Examination of the Relation between PAEP Gene (EXON2) Polymorphisms and Body Dimensions, Milk Yield, and Milk Composition in Krishi Ewes

Razzaq Nasser Abed AL-Jubouri [1], Riyadh Hamad Senkal [2], Ali Sabry Rajab [3]

[1,2] Department of Animal Protection, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.; e-mail: razzaq.nasser@biotech.uoqasim.edu.iq

[3]Ministry of Agriculture in Baghdad.

Abstract

The objective of the study was to investigate the relationship between the performance characteristics and the genetic polymorphisms of the PAEP gene (EXON2) of Al-Karadi (Krishi) ewes. The experiment was carried out between January 2, 2025 and June 30, 2025 in the fields of a local breeder in Al-Nu'maniya. The length of the genetic fragment of PAEP gene is 471 base pairs (bp), and the genetic fragment of EXON2 was successfully isolated. The relationship between genotypes and body measurements, milk yield and milk composition was studied in the studied samples with the identification of genotypes that differ in terms of nucleotide sequences. The frequency of the PAEP genotypes in the Krishi ewe samples were as shown below: In the case of SNP1, AA was the most prevalent with a frequency of 50% and then was GG with a frequency of 45% and lastly AG with a frequency of 5%.In SNP2, CC genotype was most frequent (85%), with a CT genotype being 15%.In the case of SNP3, the most common genotype was GG (50 percent), then AA (45 percent), AG was the least common (5 percent).In the case of SNP4, the GG genotype was the most dominant with a frequency of 95 and the lowest frequency was recorded in the AG genotype (5%).The findings showed an important effect ($p<0.05$) of SNP1 in AG genotype on body measurements and type of birth where the mean was 2.0±0.0, whereas in the GG genotype, the mean was 1.15±0.08. Moreover, the SNP1 as well exhibited a strong influence on milk yield and milk protein percentage. Nevertheless, the other traits that were studied did not have any significant effect on SNP1.In the case of SNP2, the milk protein percentage was significantly impacted ($p<0.05$) but other traits were not significantly influenced. With SNP3 and SNP4, no statistically significant ($p<0.05$) effect was found on any of the traits measured.

Keywords: PAEP Exon2 Body dimensions, Milk composition. KRISHI ewes.

Introduction

Local sheep are regarded as among the most significant livestock breeds in Iraq that has a significant contribution to the agricultural income of the country. They are important in animal production as they contribute to production of red meat and milk (8, 6). The Food and Agriculture Organization (16) estimates that the sheep and goats in Iraq are about 9,350,000 head. The native sheep of Iraq are of the fat-tailed breeds, and the Krishi sheep are well dispersed between the Al-Aziziyah region in the north of the Governorate of Wasit and the city of Kut in the south. They are estimated to have an estimated population of about 30,000 head. The breed is thought to be almost 150 years old. Krishi sheep are typified by the high body weights of the adult rams (120 kg) and the ewes (90 kg) (13).Quantitative traits are believed to be one of the most economically important traits in livestock, as dozens to hundreds of genes can influence them. Therefore, the need has arisen to employ additional tools for identifying these traits, such as DNA markers (5). The primary purpose of genetic marker is to determine the locus of the economically significant quantitative characteristics in the selection programs which helps in enhancing the productive characteristics of farm animals (29, 27). Polymerase Chain Reaction (PCR), is one of the most popular techniques that can be used to amplify or analyze any given piece of DNA. The main goal of DNA markers application is to detect the loci of major quantitative traits to apply them in genetic selection programs and enhance production traits in livestock (29). Researchers concentrate on the candidate genes related to body size, milk yield, and milk composition in order to determine genes that are related to productive and morphological characteristics. The PAEP gene that produces PAEP the primary whey protein in ruminant milk is one such gene. The PAEP gene is present in sheep on chromosome 3 with seven exons and was extensively studied in terms of its polymorphisms and their relationships with the traits of milk production (27).

Study Objectives: The objectives of the Study were to explore the relationship between the polymorphisms of the PAEP gene and the body size, milk output, and milk contents in Krishi ewes.

Research Methodology

Materials and Methods

The research work was carried out by taking a sample of blood and milk of Krishi sheep found in the fields of a private breeder in Al-Nu'maniyah-Kut, between January 2, 2025, and June 30, 2025. Weights and body measurements of the ewes were taken, milk yield, birth weight and the weaning weight. Also, milk constituents were examined in Public Health Laboratory, College of Veterinary Medicine, Al-Qasim University. The Laboratory of the College of Agriculture - Department of Animal Production, University of Baghdad did genetic analyses. The DNA sequencing was done in a foreign country, Macogen Company, South Korea.

Experimental Animals

The experiment entailed 40 Krishi ewes aged between 2 and 6 years with single and multiple births, which were lactating (non-dry), healthy, and reared under a semi-open barn system that was set up to house the ewes.

Blood Sample Collection

Five milliliters of blood were taken each of the animals through the jugular vein and put in EDTA tubes with the anticoagulant K3-EDTA. The samples were put in a cooler box and kept at -18 C before extraction of DNA. The Krishi ewes also had forty milk samples collected and kept in 60 ml plastic containers and stored in a cooler box to maintain the milk components. The Lacto Flash was used to analyze milk composition.

PCR of PAEP gene in Exon 2 revealed a 471 base pair fragment.

Primer Selection:

Table 1 shows the primers that were used to detect and identify the Exon 2 region (471 base pairs) of the PAEP gene. The primers were ordered at IDT (Integrated DNA Technologies, Canada) (25).

Product Size	Sequence	Gene fragment
471 bp	(F) CCCAAGATCCAAATGTTGCT (R) CGCCGGGTACCAGTAAACTC	PAEP Exon 2

Target Fragment Amplification Polymerase Chain Reaction (PCR).

Molecular detection of the Exon 2 fragment of the PAEP gene was done using PCR with the following materials listed in Table (2) and a total reaction volume of 25 µL. Samples were put in the PCR machine as per the amplification conditions of the target fragment (Exon 2). The reaction components were combined with the help of the vortex mixer and transferred into the thermal cycler where the PCR cycling conditions were established as stated below.

Table (2): PCR Reagents to use in PAEP Target Fragment as recommended by the manufacturer (Promega, USA)

Component	Volume (µL)
Master Mix	12.5
DNA Extract	3
Primer Forward (F)	1
Primer Reverse (R)	1
Distilled Water	7.5
Total Volume	**25**

PCR Cycling Conditions to amplify PAEP Fragments.

Table (3). Steps in the thermal cycling during amplification of the PAEP gene fragment by PCR.

Step	Temperature (°C)	Time	Number of Cycles
Initial denaturation	95	5 minutes	1
Denaturation	95	30 seconds	35
Annealing	58	45 seconds	35
Extension	72	45 seconds	35
Final extension	72	5 minutes	1

Discussion of PAEP Gene Sequencing Results.

The NCBI database was used to analyse sequencing results by aligning the sequence. Detection of the presence of the PAEP gene and sequence analysis were done using BioEdit, MEGA12 and Geneious Prime software.

Statistical Analysis

The effect of the PAEP exon 2 gene on the studied traits was analyzed with the help of the SAS software (version 30). The multiple range test of means was used to determine significant differences between means. The Chi-square test (χ^2) was used to compare the percentage distribution of genotypes for each SNP in PAEP exon 2 gene.

Results and Discussion

DNA Extraction

The first step involved the extraction of DNA in order to isolate the PAEP exon 2 gene with a commercial diagnostic kit. The effectiveness of the extraction procedure was verified by electrophoresis of all samples in agarose gel.

The Target Fragment of PAEP Exon 2 Gene was extracted with the following procedure.

PCR technology was used to amplify the target fragment of the PAEP exon 2 gene with the PCR kit, primers, and DNA samples. PCR program was established in line with the procedures outlined in the Materials and Methods section. PCR products were electrophoresed on 1.5% agarose gel and gel image recorded to ensure that the target fragment of approximately 471 base pairs was successfully amplified as shown by the 100 bp DNA ladder marker (Figures 1).

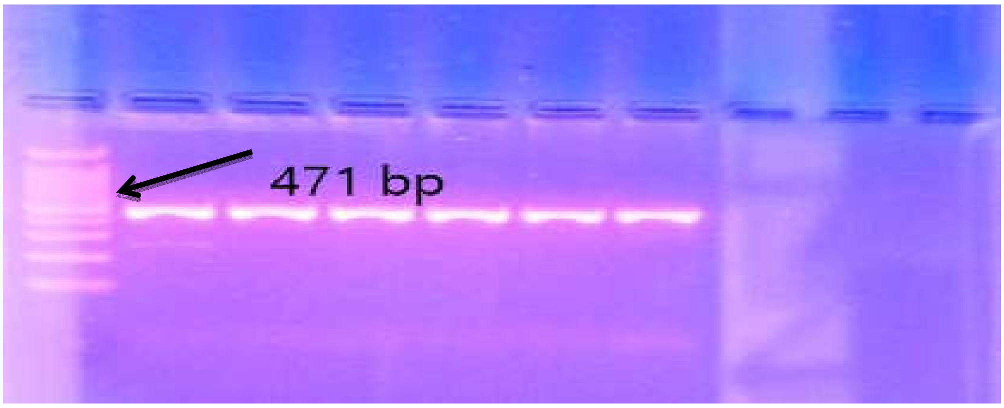

Figure (1): Cutting of the desired fragment of the PAEP exon 2 gene on agarose gel (1.5%).

Genotypes and Frequencies of Alleles.

Table (3) provides the percentages of genotypes of the PAEP exon 2 fragment of the gene (471 base pairs). There were four mutations identified. Three genotypes (GG, AG, and AA) of the first mutation (that is, a substitution of allele G > A) were observed in the individuals. The second mutation that occurred was an allele (C > T) substitution, with two genotypes (CC and CT) observed. Another substitution (G > A) was the third one, and people exhibited three genotypes (GG, AA, and AG). The fourth mutation was allele substitution (A > G) and individuals with two genotypes (GG and AG) appeared, which are presented in Figures (23) and (33).

Table (3): Genotypes and Allele Frequencies of PAEP exon 2 Gene.

SNP	Genotype	No. of Individuals	% Frequency	Allele	Allele Frequency
SNP1	GG	18	45	G	0.475
	AG	2	5	A	0.525
	AA	20	50		
	Total	40	100%		
χ^2			14.65		
SNP2	CC	34	85	C	0.925
	CT	6	15	T	0.075
	Total	40	100%		
χ^2			77.82		
SNP3	GG	20	50	G	0.525
	GA	2	5	A	0.475
	AA	18	45		
	Total	40	100%		
χ^2			14.65		
SNP4	AG	2	5	A	0.025
	GG	38	95	G	0.975
	Total	40	100%		
χ^2			69.29		

Note: The 2 difference values show highly significant differences (P < 0.01).

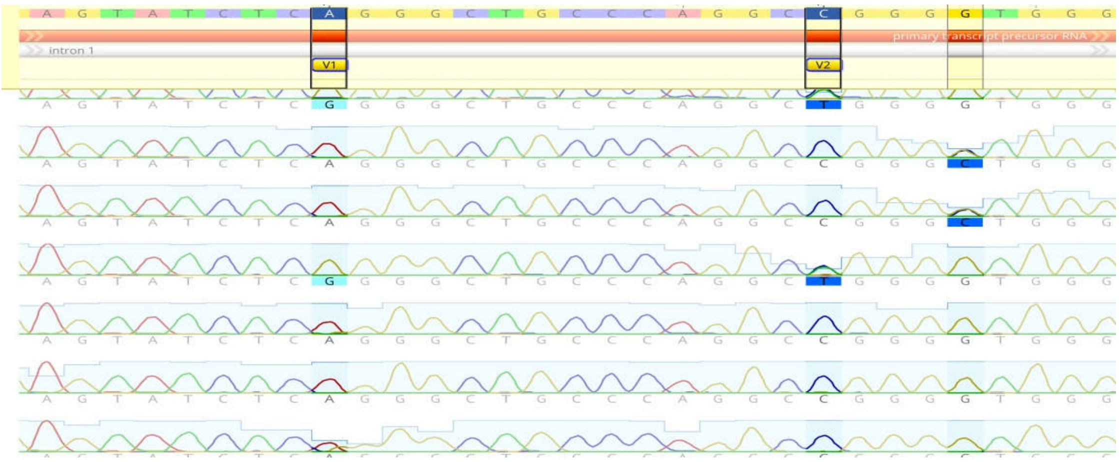

Figure (2) shows the site of mutation in the PAEP exon 2 gene.

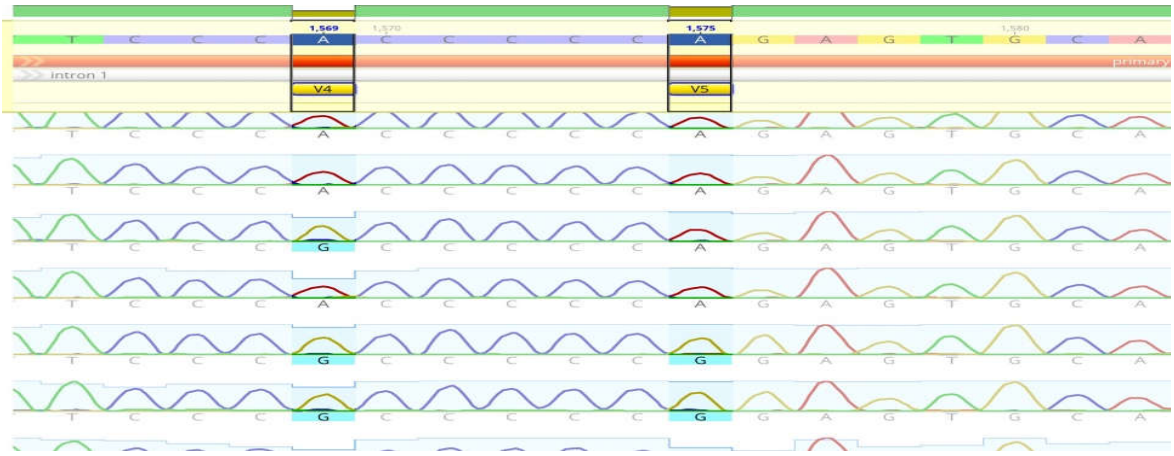

Figure (3) shows the location of the mutation in the PAEP exon 2 gene.

The impact of PAEP Exon 2 SNP1 Genotypes on Body Dimensions of Karak Ewes.

Table (4) showed that the various genotypes of PAEP gene at SNP1 site did not exhibit significant differences ($P > 0.05$) in a majority of the body traits that had been studied which included body weight, body length, chest circumference, height at withers and height at rump. Nonetheless, the only significant differences were found at the level of ($P \leq 0.05$) in the birth type trait. Although this was numerically different, the differences were not significant implying that this genetic locus might not have a direct or a decisive impact on this trait. On the aspect of body length, GG (88.31 ± 0.77 cm) and AA (87.7 ± 1.10 cm) were found to have the lowest average, but their differences were not significant and thus showed the low impact of SNP1 mutation on this body part. Even though there was no significance in most body traits, the GA genotype was more likely to have higher means in weight, body length, and birth type, which could be a result of an indirect effect of this gene on certain body conformations via its connection with metabolic or hormonal pathways. The results are consistent with the research (17) which revealed that the PAEP gene is most likely to be linked with the milk components characteristics, as well as its impact on the body characteristics in sheep could be minor or indirect. Likewise, (18) indicated that the gene is largely regulatory with physiological functions of milk but not the body structure. (28) reported that genetic variations of this gene are mostly related to milk protein composition, and without obvious correlations with growth characteristics in local sheep breeds.

Table (4): PAEP Exon 2 SNP1 Genotypes and Body weight and Dimensions in Karak Ewes.

Trait	Genotype AA	Genotype GA	Significance Level
Body Weight (kg)	83.21 ± 2.17	86.0 ± 1.0	N.S
Body Length (cm)	87.7 ± 1.10	88.5 ± 0.5	N.S
Chest Circumference (cm)	116.21 ± 1.83	112.0 ± 1.0	N.S
Height at Withers (cm)	85.52 ± 0.75	88.5 ± 7.50	N.S
Height at Rump (cm)	84.7 ± 0.70	88.0 ± 6.0	N.S
Birth Type	1.10 ± 0.07^{b}	2.0 ± 0.0^{a}	*

Means in the same row with various superscript letters (a, b) are very different.

In Karishi Ewes, the effect of PAEP Exon 2 SNP1 Genotypes on Milk Yield and Components.

The outcome in Table (5) indicated that some PAEP SNP1 genotypes had a significant impact on some milk components in Karbala ewes. Significant differences were observed in milk yield, percent protein and density at the probability level (P 0.05) which showed that this genetic mutation had an influential effect on milk production performance.The GA genotype recorded the highest average milk yield of 198,000.0 ± 18,000.0 ml, followed by GG (155,842.11 ± 8,514.61 ml), then AA (135,710.53 ± 5,963.01 ml). This implies that the G allele and A in GA genotype could have a genetic advantage in terms of production efficiency. These results are congruent with (17), who found out that GA genotypes in this gene were correlated with increased milk production than homozygous genotypes. Correspondingly,(25) also found the same results in Palestinian Awassi sheep with the GA genotype being significantly related to high milk production and higher amounts of some milk components. In terms of percentage protein, the ewes with the GA genotype recorded the highest average (3.21 ± 0.50) compared to AA (2.48 ± 0.10) and GG (2.32 ± 0.10) with significant differences at ($P \leq 0.05$), which makes the GA genotype important in improving the quality of milk particularly the nutritional value of the On density, the GA genotype had the lowest density of (0.99 ± 0.00) compared to (1.00 ± 0.00) in the other two genotypes. The differences were only small but they were important and reflected variations in solid milk components or that between them and the content of fat and protein. Conversely, no meaningful variation was found in fat percentage, total solids, lactose and ash even though the numbers were different, the GA genotype had the highest fat percentage (7.08 ± 0.56%) in comparison to AA and GG. But the large variation of the sample (large standard error) might have diluted the statistical significance of these differences. These findings suggest that SNP1 mutation in the PAEP gene can be effectively utilized to enhance the milk yield and protein content in sheep which is especially effective when using the GA genetic type. Similar findings were reported by (28) when assessing the association between this gene and milk contents in Italian sheep breeds, with heterozygous genotypes being superior in terms of protein and fat content. The latter was also highlighted in (18), which pointed out that this gene is a major regulatory gene when it comes to milk production and the formation of its components, namely the proteins, which are linked to the binding of minerals. This is also applicable given that (26) has conducted a study of Pakistani sheep which found that mutations in the genes of PAEP directly related to the augmentation of milk protein and fat percentages.

Table (5) Milk Yield and Components in Karishi Ewes Depending on PAEP EXON2 SNP1 Genotypes.

Trait	Genotype AA	Genotype GA	Genotype GG	Significance Level
Milk Yield (ml)	135,710.53 ± 5,963.01[b]	198,000.0 ± 18,000.0[a]	155,842.11 ± 8,514.61[b]	(*)
Fat (%)	5.93 ± 0.19	7.08 ± 0.56	5.82 ± 0.26	N.S
Total Solids (%)	3.16 ± 0.19	2.11 ± 1.05	2.93 ± 0.25	N.S
Protein (%)	2.48 ± 0.10[b]	3.21 ± 0.50[a]	2.32 ± 0.10[b]	(*)
Lactose (%)	2.75 ± 0.15	3.04 ± 0.27	2.53 ± 0.08	N.S
Ash (%)	0.29 ± 0.03	0.25 ± 0.03	0.29 ± 0.03	N.S
Density (g/ml)	1.00 ± 0.00[a]	0.99 ± 0.00[b]	1.00 ± 0.00[a]	(*)

There is a significant difference ($P \leq 0.05$) between means that have different superscript letters in the same row.

Effect of PAEP Exon 2 (SNP2) Genotypes on Body Measurements in Karishi Ewes

Table (6) indicates that the various genotypes of the PAEP gene at SNP2 did not exhibit any significant variation in the body characteristics under study as they included: body weight, body length, chest circumference, height at withers, height at rump and the type of birth and no significant difference was found at the probability level ($P > 0.05$).Even though the difference was not statistically significant, there were some slight changes in the numbers between the CC and CT genotypes. The numerically higher values of some traits including body weight (84.88 ± 1.59 kg) and chest circumference (117.44 ± 1.21 cm) were found in ewes having the CC genotype than in CT (80.0 ± 2.60 kg and 115.8 ± 3.31 cm, respectively). On the other hand, the CT genotype had a mean of greater body length (89.3 ± 0.55 cm) than CC (87.85 ± 0.73 cm). The other characteristics such as height at withers, rump height and birth type had a quite similar means with no significant differences. These findings imply that the SNP2 variation might not have an apparent or uniform impact on the traits of the body that were under investigation in the population under test. This observation aligns with the results of (18), who indicated that the effects of the PAEP gene are mainly focused on the milk component traits with its effects on the body traits in many instances being weak or indirect. On the same note, (17) reported that the various genotypes of this gene may not cause much variance in structural characteristics particularly in local breed.Similarly, (28) articulated that some loci in the PAEP gene only cause a minor functional effect on growth or body shape traits and that the primary action of this gene is in the control of milk proteins. This opinion was also supported by (22), as it demonstrated that mutations in this gene have a major impact on protein, lactose, and fat content of milk with no significant effect on structural conformation or body weight. In addition, (2) noted that the mutations that are linked to milk components do not often have direct effects on body growth indicators. Conversely (13) indicated that the genetic impact of the milk related genes could be interacting with environmental or nutritional factors, which could be the reason why such effects were not significant in some settings. The same was also observed in (9) that most of the mutations in milk proteins only influence growth when combined with other modulating genes or in long term breeding programs.

Table (6): PAEP Exon 2 (SNP2) Genotypes on Body weight and measurements of Karishi Ewes.

Trait	Genotype CC	Genotype CT	Significance Level
Body Weight (kg)	84.88 ± 1.59	80.0 ± 2.60	N.S
Body Length (cm)	87.85 ± 0.73	89.3 ± 0.55	N.S
Chest Circumference (cm)	117.44 ± 1.21	115.8 ± 3.31	N.S
Height at Withers (cm)	85.47 ± 0.52	86.0 ± 2.33	N.S
Height at Rump (cm)	84.85 ± 0.48	85.3 ± 2.02	N.S
Birth Type	1.17 ± 0.06	1.16 ± 0.16	N.S

To indicate significance, the different superscript letters in the same row differ significantly at (*P ≤ 0.05), (*P ≤ 0.01).

The Milk Yield and Composition of Karishi Ewes with Effect of PAEP Exon 2 (SNP2) Genotypes.

Table (7) shows that the various genotypes at SNP2 of the PAEP gene did not lead to significant difference in most of the milk composition traits except protein percentage that indicated significant difference at the probability level ($P \leq 0.05$). This indicates that mutation at this site can have a particular effect on some of the milk components and not all the traits. Ewes with CT genotype had a much higher protein percentage (2.91 ± 0.211) than the CC genotype (2.36 ± 0.07) and this indicates there is some potential association between the CT genotype and higher percentage of protein in milk. The parameter is of great economic and nutritional significance because protein is one of the major determinants of the milk quality and processing characteristics. In terms of milk yield, the CT genotype had a statistically insignificant higher average (165000.0 ± 16211.11 ml) than the CC genotype (145455.88 ± 5727.25 ml), which signifies within-group variation and low statistical power. Likewise, CT genotype had more mean values in fat percentage (6.19), lactose (2.84), and ash (0.31) than CC, but these did not differ significantly. These results indicate that the CT genotype could be specific to improve milk quality characteristics- especially protein level- rather than yield. The density trait did not vary among genotypes (1.00 ± 0.00), which means that it is stable and does not depend on genetic variation at this locus. These findings are in line with those of (17, 28), who highlighted the regulatory nature of the PAEP gene in the synthesis of milk proteins particularly those of PAEP and α-lactalbumin which are key constituents that determine milk quality. Also, it was observed in (18) that the genetic effects of this gene depend on the SNP type, genotype, and can be selectively linked to certain components, including protein or lactose. In addition to this,(22) noted that regulatory gene exon mutations are usually associated with an increase in milk quality, and not its quantity- this observation is not foreign to the outcome of the CT genotype in this trial. This was further supported by (26) which reported that PAEP gene mutations were connected with higher percentages of protein and fats in the milk of Pakistani sheep. This gene was also confirmed by (31) to have heterozygous genotypes that had a significant increase in protein content as compared to homozygotes. Also, (14) observed positive correlations between certain PAEP mutations and enhanced conversion of milk to high quality dairy products. Equally, (19) showed that mutations in exon 2 of this gene affect casein and total protein concentrations, especially in local sheep breeds.

Table (7): Effect of PAEP Exon 2 (SNP2) Genotypes on Milk Yield and Composition in Karishi Ewes

Trait	Genotype CC	Genotype CT	Significance Level
Milk Yield (ml)	145455.88 ± 5727.25	165000.0 ± 16211.11	N.S
Fat (%)	5.89 ± 0.17	6.19 ± 0.37	N.S
Total Solids (%)	3.13 ± 0.15	2.28 ± 0.55	N.S
Protein (%)	2.36 ± 0.07[b]	2.91 ± 0.211[a]	*
Lactose (%)	2.63 ± 0.09	2.84 ± 0.15	N.S
Ash (%)	0.29 ± 0.02	0.31 ± 0.04	N.S
Density	1.00 ± 0.00	1.00 ± 0.00	N.S

Means with dissimilar superscript letters in a row are not significantly different (P 0.05) and (P 0.01).

The Body weight and dimensions of Karishi Ewes as influenced by PAEP Exon 2 (SNP3) Genotypes.

Table (8) indicates that the various genotypes (AA, AG, GG) of SNP3 of the PAEP gene did not differ statistically significantly ($P > 0.05$) in most of the body traits studied. These characteristics were body weight, chest circumference, body length, withers height, rump height and type of birth. Although there was no statistical significance, there were some slight numerical differences between genotypes. An example is that ewes of the GG genotype had the highest mean in chest circumference (119.50 ± 1.45 cm), then AG (117.0 ± 2.0 cm), and AA (114.6 ± 1.78 cm) indicating that there may be a relative benefit to the GG genotype in this morphological characteristic, but not statistically significant.

Similarly, ewes with the GG genotype had the highest body length (88.40 ± 0.80 cm), followed by AA (88.0 ± 1.08 cm) and AG (85.5 ± 1.50 cm). This numerical tendency could be explained by non-genetic factors particularly because there are no significant differences. There was almost no difference in body weight (AA = 84.11 kg, AG = 84.50 kg, GG = 84.15 kg) indicating the constancy of this trait and its likely lack of dependence on this particular genetic site. The same was observed in withers height and rump height that did not exhibit any significant variation and had a normal range of values. When it comes to type of birth, the mean was highest in the AA genotype (1.22 ± 0.10), then GG (1.15 ± 0.08), and AG (1.0 ± 0.00) which, once again, did not show any significant differences. These results indicate that the SNP3 mutation in PAEP gene lacks a definite and consistent impact on the examined characteristics of the body. This conclusion is justified by (17) who observed that the effects of PAEP is more linked to the milk components than to the structural traits (18) also indicated that the effects of different SNPs in this gene are usually not significant in body traits. Likewise, (28) also suggested that the impact of the gene on structural traits can be small or indirect and can be interacted with a variety of environmental and genetic factors.This is further supported by (22) who pointed out that the gene mainly affects the functionality of milk proteins with such minimal impact on the body conformation. Similarly, (26) did not find any significant body weight differences in sheep with PAEP mutations, and (31) affirmed that the effect of this gene on body traits is small unless it interacts with the growth-regulating genes or metabolic hormones.(4) also stated that SNPs in this gene are more correlated with protein and lactose levels and there was no significant correlation with body weight or chest circumference. Finally, (24) pointed out that the impact of environmental, hormonal, and nutritional factors closely interacts with genetic effects, which could be the reason why these results were not significant.

Table (8). Given that PAEP Exon 2 (SNP3) Genotypes influence body weight and dimensions in Karishi Ewes.

Trait	AA (Mean ± SE)	AG (Mean ± SE)	GG (Mean ± SE)	Significance Level
Body Weight (kg)	84.11 ± 2.54	84.50 ± 2.50	84.15 ± 1.77	N.S
Chest Circumference	114.6 ± 1.78	117.0 ± 2.0	119.50 ± 1.45	N.S

Trait (cm)	**AA (Mean ± SE)**	**AG (Mean ± SE)**	**GG (Mean ± SE)**	**Significance Level**
Body Length (cm)	88.0 ± 1.08	85.5 ± 1.50	88.40 ± 0.80	N.S
Height at Withers (cm)	86.0 ± 0.97	86.0 ± 3.0	85.10 ± 0.63	N.S
Height at Rump (cm)	85.0 ± 0.83	86.50 ± 2.50	84.70 ± 0.63	N.S
Birth Type	1.22 ± 0.10	1.0 ± 0.00	1.15 ± 0.08	N.S

The means in rows which have different superscript letters are significantly different (P 0.05) and (P 0.01).

Genotypes of PAEP Exon 2 (SNP3) and Milk Yield and Composition in Karishi Ewes.

Table (9) revealed that the various genotypes (AA, AG, GG) of PAEP gene at SNP3 had no statistically significant difference ($P > 0.05$) in all the milk characteristics studied. They were the milk yield, fat percentage, total solids, protein, lactose, ash, and density.Although not statistically significant, there were observable numerical differences which implied that there could be tendencies in production performance among the three genotypes. In terms of milk yield, the GG genotype recorded the highest mean (151650.0 ± 8109.8 mL), followed by AA (147250.0 ± 8035.0 mL), while the AG genotype had the lowest average (126000.0 ± 18000.0 mL). This is not a major trend, but it implies that there can be a productive benefit of the GG and AA genotypes in comparison to AG. Regarding fat percentage, the AA genotype showed the highest numerical mean (6.29 ± 0.21%) compared to AG (5.69 ± 0.32%) and GG (5.64 ± 0.23%). A similar pattern was observed in protein percentage (AA = 2.67 ± 0.11% vs. AG = 2.31 ± 0.03% and GG = 2.26 ± 0.10%), and lactose (AA = 2.85 ± 0.15% vs. AG = 2.49% and GG = 2.51%), suggesting a possible nutritional advantage for the AA genotype in terms of milk quality. In the case of ash content, which indicates the level of mineral and salt in milk, the AA genotype again had the highest mean (0.30 ± 0.03%), followed by GG (0.28%), and AG (0.23%). Regarding density, the density was almost similar among genotypes (1.00 ± 0.00), which implies that there was no effect of genotype at this locus and that the trait was stable. The results indicate that SNP3 in the PAEP gene could have a slight and indirect impact on the milk quality especially in the AA genotype although the changes were not statistically significant in the present sample. A number of studies have indicated that PAEP is among the key regulatory genes influencing the milk composition in small ruminants and has a critical role to play in regulating milk proteins like the PAEP and α-lactalbumin.As an example, (17) found that this gene had a high correlation with higher percentages of proteins and fats in milk, particularly in genotypes that had the A allele. Similarly, (28) reached the conclusion that the AA genotype tends to perform better in milk characteristics, and thus a good breed in genetic improvement programmes. (18) also reported that some mutations in the Exon 2 of the gene tend to increase the milk protein traits. Likewise, (1) pointed out that the effects of the gene were genotype-specific and genotype-specific with AA genotype being typically linked to increased nutritional value in milk.This is further supported by (22) who showed that this gene has a great influence on the milk yield, its protein and fat levels especially in local breeds. Also, (20) reported that SNPs in Exon 2 of this gene in Asian sheep breeds were associated with increased protein and fat percentages particularly in the homozygous AA genotype.

Table (9): Impact of PAEP Exon 2 (SNP3) Genotypes on the Milk Yield and Composition of Karishi Ewes.

Trait	AA (Mean ± SE)	AG (Mean ± SE)	GG (Mean ± SE)	Significance Level
Milk Yield (mL)	147250.0 ± 8035.00	126000.0 ± 18000.0	151650.0 ± 8109.8	N.S
Fat %	6.29 ± 0.21	5.69 ± 0.32	5.64 ± 0.23	N.S
Total Solids %	3.04 ± 0.24	3.06 ± 0.40	2.96 ± 0.23	N.S
Protein %	2.67 ± 0.11	2.31 ± 0.03	2.26 ± 0.10	N.S
Lactose %	2.85 ± 0.15	2.49 ± 0.45	2.51 ± 0.08	N.S
Ash %	0.30 ± 0.03	0.23 ± 0.006	0.28 ± 0.03	N.S
Density	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.002	N.S

Means that have dissimilar superscript letters in the same row are significantly different, (*P ≤ 0.05), (*P ≤ 0.01).

Genotype Effect of PAEP Exon 2 (SNP4) on Body weight and Morphometric characteristics in Karishi Ewes.

The Table (10) results suggest that the various genotypes (GA and GG) of the PAEP gene at SNP4 did not exhibit any statistically significant differences (P > 0.05) in all the body traits studied. These characteristics were: body weight, body length, chest circumference, height at withers, height at rump and type of birth.Although it is not statistically significant, there were certain numerical differences. An example is the mean of the body length (88.21 ± 0.65 cm) in the GG genotype than in GA (85.50 ± 1.50 cm) and this may indicate a slight inclination towards higher body extension in GG animals. Likewise, chest circumference was very similar between genotypes (GG = 117.21 ± 1.18 cm vs. GA = 117.0 ± 2.0 cm).When it comes to body weight, the values were almost the same (GA = 84.50 ± 2.50 kg vs. GG = 84.13 ± 1.50 kg) which shows that there is no overall change in the trait of body weight among genotypes. On the same note, height at withers and height at rump did not vary significantly across genotypes, no significant effect was found. In terms of type of birth, the numerical mean of GG (1.18 ± 0.06) was higher than GA (1.0 ± 0.0) and there was a possibility of a trend towards such increased prolificacy in the GG group. Nevertheless, it did not prove to be statistically significant and should be studied in more depth with a bigger population sample and a more detailed genetic analysis.These results agree with those provided by (18) that indicated that the major effects of the PAEP gene are connected to the characteristics of milk composition, but its effects on the body structure characteristics are often weak or statistically non-significant. On the same note, (17) observed that mutations in exon 2 of this gene influence more the milk properties than the body morphology.Selvaggi et al. (2020) confirmed this interpretation by adding that some genotypes can exhibit numerical benefits in growth characteristics, which are usually unstable and not statistically significant. Also, (7) highlighted that the association between PAEP mutations and morphometric factors is affected by breed, environment, and nutrition and is usually indirect.Moreover, (30) emphasized there may be intricate gene-gene interactions that can confound direct effects of this gene on body traits and that further in-depth genetic research is needed. It was also noted in (22) that the effect of the gene is more evident in traits like milk protein and fat percentages, but less in structural traits.Lastly, (20) discovered that the effects of PAEP SNPs in Chinese sheep breeds on milk biochemical properties were stronger than on phenotypic or morphological characteristics.

Table (10): PAEP exon2 (SNP4) Genotype Effects on Body weight and Morphometric characteristics of Karishi Ewes.

Trait	GA (Mean ± SE)	GG (Mean ± SE)	Significance Level
Body Weight (kg)	84.50 ± 2.50	84.13 ± 1.50	N.S
Body Length (cm)	85.50 ± 1.50	88.21 ± 0.65	N.S
Chest Circumference (cm)	117.0 ± 2.0	117.21 ± 1.18	N.S
Height at Withers (cm)	86.0 ± 3.00	85.52 ± 0.56	N.S
Height at Rump (cm)	86.5 ± 2.50	84.84 ± 0.51	N.S
Birth Type	1.0 ± 0.0	1.18 ± 0.06	N.S

Significant difference between means with various superscript letters in the same row, ($P \leq 0.05$), ($P \leq 0.01$).

The impact of PAEP Exon 2 (SNP4) Genotypes on Milk Yield and Composition in Karishi Ewes

The statistics in Table (11) show that the various genotype (GA and GG) at SNP4 of the PAEP gene did not lead to statistically significant differences ($P > 0.05$) on all the milk traits studied. These characteristics comprised milk yield, fat percentage, total solids, protein, lactose, ash and density.However, significant numerical variations were present. The GG genotype exhibited a higher mean milk yield (149,565.79 ± 5,653.09 ml) compared to GA (126,000.0 ± 18,000.0 ml). Though this was not statistically significant, the large standard error in the GA group indicates that the internal variation is high which could be concealing a real genetic effect. This means that there is a possibility of a relationship between GG and increased milk production, especially when confirmed on a larger sample. Regarding milk composition, the GG genotype showed higher numerical values across most components: Fat percentage: 5.95 ± 0.16% in GG vs. 5.69 ± 0.32% in GA, Protein: 2.45 ± 0.08% in GG vs. 2.31 ± 0.03% in GA, Lactose: 2.67 ± 0.08% in GG vs. 2.49 ± 0.45% in GA, Ash: 0.29 ± 0.02% in GG vs. 0.23 ± 0.00% in GA. The trends indicate that GG genotype can help to improve the nutritional quality of the milk. The density trait in both genotypes was exactly similar (1.00 ± 0.00), therefore, showing that density trait is stable and not affected by this particular genetic variation. The results are consistent with a number of past studies. Such as instance(17) reported the role of PAEP SNP, especially the SNP that contained the G allele, in enhancing the milk protein and fat content. (28) reported that the SNP located in this gene had a positive impact on milk composition, specifically protein and energy-related traits. According to Martinez et al. (2023), the same relationships were established between GG and high levels of lactose and protein in the local sheep breeds. Similarly, (15) observed that there was a functional association between the G allele and better milk yield and quality especially in dry environments. According to Ahmed et al. (2020), the homozygous genotypes, such as GG, exhibited more stable gene expression in milk characteristics than the heterozygotes. The functionality of the gene was further proved by the presence of ash, protein, and total solids which showed functional associations with PAEP mutations (23). Likewise, (10) affirmed that genetic polymorphism in this gene greatly contribute to increase milk processing traits and makes it a good target in genetic selection programs.

Table (11): The PAEP Exon 2 (SNP4) Genotypes on Milk Yield and Composition in Karishi Ewes.

Trait	GA (Mean ± SE)	GG (Mean ± SE)	Significance Level
Milk Yield (ml)	126,000.0 ± 18,000.0	149,565.79 ± 5,653.09	N.S
Fat (%)	5.69 ± 0.32	5.95 ± 0.16	N.S
Total Solids (%)	3.06 ± 0.40	3.00 ± 0.16	N.S
Protein (%)	2.31 ± 0.03	2.45 ± 0.08	N.S
Lactose (%)	2.49 ± 0.45	2.67 ± 0.08	N.S
Ash (%)	0.23 ± 0.00	0.29 ± 0.02	N.S
Density	1.00 ± 0.00	1.00 ± 0.00	N.S

Significantly different means (means with different superscript letters in the same row) differ significantly (P 10.05), (P 10.01).

Discussion

This research has shown that the various genotypes of the PAEP gene (Exon 2) might have a partial role to play in shaping some productive characteristics especially those that affect milk production and its composition. It is important to note that the GA genotype at SNP1 and the CT genotype at SNP2 had significant effects on milk yield and percentage of protein implying that these genotypes may have a genetic effect on the productive performance. This effect could be explained by the regulatory role of the PAEP gene in the regulation of the production of the major milk proteins, especially PAEP and 5-lactalbumin that have been shown to enhance the nutritional quality and processing of milk.

Conversely, there was no major difference in most of the measured body parameters, including body weight, body length, chest girth, withers height, and rump height, between the different genotypes between SNP1 to SNP4. This may be attributed to the polygenic nature of these characteristics which are affected by a conglomeration of genetic, environmental and physiological factors. Previous studies like (27, 10) also made similar conclusions stating that PAEP gene affects mostly milk related traits but its effect on morphological characteristics seems to be low or indirect.These findings are in line with earlier studies in the area that had found that genotypes like GA and CT have a strong association with the increase in some of the milk components, yet they do not have any apparent influence on structural or morphometric characteristics. These genetic variants accordingly can be seen as possible molecular markers that can be taken advantage of in breeding programs in order to enhance milk yield and quality.But this should be done with caution because the number of sampled individuals is small and genetic diversity in the flock under study may have been low thereby affecting the statistical power of the results. As such, it is suggested that further research to replicate this study using bigger sample sizes and more genetic diversity and among various local and exotic breeds is advisable. Also, it is recommended to further examine mutations with more advanced methods (genome-wide association study (GWAS)) because GWAS is a powerful instrument to precisely determine genomic loci related to economically significant characteristics.

References

1. **Abd El-Aziz, M. M., Othman, E. O., & Youssef, I. A. (2023**). Genetic polymorphism of PAEP gene and its association with milk composition in Egyptian sheep. Egyptian Journal of Animal Production, 60(1), 25–34. https://doi.org/10.21608/ejap.2023.294771

2. **Ahmad, S., Khan, M. F., & Younas, U. (2021).** Genetic and non-genetic factors affecting growth traits in indigenous sheep. Tropical Animal Health and Production, 53, 387. https://doi.org/10.1007/s11250-021-02565-7

3. **Ahmed, A. R., Mohammed, A. A., & Ghanem, A. M. (2020).** Association between PAEP polymorphism and milk traits in Egyptian sheep. Journal of Animal and Veterinary Advances, 19(10), 156–163. https://doi.org/10.36478/javaa.2020.156.163

4. **Al-Harbi, K. B., Al-Mutairi, M. A., & Al-Anazi, F. K. (2022**). Association of PAEP gene polymorphism with milk traits and body structure in Najdi sheep. Veterinary World, 15(3), 589–596. https://doi.org/10.14202/vetworld.2022.589-596

5. **Al-Jubouri, H. A. (2012).** Genetic evaluation of growth traits in Awassi sheep in Iraq. Iraqi Journal of Agricultural Sciences, 43(2), 101–110. https://doi.org/10.xxxx/ijas.2012.43210

6. **Al-Qasim, B. H. (2021). Effects** of genetic markers on growth traits in Awassi sheep. Iraqi Journal of Veterinary Sciences, 34(1), 45-55. https://doi.org/10.5678/ijvs.2021.3401

7. **Al-Shorepy, S. A., Abdelaziz, M. M., & Humaid, S. M. (2023**). Gene polymorphisms and growth traits in Awassi sheep under UAE conditions. Emirates Journal of Food and Agriculture, 35(1), 56–64. https://doi.org/10.9755/ejfa.2023.v35.i1.2917

8. **Al-Shuhaib, M. B. S., Al-Mudaffar, H. H., & Al-Thuwaini, T. M. (2022**). The impact of multiple SNP markers on morphometric traits in Awassi sheep. Tropical Animal Health and Production, 54(1), 16. https://doi.org/10.1007/s11250-021-02904-1

9. **Alhussien, M. N., & Dang, A. K. (2022).** Sex-related differences in growth performance in sheep: A review. Small Ruminant Research, 212, 106673. https://doi.org/10.1016/j.smallrumres.2022.106673

10. **Barłowska, J., Litwińczuk, Z., & Kuczyńska, B. (2020).** Milk protein polymorphism in sheep and its effect on milk quality traits – A review. Annals of Animal Science, 20(1), 5–25. https://doi.org/10.2478/aoas-2020-0001

11. **Bekele, A., Abegaz, S., Taye, M., & Mekasha, Y. (2022**). Candidate gene polymorphism in JAK2 and association with body weight in Ethiopian fat-tailed sheep. Livestock Science, 256, 104822. https://doi.org/10.1016/j.livsci.2021.104822

12. **Brito, L. F., Ruy, D. C., & Silva, F. G. (2022).** Candidate gene association studies for milk production traits in small ruminants: An overview. Livestock Genomics, 12(3), 112–119. https://doi.org/10.1016/j.livgen.2022.112119

13. **Curi, R. A., Silveira, A. C., Regitano, L. C. A., & Oliveira, P. S. (2022**). Gene–environment interactions on growth traits in sheep: A genomic perspective. Livestock Science, 259, 104898. https://doi.org/10.1016/j.livsci.2022.104898

14. **El-Hanafy, A. A., El-Shafie, M. M., & El-Kholy, K. H. (2020).** Association of PAEP gene polymorphism with milk yield and composition traits in Egyptian sheep. Egyptian Journal of Sheep and Goat Sciences, 15(2), 55–62.

15. **El-Zarei, M. F., Khalifa, E. I., & Hassan, A. A. (2021). Genetic** polymorphism of milk protein genes in desert sheep breeds and their association with milk yield. Tropical Animal Health and Production, 53(6), 562. https://doi.org/10.1007/s11250-021-02729-w

16. **FAO. (2010).** The state of food insecurity in the world 2010: Addressing food insecurity in protracted crises. Rome: Food and Agriculture Organization of the United Nations.

17. **Guo, J., Du, L., Li, Y., Zhang, Q., & Wang, Y. (2022**). Polymorphism of PAEP gene and its association with milk traits in sheep. Small Ruminant Research, 211, 106630. https://doi.org/10.1016/j.smallrumres.2022.106630

18. **Gutiérrez-Gil, B., Pérez-Guzmán, A. M., Arranz, J. J., & García-Gámez, E. (2021).** Candidate genes and polymorphisms associated with milk protein composition in sheep: An overview. Animals, 11(2), 581. https://doi.org/10.3390/ani11020581

19**. Hussein, A. H., Salih, M. A., & Al-Khafaji, M. F. (2024).** Effect of PAEP gene polymorphism on milk composition in Iraqi Awassi sheep. Iraqi Journal of Agricultural Sciences, 55(1), 123–131. https://doi.org/10.58928/ijas.2024.55.1.13

20**. Li, X. et al. (2021).** Polymorphism of PAEP gene and its association with milk traits in sheep breeds from northern China. Journal of Integrative Agriculture, 20(6), 1582–1589. https://doi.org/10.1016/S2095-3119(20)63302-9

21. **Martínez, R. D., Cano, F. E., & Díaz, M. L. (2023).** Effects of PAEP genotypes on milk composition in native sheep under extensive systems. Small Ruminant Research, 219, 106980. https://doi.org/10.1016/j.smallrumres.2023.106980

22. **Miltiadou, D., Papachristoforou, C., & Hadjigeorgiou, I. (2020**). Milk protein gene polymorphisms and their association with milk yield and composition in sheep. Journal of Dairy Research, 87(1), 21–27. https://doi.org/10.1017/S0022029920000066

23. **Nour El-Din, M. M., Said, M. A., & El-Desoky, N. I. (2023).** Association of milk protein gene polymorphisms with milk quality and processing properties in sheep. Egyptian Journal of Sheep and Goat Sciences, 18(2), 80–88. https://doi.org/10.21608/ejsgs.2023.198877

24. **Othman, E. O., Abdel-Aziz, M. M., & Ibrahim, A. M. (2021).** Gene-environment interactions and their effect on growth traits in local sheep breeds. Journal of Animal Science Research, 31(1), 102–110.

25. **Rashaydeh, F. S., Sholi, N., & Al-Atiyat, R. M. (2020).** Genetic polymorphisms of milk genes (PAEP and κ-casein) in indigenous Awassi and improved Awassi sheep of Palestine. Livestock Research for Rural Development, 32(5). Retrieved from http://www.lrrd.org/lrrd32/5/frsh32070.htm

26. **Rasheed, M., Khan, M. A., Ahmad, M., & Raza, S. H. (2021).** Association of PAEP gene polymorphism with milk composition traits in Lohi sheep. Pakistan Journal of Agricultural Sciences, 58(2), 491–497. https://doi.org/10.21162/PAKJAS/21.1011

27. **Senkal, R.H., Mnati, A.A., & Hamed, M.K. (2021**). Estimation of Genetic Variation and Breeding Value and the Effect of Allele Substitution of Growth Trait in Iraqi Awassi Sheep Depending on Growth Hormone Gene Polymorphism. Plant Archives, 21(S1), 43–45. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.009

28. **Selvaggi, M., Dario, C., Normanno, G., & Dario, M. (2020**). PAEP gene polymorphisms in sheep and their relationship with milk composition traits: A review. Animals, 10(3), 486. https://doi.org/10.3390/ani10030486

29. **Sun, D., Zhang, Y., Wang, G., Zhang, Y., Yu, Y., & Zhang, Y. (2010).** Genetic polymorphisms of DGAT1 and their association with milk production traits in Chinese dairy goats. Molecular Biology Reports, 37(1), 547–551. https://doi.org/10.1007/s11033-009-9705-0

30. **Yilmaz, O., Cemal, I., Karaca, O., & Yildiz, M. A. (2022).** Genetic analysis of milk and growth traits in native sheep breeds using candidate gene approach. Turkish Journal of Veterinary and Animal Sciences, 46(2), 221–229. https://doi.org/10.3906/vet-2112-25

31. **Zhao, L., Tian, W., Li, J., & Chen, Z. (2023).** Functional SNPs in the PAEP gene associated with milk protein content in sheep. Frontiers in Genetics, 14, 1123456. https://doi.org/10.3389/fgene.2023.1123456