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ASSOCIATION BETWEEN POLYMORPHISM OF CALPAINS 1 GENE AND GROWTH TRAITS IN LOCAL AWASSI LAMBS IN IRAQ

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Abstract

Sheep have been shown to be genetically diverse but this genetic diversity has not been fully described, there are still many sheep populations which have not yet been studied. The purpose of this study were to investigate the polymorphism in the calpain gene (*CAPNS1*) in Iraqi awassi sheep by using PCR-SSCP method, and their relationship to growth traits. A 192-bp fragment of exons 5 and 6 including intron region of the *CAPNS1* gene was amplified and subjected to PCR-SSCP method to screen for polymorphisms. The observed variants had exhibited different distributions in the analyzed samples in terms of the targeted 192 bp amplicons. Were detected five *CAPNS1* genetic patterns according to SSCP technology A, B, C, D, and E**.** Results of sequence analysis revealed five nucleotide substitutions at nt 44, nt 64, nt 94, nt 103 in a part of intron 5, and nt 154 in a part of exon 6 (SNP position). The substitutions at exon 6 nt154 T > C, this SNP is a silent mutation {GGT (Glycine) > GGC (Glycine)}, which creates no substitution effect for amino acid sequence of *CAPNS1* protein. Were found Three alleles named C, G and T. Also showing insertion of 'A' nucleotide at nt (72-73) in a part of intron 5. This genetic variation is in non-coding DNA, therefore it is difficult to conclude how this polymorphism may affect the *CAPNS1* gene activity. The lambs used in the present study were weaned at three months of age. The average weight gain for weaning weight, six, nine, and twelve months were also recorded the overall average gain 21.62 ± 0.61, 31.32 ± 0.59, 37.51 ± 0.76, 41.42 ± 0.68, and 19.80 ± 0.40 kg, respectively. The results obtained in this study showed the importance of genotypes as sources of variation, where there was significant (P <0.05) effect of genotype in different economic growth traits used in the analysis. Also the study results showed that the breed is unbalanced because the calculated x^2 values are greater than the tabular in all positions of the studied *CAPNS1* gene, The imbalance, according to Hardy Weinberg rule, may be due to the possibility of out breeding. It may be the result of non-random mating for several years in awassi sheep populations. The results confirmed that the PCR-SSCP is an appropriate tool for determining genetic variability in *CAPNS1* locus. Since this gene could be considered as a candidate gene of growth and meat quality traits in sheep and may affect lamb quality parameters., further study may be conducted to ascertain the association of these genotypes with the growth and carcass/ meat quality traits.

Key words : Calpain gene (*CAPNS1*) , *Iraqi awassi lambs, PCR-SSCP,Nucleotide Sequences, growth traits.*

Introduction

Recent developments in molecular biology and statistics have provided the possibility of using genomic variation to accelerate the rate of genetic improvement of livestock (Montaldo *et al.*,1998). Calpains are calcium-dependent intracellular cysteine proteases. Two main isoforms they are μ-calpain (calpain I, CAPN1) and m-calpain (calpain II, CAPN2) are heterodimers consisting of distinct large 80-kDa catalytic subunits and identical small 28-kDa regulatory subunits calpain small subunit 1, (*CAPNS1*), which are required to maintain stability and activity of both calpains (Goll *et al.*, 2003). The calpain activity is basically required for myoblast fusion as well as cellular proliferation and growth. Furthermore, calpains participate in the muscle development and in muscle fiber determination (Sultan *et al.*, 2000). Polymorphism in *CAPNS1* gene has been identified in different breeds of sheep (Chung *et al.*, 2001; Naveen *et al.*, 2015).

The *CAPNS1* gene encodes the protein of 263–269 amino acids, with the share of more than 90% amino acids identity in humans, mice, pigs, and cattle (Juszczuk-

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Kubiak *et al.,* 2010). Ovine calpain small subunit 1 gene (*CAPNS1*), also known as *CAPN4*, is located on chromosome 14 and contains 12 exons (Chung and Davis, 2014). However there is little known about this gene in sheep, To date, only few polymorphism analyses regarding ovine *CAPNS*1 gene have been undertaken. Chung and Davis (2014) investigated genetic variants in whole *CAPNS*1 gene consisting of 12 exons. They detected SNPs, which positions were assigned to:g.45923178G>C, g.45923421G>A, g.45924950A>G, and g.45924969A>T for CAPN445 (nt115), CAPN456 (nt 71),CAPN478 (nt 25), and CAPN478 (nt 44), respectively. Shahroudi *et al.,* (2006), Nassiry *et al.,* (2007), Dehnavi *et al.,* (2012), and Azari *et al.,* (2012) analyzed polymorphisms in exons 5 and 6 (including intron region) of the *CAPNS1*gene in sheep; however, they presented only the SSCP patterns without any sequence information, therefore positions and types of polymorphisms in this DNA fragment remained unknown. Arora *et al.,* (2014) investigated genetic variability in the same fragment of the *CAPNS1* gene in Indian sheep. They sequenced PCR amplicons and observed SNP in exon 5 of this gene (*CAPNS1* g.44C>T). Kumar *et al.,* (2015) showed in the same fragment of the ovine *CAPNS1* gene the addition of an adenine in the B allele at 89bp position of PCR product. Furthermore, in cattle there is also limited knowledge about genetic variants in the *CAPNS1* gene.

As there is little information on the calpain small subunit 1 (*CAPNS1*) gene polymorphism in sheep, and no previous association studies in Iraq around this gene in sheep, the aims of the present study were to study the association between polymorphism in ovine *CAPNS1* gene and growth traits in Iraqi awassi lambs.

Materials and Methods

Animals

The present study was performed on 31 animals (males), representing Iraqi awassi lambs breed animals were reared in the experiment station, college of agriculture engineering sciences, university of Baghdad, Iraq. selected from the experimental flock reared under extensive conditions. Lambs stays with their dams up to 90 days (weaning age). The health status of the flock must be under regular observations. Lambs are weighed directly after weaning age and tagged with plastic tags. Flock is housed under semi-open sheds and can be fed on the concentrated ration for age and weight, and green roughages such as Alfalfa and clover can be added throughout the season for the period 3/10/2018 to 9/10/ 2019. Weights of lambs are measured monthly.

Blood sampling

Blood samples were isolated from randomly chosen Iraqi awassi lambs. About 3 ml of blood samples from 31 sample from Iraqi awassi lambs. by jugular vein puncture using disposable needle**.**

DNA isolation

Genomic DNA was obtained from the peripheral blood of the jugular vein of awassi lambs. DNA was isolated using genomic DNA isolation kit (Geneaid Biotech - Taiwan). After DNA extraction, the concentration and purity of DNA were measured by a nanodrop (BioDrop μLITE, Biodrop,UK). Then, the DNA degradation probability was checked bya standard 0.8% (w/v) agarose gel electrophoresis that was prestained with a higher concentration of ethidium bromide (0.7 μg/mL) in TAE (40 mMTris acetate; 2 mM EDTA, pH8.3) buffer.

PCR Amplification

The calpain gene (*CAPNS1*) was amplified by PCR using one pair of specific primers. The Primers were selected according to (Shahroudi*et al.,* 2006; Dehnavi*et al.,* 2012). The lyophilized primers were purchased from Bioneer (Korea). The sequence of Forward: 5'AAC ATT CTC AAC AAA GTG GTG 3' and Reverse: 5' ACA TCC ATT ACA GCC ACCAT 3'. The length of the only amplicon as it was determined by agarose gel electrophoresis was only 192bp, which covered exons 5 and 6 and intervening intron of *CAPNS1*gene. PCR reaction was performed using *AccuPower* PCR premix (Bioneer -Korea). Each 20μl of PCR premix was contained 1 U of *Top* DNA polymerase, 250 μM of dNTPs, 10 mM of Tris-HCl (pH 9.0), 30 mM of KCl, 1.5 mM of MgCl2. The reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic DNA. The following program was applied in gradient PCR thermocycler, version; mastercycler-nexus (Eppendorf - Germany); the amplification was began by initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 30sec, and was concluded with a final extension at 72°C for 5min. After performing PCR thermocycling, The PCR amplified products were confirmed by resolving on 1.5 percent agarose in parallel with 100 bp DNA ladder. Gel electrophoresis was carried out at a constant voltage of 100 V for 60 min in 1X TAE buffer. It was made sure that all PCR resolved bands are specific and consisted of only one band (192bp fragment each).

SSCP analysis

SSCP was performed according to Orita and his

colleges (Orita, 1989) with some modifications. Briefly, 10 µl of each amplification product was mixed with equal volume of SSCP denaturing loading buffer (95% formamide, 20 mM EDTA pH 8, 0.05%xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95°C for 5 min and chilled on ice, and loaded onto 20 x 20 x 0.1 cm gel format (Cleaver Scientific - UK). Denatured PCR products were loaded into the wells of 8% (37.5 acrylamide / 1 bis) polyacrylamide gels, containing 7% glycerol, and 1×TBE buffer. The gel was run under 30 W for about 7 hours and the resolved bands were visualized after silver staining using gel photo-documentation unit, version Chemidoc MP imaging (Bio-Rad - USA).

DNA sequencing and Statistical Analysis

Based on visualization of different band patterns, genotypes were determined. The PCR products corresponding to different patterns were custom sequenced using primers used for amplification. The sequencing results of the PCR products of different samples were edited, aligned, and analyzed as long as with the respective sequences in the reference database using BioEdit Sequence Alignment Editor Software Version 7.1 (DNA STAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in its corresponding position within the referring genome. The PCR amplified sequence of *CAPNS1* gene was used as query and subjected to nucleotide blast at NCBI (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi) for sequence homology searches in public databases. Calculation of genotypes and allele frequencies were performed in the *CAPNS1* locus using PopGene32 (Ver. 1.32) (Yeh *et al.*,, 1999). Statistical analyses were performed for different traits growth characteristics of lambs using the program (Statistical Analysis System SAS 2012), and the model used to analyze the data was as follows:

$$Y_{ijkl} = \mu + G_i + T_j + S_k + e_{ijkl}$$

As:

Y_{ijkl}:Observation values l for genotype i, type of birth j and sex of pregnancy k.

µ: General mean.

G_i: Effect of multiple genotypes of *CAPNS1* { P44 (CC, TC, TT); P64 (GG, GT, TT); P94 (CC, CT, TT), and p (72-73 Ains.)}.

T_j: Effect of type of birth (individual, twin) (For adjusted).

S_k: Effect of pregnancy sex (male, female) (For adjusted).

e_{ijkl}: Random error that is normally distributed with an average of zero and a variation of σ^2_e.

Results and Discussion

In this study, genetic polymorphism of *CAPNS1*gene was studied for the Iraqi awassi lambs breed. A 192 bp of exons 5 and 6 including intervening intron of *CAPNS1* gene was amplified by PCR (Fig. 1- A). The PCR-SSCP analysis of PCR products revealed five patterns A, B, C, D, and E was observed in all the population of sheep studied.

Within this locus, five samples were included in the present study that had shown to amplify *CAPNS1* genetic sequences in the chromosome no. 14 in sheep. The latter gene is responsible for encoding intracellular calcium-activated cysteine proteases that have been involved in several physiological, productive, and pathological processes (Mahrous *et al.*, 2016). The sequencing reactions indicated that the exact identity after performing NCBI blastn for these PCR amplicons (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Concerning the supposed 192 bp amplicons, NCBI BLASTn engine shown about 99% sequences similarities between the sequenced samples and the intended reference target sequences. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (GenBank acc. KT377442.1), the approximate positions and other details of the retrieved PCR fragments were identified. After positioning the 192 bp amplicons' sequences within the chromosome no. 14, the details of its sequences were highlighted, in terms of the positioning of both forward and reverse primers of the 192 bp amplified amplicons.

The sequencing chromatogram of the observed substitution SNPs, as well as their detailed annotations, were documented, and the chromatogram details of the observed SNPs were shown according to their positions in the PCR amplicons (Fig. 1-B).

Moreover the obtained sequences were submitted and accepted at the international gene bank and got accession numbers: {Banklt 2248616 seq. 1- 5 {(MN135979, MN135980, MN135981, MN135982, and MN135983)}. Because of the observed variably distributed SSCP banding pattern in each genetic group, it is possible therefore to attribute S1, S2, S3, S4, and S5 genetic groups to SSCP banding pattern A, B, C, D, and E respectively. And to summarize all the results obtained from the sequenced 192 bp fragments, the exact positions of the observed variations were described in the NCBI reference sequences (Table 1).

The PCR-SSCP analysis of PCR products revealed

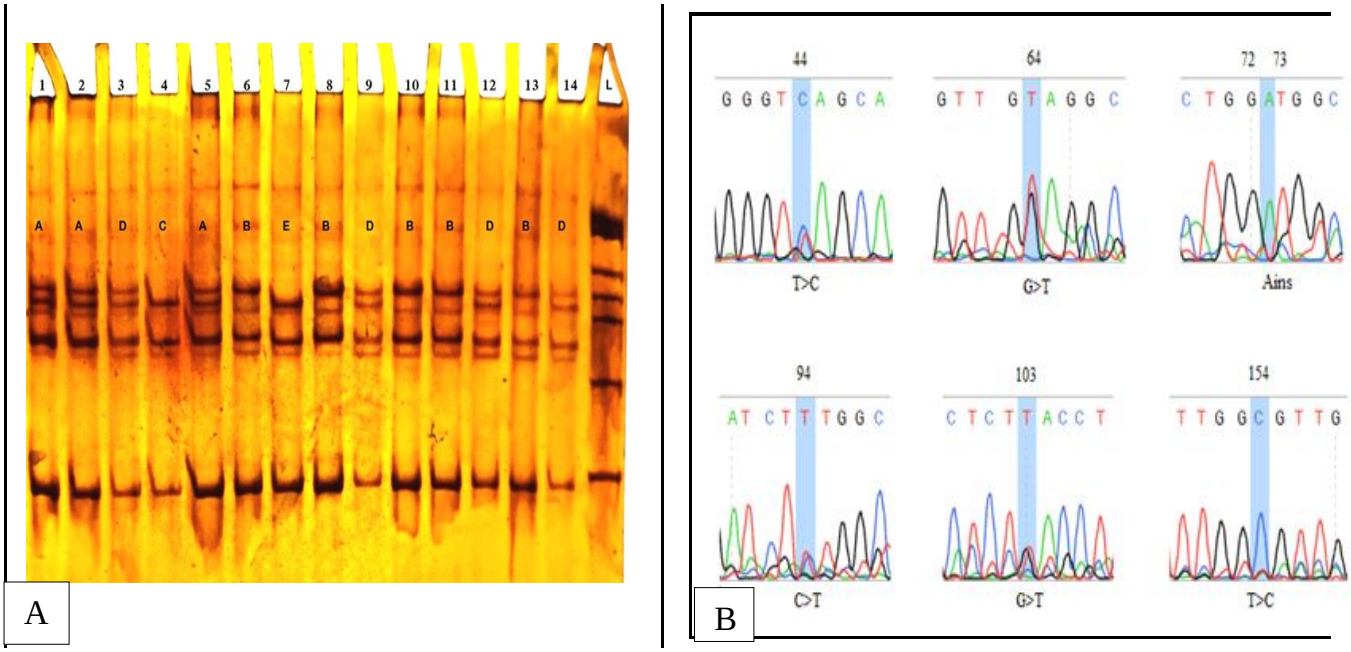

Fig.1: A: PCR-SSCP analysis of CAPNS1 gene in sheep. Lane L 100 bp DNA ladder, Lane 1 to 14 are the genotypes(A, B, C, D, and E).

B: The pattern of the observed substitution mutations within the DNA chromatogram of the targeted 192 bp amplicons within the ovine CAPNS1 gene. The observed substitution mutations were highlighted according to their positions in the PCR products. S1 – S5 refer to the studied no. 1 to no. 5 samples. The symbol ">" refers to the mutation event, while the phrase "ins" refers to insertion mutation.

Table 1: Genotypes corresponding to the SSCP patterns of the *CAPNS1* gene in the local Iraqi awassi sheep breed by sequence technique for each locus compared to the corresponding NCBI reference sequence (Gen Bank acc. no. KT377442.1).

Sample No.	SSCP bending Pattern	Variant summary					
		g.44T>C	g.64G>T	g.72-73A ins	g.94C>T	g.103G>T	g.155T>C
S1MN135979	A	TC	GT	-	CC	GT	CC
S2MN135980	B	TT	GT	IN	CT	GT	CC
S3MN135981	C	TC	GT	IN	CT	GT	CC
S4MN135982	D	CC	GT	IN	CC	GT	CC
S5MN135983	E	TT	GG	-	CC	GT	CC

Table 2: Number and frequency of patterns of the Calpain gene (*CAPNS1*) for local Iraqi awasi lambs according to the results of SSCP technology.

Percentage %	The number	SSCP bending Pattern
12.90	4	A
19.36	6	B
9.68	3	C
45.16	14	D
12.90	4	E
100 %	31	Total

SSCP : Single strand conformation polymorphism.

five patterns, A,B,C,D, and E, with Percentage (%) of 12.90, 19.36, 9.68, 45.16, and 12.90, respectively, (Table 2).

The genotype and allele frequencies for *CAPNS1* loci are presented in table 3 showed that recurrence of genotype patterns CC, GG, CT, GT, and TT were 43.23, 2.58, 10.32, 37.42, and 6.45 respectively, and allele frequencies C, G, and T were 0.4839, 0.2129, and 0.3032 respectively, Furthermore, From the above results, genotype CC, and the allele C was the highest frequency and genotype GG and the allele G was the less frequency.

The exon 5 and 6 as well as intron 5 in ovine *CAPNS1* gene were amplified and produced a 192 bp fragment.

Table 3: Genotype and allele frequencies for *CAPNS1* loci.

Genotype frequencies(%)					Allelic frequencies(%)			x^2
CC	GG	CT	GT	TT	C	G	T	
43.23	2.58	10.32	37.42	6.45	0.4839	0.2129	0.3032	151.659

Table 4: Association of *CAPNS1* genotypes with growth traits according to the results of SSCP technique.

Significantly	Genetic Pattern					Growth traits(kg)
	E	D	C	B	A	
*	20.82 ± 0.88 ab	22.38 ± 0.48 a	20.21 ± 1.05 b	21.84 ± 0.75 ab	20.49 ± 0.86 b	Weaning weight
*	31.38 ± 0.98 ab	31.83 ± 0.54 a	30.96 ± 1.17 ab	31.37 ± 0.84 ab	29.63 ± 0.96 b	Six months weight
*	37.64 ± 1.52 ab	37.13 ± 0.83 ab	39.56 ± 1.81 a	38.32 ± 1.28 a	35.90 ± 1.47 b	Nine months weight
*	42.89 ± 1.36 a	41.13 ± 0.75 ab	41.51 ± 1.62 ab	41.97 ± 1.15 ab	40.08 ± 1.32 b	Twelve months weight

Averages with different characters within a row are significantly different * (P <0.05).

Table 5: Association of *CAPNS1* genotypes with weight gain according to the results of SSCP technique.

Significantly	Genetic Pattern (SSCP)					Growth traits(kg)
	E	D	C	B	A	
NS	10.55± 0.61 a	9.45± 0.34 a	10.7± 0.73 a	9.52 ± 0.52 a	9.1 ± 0.59 a	Weight gain(1)
NS	6.25 ± 1.28 a	5.29± 0.70 a	8.59± 1.53 a	6.95 ± 1.08 a	6.27 ± 1.25 a	Weight gain(2)
*	5.24 ± 1.06 a	3.99± 0.58 ab	1.95± 1.26 b	3.64 ± 0.90 ab	4.18 ± 1.03 a	Weight gain(3)
NS	22.06± 1.07 a	18.7± 0.59 a	21.3± 1.27 a	20.12± 0.90 a	19.59±1.04 a	Weight gain(4)

Weight gain(1): between weaning and six months. Weight gain(2) : between six and nine months. Weight gain(3) : between nine and twelve months. Weight gain(4) : between weaning and twelve months. The averages with different characters within a row are significantly different (P<0.05)*. NS : No significant.

Table 6: Association of *CAPNS1* genotypes with growth traits according to the results of sequence technique.

Weights (kg)				Genotypes	Position
Twelve months weight	Nine months weight	Six months weight	Weaning weight		
42.36 ±0.86a	38.07 ±1.00a	31.38 ±0.62 a	21.42±0.56a b	TT	P44T>C
0.99 ±40.63a	37.39 ±1.15a	30.16 ±0.72a	20.41 ±0.64b	TC	
0.99 ±40.63a	0.84 ±37.16a	0.53 ±31.84 a	0.47 ±22.37a	CC	
1.62 ±42.51a	1.85 ±36.75a	1.22 ±31.33a	1.12 ±20.49a	GG	P64G>T
0.49 ±41.31 a	0.56 ±37.58 a	0.37±31.32 a	0.33 ± 21.74 a	GT	
0.56 ±41.28a	0.61 ±36.99b	0.42±31.33a	0.39 ±21.72 a	CC	P94C>T
0.89 ±41.76a	0.97 ±38.75a	0.67±31.29a	0.62 ±21.38a	CT	
41.43±0.42a	0.61 ±37.80a	0.39 ±31.59a	0.36 ±21.93a	Insertion	P(72-73)A Ins
0.94 ±41.42a	1.04±36.64a	0.68 ±30.52a	0.62 ±20.73b	Null	

The averages with different characters within one column for each position are significantly (P <0.05)* different.

Part of this fragment from nt-1 to nt-28 corresponds to exon 5, from nt-29 to nt-127 corresponds to intron 5 and from nt-128 to nt- 190 is part of exon 6 (Chung *et al.*, 2001). Our results of sequence analysis revealed five nucleotide substitutions at nt 44, nt 64, nt 94, nt 103 in a part of intron 5, and nt 154 in a part of exon 6 (SNP position).The substitutions at exon 6 nt154 T >C, this SNP is a silent mutation {GGT (Glycine) > GGC (Glycine)}, which creates no substitution effect for amino acid sequence of *CAPNS1* protein. Also showing insertion of 'A' nucleotide at nt (72-73) in a part of intron 5.This genetic variation is in non-coding DNA, therefore it is difficult to conclude how this polymorphism may affect the CAPNS1 gene activity. It should be mentioned that it may affect mRNA splicing, or may be linked to polymorphism elsewhere in the coding sequence, which may have an impact on expression of this gene. Also the study results showed that the breed is unbalanced because the calculated values are greater than the tabular in all positions of the studied *CAPNS1* gene, The imbalance,

Table 7: Association of *CAPNS1* genotypes with weight gain according to the results of sequence technique.

Growth traits (kg)				Genotypes	Position
Weight gain(4)	Weight gain(3)	Weight gain(2)	Weight gain(1)		
0.71 ±20.94 a	0.71 ±4.29 a	0.83 ±6.68 ab	9.96±0.41 a	TT	P44T>C
0.81 ±20.22 ab	0.82 ±3.24 a	0.96 ±7.22 a	0.48 ±9.75 a	TC	
0.60 ±18.77 b	0.60 ±3.98 a	0.70 ±5.31 b	0.35 ±9.47 a	CC	
1.35 ±22.01 a	1.28 ±5.75 a	1.60 ±5.42 a	0.74±10.83 a	GG	P64G>T
0.41 ± 19.56a	0.38 ± 3.72b	0.48 ±6.26 a	0.22 ±9.57 a	GT	
0.48 ± 19.56a	0.43 ± 4.29a	0.52 ±5.66 b	0.26 ± 9.61a	CC	P94C>T
0.76 ±20.38 a	0.69 ±3.01 a	0.83 ±7.46 a	0.42 ±9.91 a	CT	
0.46 ±19.49 a	0.43 ±3.62 a	0.53 ± 6.1 a	0.25 ±9.66 a	Insertion	P(72-73)A Ins
0.79 ±20.68 a	0.74 ± 4.77a	0.92 ± 6.12a	0.44 ±9.78 a	Null	

Weight gain(1): between weaning and six months. Weight gain(2) : between six and nine months. Weight gain(3) : between nine and twelve months. Weight gain(4) : between weaning and twelve months. The averages with different characters within one column for each position are significantly different(P <0.05)*.

Table 8: General mean ± Standard error for the studied growth traits of *CAPNS1* gene for Iraqi Awassi lambs (N = 31).

Weaning weight (kg)	Six months weight (kg)	Nine months weight (kg)	Twelve months weight (kg)	Overall average gain (kg)
21.62 ± 0.61	31.32 ± 0.59	37.51 ± 0.76	41.42 ± 0.68	19.80 ± 0.40

according to Hardy Weinberg rule, may be due to the possibility of out breeding. It may be the result of non-random mating for several years in awassi sheep populations.

Zhou *et al.*, (2007) also Identified SNP g.44C > T in CAPN4 gene of Indian sheep breeds. Consistent to the present study Grochowska *et al.*, (2017) indicated that Positions 44 and 154 were placed in intron 5 and exon 6 respectively. Variation at position 154 covered the third nucleotide in the codon GGC for glycine, and was synonymous. Variations in these two positions (44 and 154) resulted in the occurrence of three alleles, named A1, B1 and C1. Consequently, the analysis revealed six *CAPNS1* genotypes: A1A1, A1B1, B1B1, A1C1, B1C1 and C1C1.

Growth traits

Growth traits such as weaning weight, six, nine, and twelve months body weight were recorded on 31 lambs that were randomly selected for genotyping of *CAPNS1* gene.

Association of *CAPNS1* genotypes with growth traits According to the SSCP technique

The *CAPNS1* genotypes recorded in the present study (A, B, C, D, and E) had significant association with growth traits (P<0.05), where the superiority of the genetic pattern D on the genotype A at weighing weaning (22.38 ± 0.48, 20.49 ± 0.86 kg respectively), also and at six months weight was the superiority of the genetic pattern D On the genotype A(31.83 ± 0.54, 29.63 ± 0.96 kg respectively), At the age of nine months genotypes significantly superiority (P<0.05, were the superiority of the genetic pattern C, and B On the genotype A (39.56 ± 1.81, 38.32 ± 1.28, and 35.90 ± 1.47 kg respectively), and was the superiority of the genetic pattern E On the genotype A at twelve months (42.89 ± 1.36, 40.08 ± 1.32 kg respectively), (Table 4).

Regarding the mean weight gain, there were significant differences (P <0.05) for ages nine and twelve months between genotypes, where genotypes A and E were superior to genotypes C (4.18, 5.24, and 1.95 kg respectively). However, the results of the present study did not record significant differences between genotypes in other weight gain values, (Table 5).

Association of *CAPNS1* genotypes with growth traits according to the Sequence technique

Significant differences (P <0.05) were reported according to the sequencing technique at position 44 at weaning weight between CC and TC genotypes, where CC genotype exceeded TC genotype, and weights averages for genotypes were 22.37 ± 0.47 and 20.41 ± 0.64 kg, respectively, as well as weaning at position P(72-73) recorded a significant superiority in favor of the insertion mutation, However, there was no significant effect of genotype on growth traits elsewhere, (Table 6).

Regarding the mean weight gain there were significant differences (P <0.05) between different ages, (Table 7).

The results obtained in this study showed the importance of genotypes as sources of variation, where there was significant (P <0.05) effect of genotype in different economic growth traits used in the analysis, and There were no earlier reports or studies on association between *CAPNS1* genotypes weight body measurement in Iraqi awassi and other sheep breeds in Iraq for comparison. However, these results are in line with Mahrous *et al.,* (2016), where he indicated that animals with TT genotype were the highest weight followed by those with CT and CC genotypes, and that the genotype had a significant impact on the average of weight gain, where animals were TT genotypes gave the highest weight gain compared with those of CT and CC genotypes at position P44 and P154 in three Egyptian sheep breeds: Barki, Rahmani and Ossimi.

General mean ± Standard error for the studied growth traits of *CAPNS1* gene for Iraqi Awassi lambs

The lambs used in the present study were weaned at three months of age. The average weight gain for weaning weight, six, nine, and twelve months were also recorded the overall average gain 21.62 ± 0.61, 31.32 ± 0.59, 37.51 ± 0.76, 41.42 ± 0.68, and 19.80 ± 0.40 kg, respectively are presented in (Table 8).

They are among the ranges indicated by Jafari *et al.,* (2012) (21.26, 29.22, 30.75, 40.00 kg respectively), and Rahimi *et al.,* (2014) (21.50, 27.18, 28.27, 34.21 kg, respectively) of the Iranian Makuie breed, and above the averages reached by Mallick *et al.,* (2017) when studying weights for the Indian Merino sheep breed (weaning weight (3 months, 6 months, and 12 months) 19.08 ± 0.23, 25.00 ± 0.35 and 34.79 ± 0.59 kg, respectively, As for weaning weight, this rate was higher than that found by Al-Rawi *et al.,* (1982), Said *et al.,* (2000), and Alkass *et al.,* (2005) 17.70, 19.7, and 17.93 kg respectively in Awassi sheep. The reason for the variation in averages in the weaning weight of the same strain is due to the difference in genotypes between herds from one country to another and in breeding and management conditions.

These results are consistent with Zamani *et al.,* (2015) in the presence of significant variation in growth characteristics, including body weights of different ages in lambs with different genotypes of genes. The results of this study significantly support the possibility of adopting the genetic analysis of *CAPNS1* gene in selection programs if the aim is to improve the growth characteristics of lambs in sheep herds, especially weight at weaning and the rate of weight gain between them, note that the body weights at an early age with a positive and high moral correlation with weights and subsequent body measurements, which supports the possibility of adopting these results in accelerating improvement programs to maximize Economic return of the herd, the growth qualities are one of the most important economic qualities in sheep breeding projects.

The results confirmed that the PCR-SSCP is an appropriate tool for determining genetic variability in *CAPNS1* locus. Since *CAPNS1*gene is considered as candidate gene for growth and carcass/ meat quality traits, further study may be conducted to ascertain the association of these genotypes with the growth and carcass/ meat quality traits.

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