 - 8	705.65^{a}±7.77	649.75^{b}±16.71	644.81±8.34	594.77^{c}±6.40	630.92±6.15	0.0345*
WG 8 - 11	621.60^{a}±6.73	626.50^{a}±13.28	553.38^{b}±15.8	534.15^{b}±7.57	569.76±6.42	0.033*
WG 11 - 14	497.90^{a}±33.8	425.58^{b}±7.83	422.56^{b}±15.2	434.35^{b}±5.64	441.62±7.08	0.038*
WG 5 - 14	1825.2^{a}±28.3	1701.8^{b}±8.57	1620.8^{c}±13.4	1563.3^{d}±15.46	1642.3±12.8	0.001**
DWG 5 - 8	33.60^{a}±0.37	30.94^{b}±0.80	30.71^{b}±0.40	28.32^{c}±0.30	30.04±0.29	0.020*
DWG 8 - 11	29.60^{a}±0.32	29.83^{a}±0.63	26.35^{b}±0.76	25.44^{b}±0.36	27.13±0.31	0.034*
DWG 11 - 14	23.71^{a}±1.61	20.27^{b}±0.37	20.12^{b}±0.73	20.68^{b}±0.27	21.03±0.34	0.029*
DWG 5 - 14	28.97^{a}±0.45	27.01^{b}±0.14	25.73^{c}±0.21	24.81^{d}±0.25	26.07±0.20	0.006**

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 week, GV1-Lep: Group SNP 1 for *leptin* in V line breed, GV2-Lep: Group SNP 2 for *leptin* in V line breed, GV3-Lep: Group SNP 3 for *leptin* in V line breed, GB1-Lep: Group SNP 1 for *leptin* in Baladi Black breed. According to LSD's test, means of different levels having different superscripts are significantly different at P<0.05.

DISCUSSION

The candidate gene associated with meat quality and quantity encodes leptin, a 16 kDa polypeptide hormone that is mainly synthesized and secreted in fat cells. Infertility and obesity have been linked to leptin based on animal models and human research (MIGDAL *et al*., 2018).

In this context, DNA sequencing of *leptin* (202-bp) revealed four non-synonymous SNPs in a number of V line rabbits. The identified SNPs were associated with growth and carcass traits, both within and between the two rabbit breeds. DNA sequencing revealed four novel SNPs (submitted to GenBank with accession numbers gb|MT832142|, and gb|MT832143|), compared with the matched reference sequence JX868865.1 (Figure 1).

To the best of our knowledge, there is little information on *leptin* polymorphisms and their association with rabbit feed intake and feed conversion ratio. In a study conducted by MIGDAL *et al*. (2018), the authors examined the associations between SNPs in the rabbit *leptin* gene and growth traits, slaughter traits, and physicochemical parameters. Animals were genotyped for polymorphisms within exon 2 (g.16081633T>C), intron 1_2 (g.16081420C>T), and within the UTR (g.16079636C>G) for the association analysis. Identified polymorphisms within the rabbit *leptin* gene showed significant differences in dissectible fat percentage in both the carcass and intermediate part (g.16081633T>C). Moreover, meat traits like protein content (g.16081633T>C; g.16079636C>G), intramuscular fat content (g.16081633T>C; g.16079636C>G, g.16081420C>T), dry matter (g.16081420C>T), ash (g.16081420C>T), water (g.16081420C>T), and cohesiveness (g.16081420C>T, g.16079636C>G) were affected by polymorphisms in the leptin gene. In another study, LUO *et al*. (2019) reported that genetic polymorphisms and the gene expression profile of *leptin* in rabbit tissues were correlated with meat quality.

Biochemical and hematological examinations provide valuable information on objective assessment of health status, in order to detect health disorders or for monitoring stress factors already at preclinical stage (HINTON *et al*., 1982). Changes in physiological biochemical and hematological values can also be used as indicators of welfare in rabbit breeding (HOY and VERGA, 2006). Differences between rabbit genotypes in biochemical and hematological values

were not studied in details with the exception of status in Watanabe heritable hyperlipaemic rabbits **(**KONDO and WATANABE, 1975), and similar model rabbits (KUROSAWA *et al*., 1995). The effect of rabbit genotype on blood picture and serum biochemical indicators were demonstrated in crossbreeds of Californian, Checkered giant and New Zealand White (NZW) rabbits (BURNETT *et al*., 2006).

In this context, results of hormonal assays demonstrated a difference in TSH across the V line and Baladi Black. Conversely, there was no noticeable difference between the levels of GH and leptin across the studied groups. The results of the studied metabolic biochemical parameters could be affected by rabbit genotype (MARTINEC *et al*., 2012). Thyroid Hormones (TH) are essential for the control of many fundamental physiological processes, such as development, differentiation, growth, metabolism and thermoregulation (MCNABB, 1992).

The results of this study suggest that the major effect of TSH may vary from that provided by BENSO *et al*. (2007), who demonstrated an increase in free thyroxine and a decrease in free triiodothyronine, but no changes to TSH. EL-WERDANY *et al*. (2016) found that Baladi Black rabbits had negligibly higher levels of leptin hormone than New Zealand White rabbits. Broiler breeds may have a significant effect on serum T3 and T4 levels, and these findings may be consistent with growth and body composition data (GHANEM *et al*. 2016).

The current results demonstrate that there is genetic variation between the V line and Black Baladi breeds, as well as within the V line among SNP groups. Based on these findings, rabbits with weight problems can be knocked out early, i.e. up to the ninth week, as in the first formula and/or eleventh week after birth, as in the second and third formula instead of waiting until the end of the fattening period. In the same line, DIMITROVA *et al*. (2015) conducted a comparative study among various rabbit breeds and identified a highly significant difference in live weight trait at two, three, and four months of age between the V line, New Zealand White, and Californian breeds, with the V line outperforming both the New Zealand White and Californian breeds.

Similarly, SAKR *et al*. (2020) revealed a substantial difference ($P>0.0001$) in mean body weight at weaning (5 weeks) between V line and Baladi Black and their crossbreds, with V line (774.62±5.03) outperforming Baladi Black (565.00± 6.52) and their crossbred B♂ x V♀ (696.92±6.28) and V♂ x B♀ (645.96± 4.90).

Through the results of the current study, it is possible to select the carcass characteristics in rabbits by judging liver weight, carcass weight, and heart percentages, as in the first, second and third functions, respectively. MIGDAL *et al*. (2018) proposed that essential carcass and meat traits of New Zealand White × Belgian Giant Gray crossbreeds are compromised by polymorphisms in the rabbit *leptin* gene. SNPs that affect essential carcass and meat characteristics may be beneficial in rabbit breeding programs.

CONCLUSION

The current findings revealed an important connection between *leptin* gene polymorphisms and rabbit growth, carcass measurements, and feed consumption. SNP findings indicated the potency of the candidate genes with respect to their effect on genetic features. These findings were also useful for promoting a high body weight at market age. Consequently, *leptin* SNPs

allowed for the estimation of individuals' genetic potential, combined with the promise to meet the demand of the market for high-quality rabbit meat.

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Ethical Statement

The protocol of the study and animals were managed in compliance with the 'Guide for the Care and Use of Laboratory Animals' approved by the Ethics Committee of the Faculty of Veterinary Medicine, Mansoura University, Egypt.

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EFIKASNOST POLIMORFIZMA *LEPTIN* GENA U EVAULACIJI OSOBINA RASTA I TRUPA V-LINIJA I BALADI CRNIH ZEČEVA

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Izvod

Ova studija je istraživala povezanost između polimorfizma i promena metaboličkih markera u leptin genu, i telesne težine, prirasta, osobina trupa, unosa hrane i odnosa konverzije hrane. Uzorci krvi su sakupljeni od 60 V-line i 60 Baladi crnih zečeva za ekstrakciju DNK i biohemijsku analizu. DNK sekvencioniranje leptina (202-bp) otkrilo je četiri SNP-a koji su karakterisali veliki broj zečeva V-linije. Statistička analiza je otkrila da su identifikovani SNP povezani sa rastom kunića i merenjem trupa ($p < 0,05$). Model diskriminantne analize pokazao je visok procenat klasifikacije za identifikovane SNP unutar i između rasa koristeći telesnu težinu u dobi od 5-14 nedelja (91,7%), osobine klanja (91,6%), unos hrane, dnevni unos hrane i odnos konverzije hrane (76,7%). Ova studija otkriva da bi leptin gen mogao biti kandidat za osobine rasta kod zečeva koji omogućavaju razvoj selekcije uz pomoć markera (MAS) kod različitih rasa zečeva.

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