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THE PRKAG3 GENE POLYMORPHISMS AND THEIR ASSOCITATIONS WITH GROWTH PERFORMANCE AND BODY INDICES IN BARKI LAMBS

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ABSTRACT

In this study the polymorphisms for a variable fragment located between exon 4 and exon 6 of the ovine protein kinase adenosine mono-phosphate activated gamma 3 (PRKAG3) gene were detected in 59 males and 62 females of Barki lambs using the polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis, followed by cloning and sequencing the detected SSCP banding patterns.

The associations of PRKAG3 gene polymorphisms with growth traits (birth weight, weaning weight, pre-weaning daily gain, marketing weight and post-weaning daily gain) and body indices (body mass index, skeletal muscle index, body index and relative body index) were tested using general linear models of **SAS (2000)**. The models included animal genotype, gender of lamb and parity of ewe as fixed effects. Age at weaning was included as a covariate in the models assessing the effect of PRKAG3 genotype on pre-weaning daily gain and weaning weight as well as age at marketing was included in the models assessing the effect of PRKAG3 genotype on post-weaning daily gain, marketing weight and body indices.

Three genotypes (AA, AB and BB) were identified with frequencies of 0.26, 0.50 and 0.24, respectively. These genotypes were derived from two alleles (A and B) with frequencies of 0.51 and 0.49, respectively. The variation in PRKAG3 gene showed significant associations with marketing weight ($P < 0.05$), post-weaning daily gain ($P < 0.01$), skeletal muscle index ($P < 0.05$) and body mass index ($P < 0.01$). The presence of B allele was associated with heavier marketing weight ($P < 0.05$), faster post-weaning daily gain ($P < 0.01$) and higher body mass index ($P < 0.01$) and skeletal muscle index ($P < 0.05$).

The presented results give valuable information to select for B allele and against A allele of PRKAG3 gene to improve marketing weight and muscularity of Barki lambs.

Keywords: *Barki sheep, PCR-SSCP, PRKAG3 gene polymorphisms, growth traits, body indices.*

INTRODUCTION

Sheep growth traits represent economic importance for both breeders and industry due to their association with meat production. Fast growing lambs need less feed for maintenance requirements because they reach their market weights faster than the slow growing lambs.

Body measurements and conformation indices could be equally important because they are related to feed intake, body weight and fat and muscle percentages **(Cam *et al.*, 2010; Musa *et al.*, 2012; Tariq *et al.*, 2012; Younas *et al.*, 2013)**. Body indices are relevant to establish a morphological based standard with visual conformation appraisal, which is most likely the oldest method of information collection for the purpose of selection in many sheep breeding associations **(Janssen and Vandepitte, 2004)**.

Recently, there is a great interest to identify molecular markers controlling economically important traits of farm animals. Molecular markers are not affected by environmental factors and provide more accurate and reliable criteria to assess the true genetic merit of animals **(Beuzen**

***et al.*, 2000**). There are two common approaches used to identify the molecular markers: the genome scan approach and the candidate gene approach. In the genome scan approach, the whole genome is searched to identify where such gene(s) affecting the desired trait may lie. In the candidate gene approach, the purpose is to identify gene(s) that are thought to be responsible for the phenotypic variance of the desired trait **(Rothschild and Sölkner, 1997)**.

The 5' adenosine monophosphate-activated protein kinase (AMPK) is an important energy-sensing enzyme that plays a crucial role in regulating the intracellular energy metabolism through being involved in the metabolism of carbohydrate and fatty acids in adipose tissues, liver, pancreatic beta cells and skeletal muscles and also protecting cells from depletion of adenosine tri-phosphate (ATP) in response to cellular metabolic stresses by enabling the metabolism of energy reverse **(Yang *et al.*, 2015)**. It consists of three proteins that together make the function of enzyme; catalytic alpha subunit (composed of α1 and α2 subunits), non-catalytic beta subunit (composed of β1 and β2 subunits) and non-catalytic gamma subunit (composed of γ1, γ2 and γ3 subunits).

The AMPK γ3 subunit, also called protein kinase AMP-activated gamma 3 (PRKAG3), is encoded by PRKAG3 gene and is predominantly expressed in skeletal muscle mostly in type II glycolytic fiber types **(Cheung *et al.*, 2000)**. It has been reported that the PRKAG3 plays a crucial role in the activity of AMPK through binding to the adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP; **Cheung *et al.*, 2000)**.

There are few studies identified the variation in PRKAG3 gene and evaluated only its effect on carcass traits of farm animals.

In sheep, **Yang *et al.* (2015)** investigated the variation in two regions (exon 3 and exons 4-6) of PRKAG3 gene in the New Zealand Suffolk sheep using the polymerase chain reaction- single strand conformational polymorphism (PCR-SSCP), and identified two alleles in exon 3 and three alleles in exons 4-6. They detected three nucleotide substitutions that were located in exon 4 (g. 2399 C > T), intron 4 (g. 2656 G > A) and intron 5 (g.2690 C > T). The nucleotide substitution, that was detected in the exon 4 (g.2656 C > T), caused an amino acid substitution of tryptophan to arginine at position 230 (R230W) of the ovine PRKAG3 amino acid sequence.

In cattle, **Roux *et al.* (2006)** reported that, the bovine PRKAG3 has a total length of 8048 bp and contains thirty-two SNPs. Among which, thirteen are in the exons, one is in the 3' UTR and eighteen are in the introns. Five of them change an amino acid in the PRKAG3 protein sequence. The variation in bovine PRKAG3 has been found to affect carcass traits in beef cattle **(Li *et al.*, 2012)**.

In goats, the allelic polymorphisms in 5` regulatory region and exon 13 of PRKAG3 gene in Anhui white, Matou, Boer, Xiang dong and Sanen breeds were detected by **Jin *et al.* (2012)**. They found two SNPs in the 5' regulatory region (C-525A and C-225T), located at 525 and 225 bp upstream of the start codon and two SNPs in the exon 13 (T90C and C102T), located at 90 bp and 102 bp of the exon 13. The mutations at T90C and C102T didn't cause the substitution of corresponding amino acids in the AMPK protein. The results revealed that the lipoidosis ability of goat breeds may be associated with C-525A and C-225T loci of PRKAG3 gene.

In pigs, several mutations have been identified in the porcine PRKAG3 gene **(Ryan *et al.*, 2012)**. Of these mutations, the c.595A > G and c.599G > A single nucleotide polymorphisms (SNPs) caused the p.I199V and p.R200Q amino acid substitutions, respectively. These two SNPs were the most studied in different pig breeds and populations. The c.595A > G SNP has been associated with variation of carcass traits and composition. The pigs that carry the c.599A (p.200Q) allele have higher skeletal muscle compared to animals that do not carry this allele **(Gou *et al.*, 2012; Santé-Lhoutellier *et al.*, 2012; Škrlep *et al.*, 2012)**.

To date, there is no study investigated the allelic and genotypic polymorphisms of PRKAG3 gene and their associations with growth performance and body indices in Barki sheep. Therefore, the aims of the present study are to detect the PRKAG3 gene polymorphisms at a variable region located between exon 4 and exon 6 by PCR-SSCP and DNA sequencing methods and to test the association of these polymorphisms with growth traits and body indices of Barki lambs.

MATERIALS AND METHODS

Animals and phenotypic data

In total, 59 males and 62 females of Barki lambs, reared at Maryout Research Station, Desert Research Center, were investigated. At birth, lambs were ear-tagged, weighed and allowed to suckle their ewes until weaning at about three months of age. After weaning, animals were fed 0.5 to 1.0 kg/head/day concentrate mixture according to their physiological status, in addition to Berseem (*Trifolium alexandrinum*) hay *ad-libitum*. The concentrate mixture consisted of 50% cotton seed cake, 18% wheat bran, 15% yellow maize, 11% rice polish, 3% molasses, 2% limestone and 1% salt. The live weights at weaning (3 months) and marketing (9 months) were recorded. From the recorded weights, pre- and post-weaning daily gains were calculated.

At marketing age, five body measurements were taken for each animal: body length, heart girth, height at withers, height at hips and thigh circumference. Body length was considered as the distance between the point of shoulder and pinbone; heart girth was measured as the circumference of the chest of animal; height at wither was measured as the distance from the floor to the point between the shoulders; height at hips was measured as the distance from the floor to the back of animal; thigh circumference was measured as the circumference of the hind leg as close as the abdomen of animal. From these body measurements, 4 conformational indices were calculated according to **Salako (2006).**

- Body mass index = (marketing weight × 100) / height at withers.

- Skeletal muscle index = (thigh circumference × 100) / height at withers.

- Body index = (body length × 100) / heart girth.

- Relative body index = (body length × 100) / height at withers.

Polymerase chain reaction (PCR)

Blood samples were collected on FTA cards (Whatman Bio Science, Middlesex, UK) and genomic DNA was purified using a two-step washing procedure as described in **Zhou *et al.* (2006)**.

A variable fragment (485 pb) from exon 4 to exon 6 of the ovine PRKAG3 gene (GenBank accession No. FJ685774) was amplified using a pair of specific primers suggested by **Yang *et al.* (2015)**. The primer sequences were as follows: (F: 5`-TCTGCATCGCTATTACCG-3` and R: 5`-AGGAACGGGACGTGTCT-3`). The polymerase chain reaction (PCR) mixture contained the genomic DNA on one 1.2-mm punch of FTA card, 0.25 µM of each primer, 150 µM dNTPs (Eppendorf, Hamburg, Germany), 1x polymerase buffer (including 1.5 µM MgCl2), 0.5 U Taq DNA polymerase (Qiagen, Hilden, Germany) and some deionized water up to final volume of 20 µl. The thermal cycling was carried out using a Bio-Rad C 1000 touch thermal cycler (Bio-Rad, Hercules, CA, USA). The program conditions were 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 59°C for 30s and 72°C for 30s. The final step prolonged 10 min at 72°C.

Single strand conformational polymorphism analysis

A 2 µl aliquot of each PCR amplicon was mixed with 8 µl of loading dye (98% formamide, 0.025% bromophenol blue, 0.025% xylene cyanol, 10 mM EDTA (Eppendorf, Hamburg, Germany), denatured at 105 °C for 6 min, rapidly chilled on wet ice and loaded on 16 × 18 cm; 12% acrylamide: bisacrylamide (37.5: 1; Bio-Rad, USA) gels. The electrophoresis was run in 0.5 x TBE buffer for 18 h at 280V and 22 ºC,

using a Protean II xi cells electrophoresis apparatus (Bio-Rad, Hercules, CA, USA). Gels were silver stained using the method of **Byun *et al.* (2009)**.

Sequencing and analysis of allelic polymorphisms

Three PCR amplicons of each homozygous banding pattern (AA and BB) were directly sequenced. As well as, three PCR amplicons of the heterozygous banding pattern (AB) were sequenced using a rapid sequencing approach that has been described by **Gong *et al.* (2011)**. Briefly, a band corresponding to the allele was excised as a gel slice from the polyacrylamide gel, washed twice with 200 µl 1x TE buffer in a 1.5 ml tube, mashed up with a pipette tip in 50 µl 1x TE buffer, incubated for 1 h at 55 ºC., and then used as a template for re-amplification with the original primers. Those second amplicons were purified and then sequenced in both directions.

Sequences, alignments, translations and comparisons were carried out using DNASTAR (Madison, WI, USA) and DNAMAN (Version 5.2.10, Lynnon BioSoft, Vaudreuil, Canada). The BLAST algorithm was used to search the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) and Ensembl (http://www.ensembl.org) databases for homologous sequences of PRKAG3 in cattle.

Statistical analysis

Hardey-Weinberg equilibrium was tested by comparing the observed and expected genotypic frequencies using χ^2.

The effects of variation in PRKAG3 gene on growth traits and body indices were undertaken using the general linear model (GLM) of **SAS (2000).**

Two different sets of modeling approaches were used to test these effects. The first set of GLMs was used to assess the effect of PRKAG3 genotypes on growth traits and body indices and the second set of GLMs was used to explore the effect of the presence/absence of each PRKAG3 allele on growth traits and body indices.

Variation in PRKAG3 gene, gender of lamb and parity of ewe were fitted as fixed factors. Weaning age was included as a covariate in the models assessing the effect of variation in PRKAG3 gene on weaning weight; while, marketing age was included as a covariate in the models assessing the effect of variation in PRKAG3 gene on marketing weight and body indices.

If significant results were obtained, these were further explored using pairwise comparisons (Duncan test; $P \leq 0.05$).

The generalized statistical models were as follows:

$$Y1_{ijkl} = \mu + G_i + S_j + P_k + \varepsilon_{ijKl}$$

$$Y2_{ijklm} = \mu + G_i + S_j + P_k + bWA_l + \varepsilon_{ijKlm}$$

$$Y3_{ijklm} = \mu + G_i + S_j + P_k + bMA_l + \varepsilon_{ijKlm}$$

Where,

$Y1$ = the observed records on birth weight,

$Y2$ = the observed records on weaning weight and pre-weaning daily gain,

$Y3$ = the observed records on marketing weight, post-weaning daily gain and body indices,

μ = the overall mean,

G_i = the fixed effect of i^{th} PRKAG3 genotype (i = 1, 2, 3) in the first set of GLMs, or the fixed effect of the presence/ absence of each detected PRKAG3 allele in the second set of GLMs (i = 0, 1),

S_j = the fixed effect of j^{th} of gender of lamb, j = 1, 2,

P_k = the fixed effect of k^{th} parity of ewe, k = 1, …5,

bWA_l = the partial regression coefficient of weaning weight and pre-weaning daily gain on age at weaning as a covariate,

bMA_l = the partial regression coefficient of marketing weight, post-weaning daily gain and body indices on age at marketing as a covariate and

e_{ijklm} = Random error; assumed N.I.D. $(0, \sigma^2 e)$.

RESULTS AND DISCUSSION

Allelic and genotypic polymorphisms

Three different SSCP banding patterns were observed from amplicons of the amplified region of PRKAG3 gene (Fig. 1) and exhibited three genotypic polymorphisms (coded as: AA, AB and BB with frequencies of 0.26, 0.50 and 0.24, respectively) representing two allelic polymorphisms A and B with frequencies of 0.51 and 0.49, respectively). Chi-square (χ^2) test confirmed Hardy-Weinberg equilibrium for the detected alleles in the studied locus**,** which could be mainly the result of non gene flow within the population through migration or transfer of gametes.

At the same region in Suffolk sheep, **Yang *et al.* (2015)** detected three alleles: A, B and C with frequencies of 0.67, 0.27 and 0.06, respectively. This inconsistency might be due to the breed difference and/or the number of genotyped animals.

Sequence variation in the PRKAG3 gene

Cloning and sequencing of PCR amplicons representative of the detected SSCP banding patterns, confirmed two different DNA sequences (Fig. 2). The results revealed two substitutions in exon 4 (g. 2399 C > T) and intron 4 (g. 2600 A > G) of the PRKAG3 gene. Whereas the first nucleotide substitution does not result in any change for the amino acid chain, and the second nucleotide substitution is an intronic substitution, they may nevertheless be linked to other nucleotide changes in the coding regions or to sequence variation elsewhere in the gene **(Byun *et al.*, 2012)**. This may affect the expression and/or the function of PRKAG3 gene and hence affect sheep growth and body indices. These two sequences shared high homology (95%) to the reported cattle sequence (Gen Bank accession number AY692035.1). The obtained results are partially consistence with those results obtained by **Yang *et al.* (2015)**.

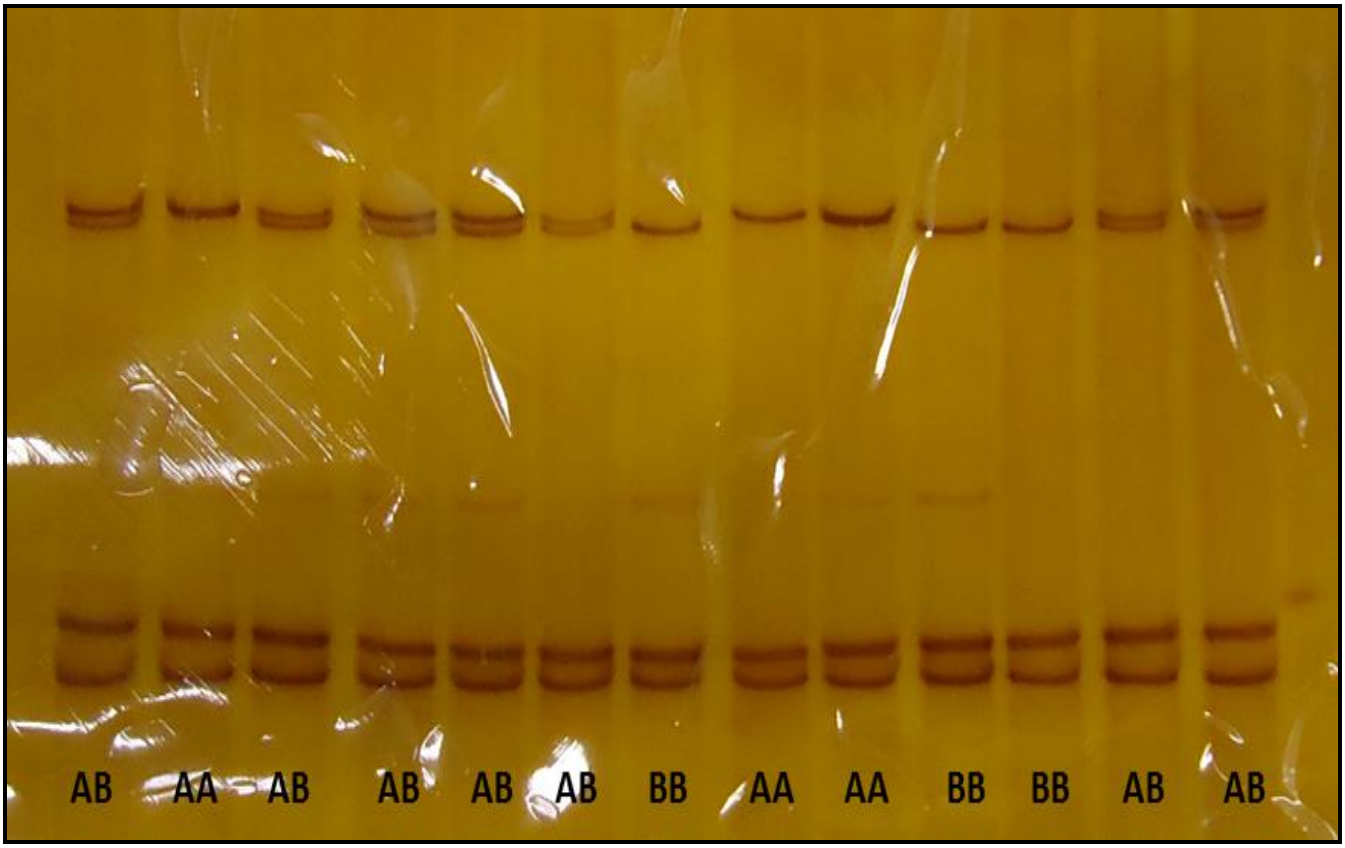

Figure (1). PCR-single strand conformational polymorphism of PRKAG3 gene in Barki sheep.

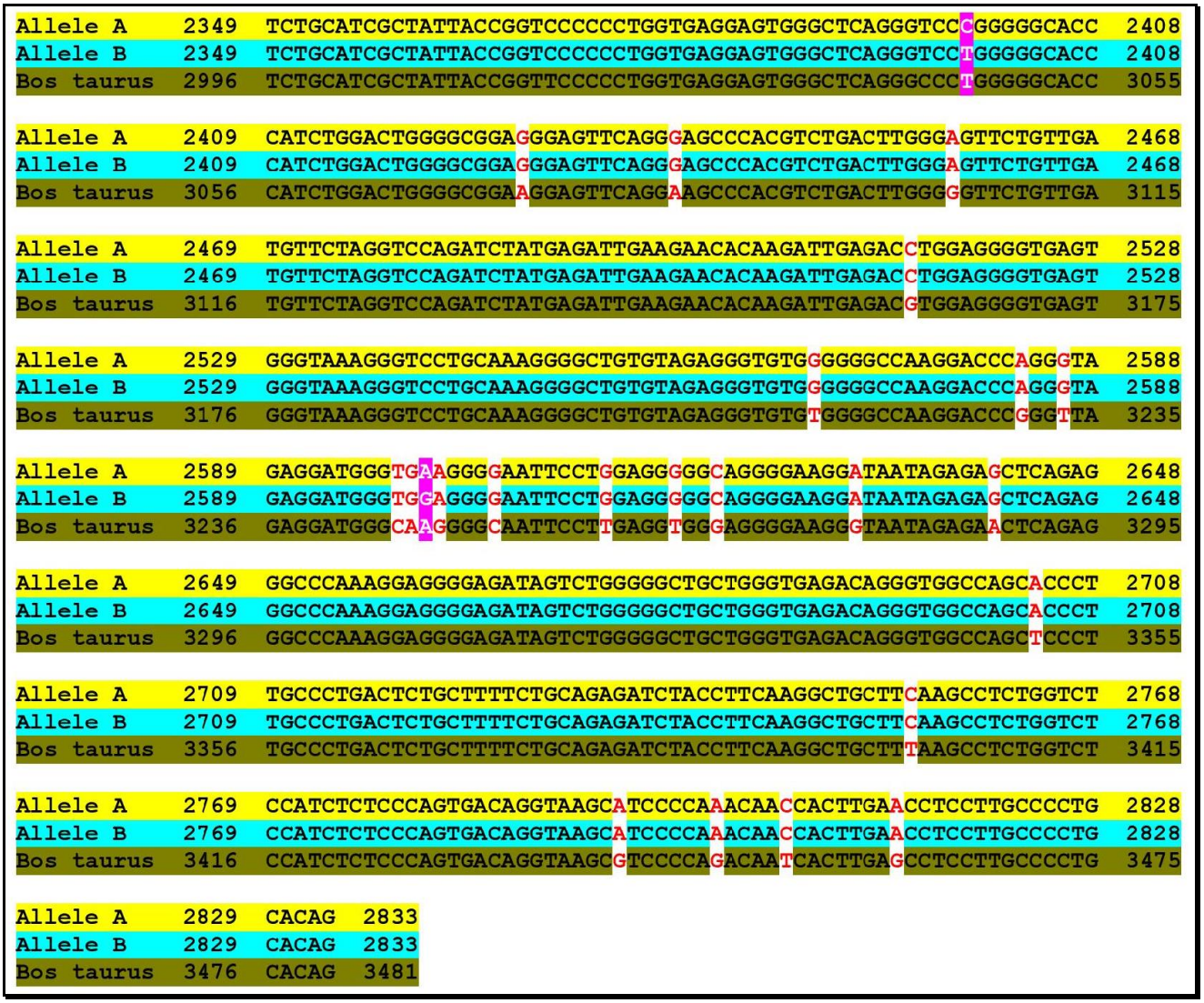

Figure (2). Sequences of the two detected alleles of the PRKAG3 gene in Barki sheep and their corresponding region in *Bos taurus* specie.

Effect of non-genetic factors on growth traits and body indices

The significant effects of non-genetic factors on growth traits and body indices are shown in Table (1). Gender of lamb had a significant effect ($P < 0.05$) on body weights at birth, weaning and marketing and a high significant effect ($P < 0.01$) on skeletal muscle index. Heavier body weights at birth, weaning and marketing as well as higher skeletal muscle index were found in males. This is mainly due to the physiological differences between the two genders. Similar findings were reported by **Mousa *et al*. (2006); Petrovic *et al*. (2011); Roshanfekr *et al*. (2011) and Abbasi *et al*. (2012).**

Parity of ewe had a high significant ($P < 0.01$) effect on lamb's birth weight. It could be attributed to the development of ewes' uterine system with age. This could be further explained as the results of systematic environmental changes in ewes with time **(Falconer, 1989)**. The significant influence of parity of ewe on birth weight observed in the present study is in agreement with many workers (**Thiruvenkadan *et al.*, 2011; Shokrollahi and Zandieh, 2012 and Simeonov *et al.*, 2015).**

Age at weaning showed high significant effect ($P < 0.01$) on weaning weight. As well as, age at marketing showed significant effect ($P < 0.05$) on marketing weight.

Table (1). Significance effect of non-genetic factors on growth traits and body indices of Barki lambs.

Trait	Non-genetic factor			
	Gender of lamb	Parity of ewe	Age at weaning	Age at marketing
BW (kg)	*	**	-	-
WW (kg)	*	ns	**	-
ADG1 (gm/d)	ns	ns	ns	-
MW (kg)	*	ns	-	*
ADG2 (gm/d)	ns	ns	-	ns
BMI	ns	ns	-	ns
SMI	**	ns	-	ns
BI	ns	ns	-	ns
RBI	ns	ns	-	ns

BW: birth weight; WW: weaning weight; ADG1: pre-weaning daily gain; MW: marketing weight; ADG2: post-weaning daily gain; BMI: body mass index; SMI: skeletal muscle index; BI: body index; RBI: relative body index; ns: refers to non significance (P > 0.05); *: refers to significance at (P < 0.05); **: refers to significance at (P < 0.01).

Effect of PRKAG3 genotypes on growth traits and body indices

Association of the detected PRKAG3 genotypes with growth traits and body indices was analyzed as shown in Table (2). Significant association (P < 0.05) was observed for PRKAG3 genotype with marketing weight and skeletal muscle index. In addition, high significant association (P < 0.01) was found for PRKAG3 genotype with post-weaning daily gain and body mass index. No associations were found between the rest of traits and PRKAG3 genotype.

The obtained results also showed that lambs with BB genotype had superior performance for marketing weight, post-weaning daily gain, body mass index and skeletal muscle index; however lambs with AA genotype had the lowest performance for the same traits.

Table (2). Least square means and their standard errors for growth traits and body indices in Barki lambs according to the PRKAG3 genotypes.

Trait	Genotype			Significance
	AA	AB	BB	
BW (kg)	3.49 ± 0.11	3.59 ± 0.07	3.43 ± 0.11	ns
WW (kg)	19.62 ± 0.64	19.82 ± 0.46	20.13 ± 0.68	ns
ADG1 (gm/d)	172.85 ± 6.07	175.85 ± 4.46	180.49 ± 6.85	ns
MW (kg)	40.52^{b} ± 1.45	43.16^{ab} ± 1.02	46.66^{a} ± 1.18	*
ADG2 (gm/d)	76.42^{b} ± 4.59	85.68^{b} ± 3.01	97.39^{a} ± 3.21	**
BMI	56.95^{b} ± 1.81	60.22^{b} ± 1.24	65.41^{a} ± 1.43	**
SMI	39.60^{b} ± 0.62	40.44^{b} ± 0.44	42.41^{a} ± 0.64	*
BI	80.18 ± 0.73	79.31 ± 0.71	78.77 ± 0.84	ns
RBI	101.27 ± 1.03	99.97 ± 0.78	102.38 ± 1.29	ns

BW: birth weight; WW: weaning weight; ADG1: pre-weaning daily gain; MW: marketing weight; ADG2: post-weaning daily gain; BMI: body mass index; SMI: skeletal muscle index; BI: body index; RBI: relative body index; ns: refers to non significance (P > 0.05); *: refers to significance at (P < 0.05); **: refers to significance at (P < 0.01); Means of the same trait with the same letter do not significantly (P < 0.05) differ from each other.

Effect of the presence/ absence of PRKAG3 alleles in animal genotype on growth traits and body indices

The results of testing the association between the presence/ absence of the detected PRKAG3 alleles in animal genotype and the studied traits are presented in Table (3). These results showed that, the presence of B allele in animal genotype was significantly associated with heavier marketing weight ($P < 0.05$), faster post-weaning daily gain ($P < 0.01$) and higher body mass index ($P < 0.05$) and skeletal muscle index ($P < 0.05$). In contrast, the presence of A allele in animal genotype was significantly associated with lighter marketing weight ($P < 0.05$), slower post-weaning daily gain ($P < 0.01$) and lower body mass index ($P < 0.01$) and skeletal muscle index ($P < 0.05$).

In this study, the association analysis showed significant effects for the PRKAG3 genotype on two of the studied growth traits (marketing weight and post-weaning daily gain) and two of the studied body indices (body mass index and skeletal muscle index). These traits are the most important traits in sheep industry, where the value of lamb bases on its weight and muscularity at harvest and the buyer and seller do not have to estimate any other traits.

Table (3). Association of the presence / absence of PRKAG3 alleles with growth traits and body indices in Barki lambs.

Trait	Allele being assessed	LSM ± SE				Significance
		N	Absent allele	N	Present allele	
BW (kg)	A	30	3.43 ± 0.10	91	3.55 ± 0.06	ns
	B	31	3.49 ± 0.10	90	3.53 ± 0.06	ns
WW (kg)	A	30	20.13 ± 0.68	91	19.75 ± 0.36	ns
	B	31	19.62 ± 0.63	90	19.92 ± 0.37	ns
ADG1 (gm/d)	A	30	180.49 ± 6.85	91	174.83 ± 3.57	ns
	B	31	172.85 ± 6.07	90	177.39 ± 3.73	ns
MW (kg)	A	30	46.66 ± 1.17	91	42.26 ± 0.83	*
	B	31	40.52 ± 1.44	90	44.32 ± 0.79	*
ADG2 (gm/d)	A	30	97.39 ± 3.20	91	82.53 ± 2.55	**
	B	31	76.42 ± 4.58	90	89.58 ± 2.33	**
BMI	A	30	65.41 ± 1.43	91	59.11 ± 1.03	**
	B	31	56.95 ± 1.81	90	61.95 ± 0.98	*
SMI	A	30	42.41 ± 0.63	91	40.15 ± 0.35	*
	B	31	39.60 ± 0.62	90	41.09 ± 0.37	*
BI	A	30	78.77 ± 0.84	91	79.60 ± 0.53	ns
	B	31	80.18 ± 0.73	90	79.13 ± 0.54	ns
RBI	A	30	102.38 ± 1.29	91	100.41 ± 0.62	ns
	B	31	101.27 ± 1.02	90	100.77 ± 0.68	ns

BW: birth weight; WW: weaning weight; ADG1: pre-weaning daily gain; MW: marketing weight; ADG2: post-weaning daily gain; BMI: body mass index; SMI: skeletal muscle index; BI: body index; RBI: relative body index; ns: refers to non significance ($P > 0.05$); *: refers to significance at ($P < 0.05$); **: refers to significance at ($P < 0.01$).

The role of PRKAG3 on growth traits and body indices associated with muscularity of meat animals might be due to its indirect effect on energy metabolism and skeletal muscle cells. A positive balance results in energy being stored as fat and/or muscle, causing weight gain. The consequences of negative energy balance on total body and skeletal muscle mass are well established. In general, total body mass decreases in response to sustained periods of negative energy balance, and the proportion of body mass loss is ~75% adipose tissue and 25% fat-free mass **(Weinheimer *et al.*, 2010)**.

As cited, the PRKAG3 (AMPK γ3) plays a crucial role in the activity of AMPK. Over the last decade, many studies have emerged the AMPK as a central integrator of signals controlling energy metabolism that correlates with the growth rate in a wide variety of organisms, from yeast to mammals. **Mounier *et al.* (2011)** extended this notion by showing that AMPK serves an essential first step in the regulation of energy metabolism within all cells in nature. In eukaryotic cells, AMPK activation has pleiotropic effects in many tissues, including adipose tissue, liver and skeletal muscle. AMPK acts as a "metabolic master switch" that serves an essential role in intracellular energy-sensing by detecting cellular energy status in order to maintain energy balance within every cell **(Hardie, 2004)**. AMPK is an intracellular energy sensor that, when activated, induces catabolic processes to rapidly produce more ATP **(Mhairi *et al.*, 2007).** AMPK stimulates fatty acid oxidation, improves insulin sensitivity and glucose metabolism **(Yamauchi *et al.*, 2002)**, and acts as a direct endogenous inhibitor of inflammation and angiogenesis **(Brakenhielm *et al.*, 2004; Yamaguchi *et al.*, 2005)**.

Studies regarding the effect of PRKAG3 genotypes on animal muscularity are missing from the literature, however, many recent evidences indicated that AMPK represents one of the major antagonistic forces governing muscle adaption to nutrition, starvation and growth stimulation. **Mounier *et al.*, 2009 and Lantier *et al.*, 2010** have cited that the AMPK has emerged as a key player in controlling muscle cell size. **Paturi *et al.* (2010)** have reported that the decreases in the ability of muscle to undergo hypertrophy were associated with increased AMPK phosphorylation. It has also been reported that chronic AMPK activation inhibits overload-induced muscle hypertrophy **(Gordon *et al.*, 2008)**. Moreover, knockdown of p70S6K in myotubes induces AMPK activation and a concurrent decrease in cell size, indicating that the activation of AMPK is accountable for muscle cell atrophy **(Aguilar *et al.*, 2007)**. Similarly, the muscle-specific knockout of IRS1/2 exhibits increased AMPK phosphorylation, associated with increased phosphorylation of ACC and raptor **(Long *et al.*, 2011)**. All together, these results indicate that, AMPK promotes cell growth and protein synthesis in muscle.

CONCLUSION

This is the first report suggesting a relationship between the variation in PRKAG3 gene and growth performance and body indices in sheep. The variation in PRKAG3 gene significantly associated with post-weaning daily gain, marketing weight, body mass index and skeletal muscle index. These traits are positively correlated with muscularity of meat animals. If the breeding program will be done for improving meat percentage in Barki lambs based upon the PRKAG3 polymorphisms, the BB genotype is recommended to be increased in frequency through the marker assisted selection. However, further studies are needed on a large population of Barki sheep and/or other local breeds of sheep to assure our findings.

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REFRENCES

Abbasi M. A., R. Abdollahi-Apanahi, A. Maghsoudi, R. VaezTorshizi and A. Nejati-Javaremi (2012). Evaluation of models for

estimation of genetic parameters and maternal effects for early growth traits of Iranian Baluchi sheep. Small Ruminant Research, 104: 62–69.

Aguilar V., S. Alliouachene, A. Sotiropoulos, A. Sobering, Y. Athea and F. Djouadi (2007). S6 kinase deletion suppresses muscle growth adaptations to nutrient availability by activating AMP kinase. Cell Metabolism, 5: 476–87.

Beuzen N. D., M. J. Stear and K. C. Chang (2000). Molecular markers and their use in animal breeding –review. The Veterinary Journal, 160: 42–52.

Brakenhielm E., N. Veitonmaki, R. Cao, S. Kihara, Y. Matsuzawa, B. Zhivotovsky, T. Funahashi and Y. Cao (2004). Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. The Proceeding of National Academy of Science, USA, 101: 2476–2481.

Byun S. O., Q. Fang, H. Zhou and J. G. H. Hickford (2009). An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Analytical Biochemistry, 385:174–175.

Byun S. O., R. H. Forrest, C. M. Frampton, H. Zhou and J. G. H. Hickford (2012). An association between lifespan and variation in insulin-like growth factor I receptor in sheep. Journal of Animal Science, 90: 2484–2487.

Cam M. A., M. Olfaz and E. Soydan (2010). Body measurements reflect body weights and carcass yields in Karayaka sheep. Asian Journal of Animal Veterinary Advances, 5: 120–127.

Cheung P. C., I. P. Salt, S. P. Davies, D. G. Hardie and D. Carling (2000). Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochemistry Journal, 346: 659–669.

Falconer D. S. (1989). Introduction to Quantitative Genetics. Third edition, Longman, London, UK, pp. 438.

Gong H., H. Zhou and J. G. H. Hickford (2011). Diversity of the glycine/tyrosine-rich keratin-associated protein 6 gene (KAP6) family in sheep. Molecular Biology Reports, 38: 31–35.

Gordon S. E., J. A. Lake, C. M. Westerkamp and D. M. Thomson (2008). Does AMP-activated protein kinase negatively mediate aged fast-twitch skeletal muscle mass? Exercise and Sport Sciences Reviews**,** 36:179–86.

Gou P., Z. Y. Zhen, M. Horos, J. Arnau, A. Diestre, N. Robert, A. Claret, M. Čandek-Potokar and V. Santé-Lhoutellier (2012). PRKAG3 and CAST genetic polymorphisms and quality traits of dry-cured hams-I. Associations in Spanish dry-cured ham Jamon Serrano. Meat Science 92: 346–353.

Hardie D. G. (2004). The AMP-activated protein kinase pathway--new players upstream and downstream. Journal of Cell Science, 117: 5479–87.

Janssens S. and W. Vandepitte (2004). Genetic parameters for body measurements and linear type traits in Belgian Blue du Maine, Suffolk and Texel sheep. Small Ruminant Research, 54: 13–24.

Jin H., H. Chen, J. Qin, Y. Zhu, H. Chen, G. Chen, Y. Xie, Z. Pan, M. Jiao, S. Huang and M. Chu (2012). The Polymorphism in 5' Regulatory Region and Exon 13 of *PRKAG3* Gene and its Distribution Pattern in Different Goat Breeds. Asian Journal of Animal and Veterinary Advances, 7: 568–577.

Lantier L., R. Mounier, J. Leclerc, M. Pende, M. Foretz and B. Viollet (2010). Coordinated maintenance of muscle cell size control by AMP-activated protein kinase. The FASEB Journal, 24: 3555–3561.

Li W. F, J. Y. Li, X. Gao, S. Z. Xu and W. B. Yue (2012). Association analysis of PRKAG3 gene variants with carcass and meat quality traits in beef cattle. African Journal of Biotechnology, 11: 1855–1861.

Long Y. C., Z. Cheng, K. D. Copps and M. F. White (2011). Insulin receptor substrates Irs1 and Irs2 coordinate skeletal muscle growth and

metabolism via the Akt and AMPK pathways. Molecular and Cellular Biology, 31: 430–441.

Mhairi C., D. Towler and G. Hardie (2007). AMP-Activated Protein Kinase in Metabolic Control and Insulin Signaling. Circulation Research, 100: 328–341.

Mounier R., L. Lantier, J. Leclerc, A. Sotiropoulos, M. Pende, D. Daegelen, K. Sakamoto, M. Foretz, and B. Viollet (2009). Important role for AMPKalpha1 in limiting skeletal muscle cell hypertrophy. The FASEB Journal, 23: 2264–2273.

Mounier R., L. Lantier, J. Leclerc, A. Sotiropoulos, M. Fortez and B. Viollet (2011). Antagonistic control of muscle cell size by AMPK and mTORC1. Cell Cycle, 15: 2640–2646.

Mousa E., M. A. Osman and U. M. EL-Saied (2006). Genetic parameters for body weight of Egyptian Farafra lambs with random regression model. Egyptian Journal of Animal Production, 43: 57–69.

Musa A. M., N. Z. Idam and K. M. Elamin (2012). Heart girth reflects live body weight in Sudanese Shogur Sheep under field conditions. World's Veterinary Journal, 2: 54–56.

Paturi S., A. K. Gutta, A. Katta, S. K. Kakarla, R. K. Arvapalli, M. K. Gadde, S. K. Nalabotu, K. M. Rice, M. Wu and E. Blough (2010). Effects of aging and gender on muscle mass and regulation of Akt-mTOR-p70s6k related signaling in the F344BN rat model. Mechanisms of Ageing and Development 131: 202–209.

Petrovic M. P., D. R. Muslic, V. C. Petrovic and N. Maksimovic (2011). Influence of environmental factors on birth weight variability of indigenous Serbian breeds of sheep. African Journal of Biotechnology, 10: 4673–4676.

Roshanfekr H., M. Mamouei, K. Mohammadi and E. Rahmatnejad (2011). Estimation of genetic and environmental parameters affected pre-weaning traits of Arabi lambs. Journal of Animal and Veterinary Advances, 10: 1239–1243.

Rothschild M. and M. Sölkner (1997). Candidate gene analysis to detect genes controlling traits of economic importance in domestic livestock. Probe, 8: 13–20.

Roux M., A. Nizou, L. Forestier, A. Ouali, H. Levéziel and V. Amarger (2006). Characterization of the bovine PRKAG3 gene: structure, polymorphism, and alternative transcripts. Mammalian Genome, 17: 83–92.

Ryan M. T., R. M. Hamill, A. M. O'Halloran, G. C. Davey, J. McBryan, A. M. Ullen, C. McGee, M. Gispert, O. I. Southwood and T. Sweeney (2012). SNP variation in the promoter of the *PRKAG3* gene and association with meat quality traits in pig. BMC Genetics, 13: 66.

SAS (2000). SAS Online Doc, Version 8, SAS Institute Inc. Cary, NC, USA.

Salako A. E. (2006). Application of morphological indices in the assessment of type and function in sheep. International Journal of Morphology, 24: 13–18.

Santé-Lhoutellier V., N. Robert, J. F. Martin, P. Gou, M. Hortos, J. Arnau, A. Diestre and M. Čandek-Potokar (2012). PRKAG3 and CAST genetic polymorphisms and quality traits of dry-cured hams--II. Associations in French dry-cured ham Jambon de Bayonne and their dependence on salt reduction. Meat Science, 92: 354–359.

Shokrollahi B. and M. Zandieh (2012). Estimation of genetic parameters for body weights of Kurdish sheep in various ages using multivariate animal models. African Journal of Biotechnology, 11: 2119–2123.

Simeonov M. S., D. L. Harmon and K. V. Nedelkov (2015). Non-genetic factors affecting birth weight in the lambs of Blackheads Pleven breed. Journal of Animal Science and Advances, 5: 1208–1217.

Škrlep M., M. Čandek-Potokar, B. Žlender, N. Robert, V. Santé-Lhoutellier and P. Gou (2012). PRKAG3 and CAST genetic polymorphisms and quality traits of dry-cured hams--III. Associations in Slovenian dry-cured ham Kraški

pršut and their dependence on processing. Meat Science, 92: 360–365.

Tariq M. M., E. Eyduran, M. A. Bajwa, A. Waheed, F. Iqbal and Y. Javed (2012). Prediction of body weight from testicular and morphological characteristics in indigenous Mengali sheep of Pakistan: using factor analysis scores in multiple linear regression analysis. International Journal of Agriculture and Biology, 14: 590–594.

Thiruvenkadan A. K., K. Karunanithi, J. Muralidharan and B. R. Narendra (2011). Genetic analysis of pre-weaning and post-weaning growth traits of Mecheri sheep under dry land farming conditions. Asian - Australian Journal of Animal Science, 24: 1041–1047.

Weinheimer E. M., L. P. Sands and W. W. Campbell (2010). A systematic review of the separate and combined effects of energy restriction and exercise on fat free mass in middle-aged and older adults: implications for sarcopenic obesity. Nutrition Review, 68: 375–88.

Yamauchi T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, E. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn and T. Kadowaki (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature Medicine, 8: 1288–1295.

Yamaguchi S, H. Katahira, S. Ozawa, Y. Nakamichi, T. Tanaka, T. Shimoyama, K. Takahashi, K. Yoshimoto, M. O. Imaizumi and S. Nagamatsu (2005). Activators of AMP-activated protein kinase enhance GLUT4 translocation and its glucose transport activity in 3T3-L1 adipocytes. American Journal of Physiology -Endocrinology and Metabolism, 289: E643–E649.

Yang G., H. Zhou, R. Wang and J. Hickford (2015). Variation in the ovine PRKAG3 gene. Gene, 567: 251–254.

Younas A., M. Abdullah, J. A. Bhatti, T. N. Pasha, N. Ahmad, M. Nasir and A. Hussain (2013). Inter-relationship of body weight with linear body measurements in Hissardale sheep at different stages of life. The Journal of Animal and Plant Science, 23: 40–44.

Zhou H., J. G. Hickford and Q. Fang (2006). A two-step procedure for extracting genomic DNA from dried blood spots on filter paper for polymerase chain reaction amplification. Analytical Biochemistry, 354: 159–161.

الأشكال المختلفة لجين PRKAG3 وارتباطها مع أداء النمو وأدلة الجسم لحملان أغنام البرقي

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تعتبر صفات النمو من أهم الصفات ذات القيمة الاقتصادية في الأغنام حيث أن الحملان ذات معدل النمو السريع يكون لها معدل تحويل غذائي مرتفع وأقل عرضة للاصابة بالأمراض ومن ثم تكون عملية الانتاج أقل تكلفة. كما تعتبر أدلة الجسم من الصفات الهامة لما لها من ارتباط بمعدل تكوين العضلات وترسيب الدهن بالجسم.

هذه الصفات ذات طبيعة كمية، وحتى الآن تتم عملية التحسين الوراثي لها باستخدام الطرق التقليدية مثل الانتخاب والخلط، ويعاب على هذه الطرق التكلفة العالية والبطء، خاصة في الأنواع ذات مدة الجيل الطويلة مثل الأبقار والأغنام.

حديثا قام علماء الوراثة الجزيئية باستنباط بعض التقنيات التي يمكن استخدامها في اجراء عملية التحسين الوراثي لحيوانات المزرعة، ومن أهم هذه التقنيات الانتخاب باستخدام الأدلة الوراثية (Marker assisted selection) والذي يعتبر أكثر كفاءة وسرعة ودقة مقارنة بالطرق التقليدية حيث أنه يمكن انتخاب الحيوان في مرحلة مبكرة من العمر اعتمادا على تركيبه الوراثي فقط .

في هذه الدراسة تم اختيار طريقة الجين ذات التأثير واسع المدى (candidate gene approach) لتحديد أدلة وراثية انتخابية لصفات النمو وأدلة الجسم في حملان أغنام البرقي.

يعتبر العامل PRKAG3 أحد المكونات الرئيسية لبروتين AMPK الذي يلعب دورا مهما في عملية تمثيل الكربوهيدرات والأحماض الدهنية في أنسجة الجسم المختلفة خاصة الأنسجة الدهنية ، الكبد ، البنكرياس ، العضلات الهيكلية، ويتم التشفير لهذا العامل بواسطة جين PRKAG3 ، لذا وجد من المهم دراسة تأثير التباين في هذا الجين على الصفات المدروسة.

تم تحديد الأشكال الأليلية والتراكيب الوراثية في المنطقة الواقعة بين الاكسون 4 والاكسون 6 من جين PRKAG3 لعدد 121 من حملان أغنام البرقي باستخدام تقنية PCR-SSCP ، وتلى ذلك تحديد التتابعات النيوكلوتيدية للأليلات المكتشفه.

تم دراسة تأثير الجين على صفات النمو (الوزن عند الميلاد – معدل النمو من الميلاد للفطام - الوزن عند الفطام – معدل النمو من الفطام للتسويق – الوزن عند التسويق) وأدلة الجسم (دليل كتلة الجسم – دليل العضلات الهيكلية - دليل الجسم - دليل الجسم النسبي).

تم اجراء عملية التحليل الاحصائي باستخدام برنامج (SAS, 2000) ، حيث اشتمل النموذج الاحصائي على التباين في الجين (التركيب الوراثي ووجود الأليل من عدمه في التركيب الوراثي) ، جنس الحمل ، ترتيب الولادة ، كعوامل ثابته ، كما اشتمل النموذج على : العمر عند الفطام كعامل مغاير، عند دراسة التأثير على: الوزن عند الفطام، معدل النمو من الميلاد للفطام ، كما اشتمل على العمر عند التسويق كعامل مغاير، عند دراسة التأثير على كل من: معدل النمو من الفطام للتسويق ، الوزن عند التسويق ، أدلة الجسم.

وكانت النتائج المتحصل كالتالي

1. تم تحديد عدد 2 أليل لهذا الجين ورمز لهما بـ (A ، B) وكان تكراراهما (0.51 ، 0.49) على التوالي ، كما تم تحديد عدد 3 تراكيب وراثية هي (AA ، AB ، BB) وكانت تكراراتهم (0.26 ، 0.50 ، 0.24) على التوالي.

2. أثبتت نتائج التحليل الاحصائي الآتي:

أ. كان للتركيب الوراثي الخاص بجين PRKAG3 تأثيرا معنويا ($P < 0.05$) على كل من الوزن عند التسويق ودليل العضلات الهيكلية وتأثيرا عالي المعنوية ($P < 0.01$) على كل من معدل النمو من الفطام للتسويق ودليل كتلة الجسم.

ب. كان لوجود الأليل B في التركيب الوراثي تأثيرا معنويا ($P < 0.05$) على زيادة كل من الوزن عند التسويق ودليل العضلات الهيكلية وتأثيرا عالي المعنوية ($P < 0.01$) على زيادة كل من معدل النمو من الفطام للتسويق ودليل كتلة الجسم.

التوصيات:

وفقا للنتائج المتحصل عليها في هذه الدراسة يوصى بالانتخاب للحملان الحاملة للتركيب الوراثي BB الخاص بالجين PRKAG3 ، وذلك للحصول على حملان ذات (معدل نمو أسرع - وزن تسويق أكبر – نسبة عضلات أعلى).