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GENETIC DIVERSITY INDICATORS IN SPP1 GENE OF LOCAL AWASSI EWES IN MIDDLE OF IRAQ

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ABSTRACT

This study was conducted to identify the genetic diversity indicators of the secreted phosphoprotein 1 (SPP1) gene in flock of local ewe's sheep in middle of Iraq. The sample was made up of 48 ewes and the duration of the study was six months. Genetic polymorphisms were identified and four SNPs (4) variants were obtained. The average allelic abundance (Na) was 2.00, the average number of alleles affected (Ne) was 1.0539, Shannon Guide (I) 0.1163. The average allelic abundance was equal in all the variations studied (2.00) and the number of alleles affected was higher in SNP2 and SNP3, both at (1.0425) compared to SNP1 (1.0211) and SNP4 (1.1096). Obs-Hom and (0.9479) are very close to Exp- Hom and (0.9492). The individual's evidence of persistence within the Fis clan of the G, A, C and T allels of local sheep were -0.0367. The Fit's aggregate stability manual obtained according to SPP1 gene analysis is also -0.0367. Therefore, we can conclude that there is no genetic drift that causes genetic differentiation of the local sheep's sample and this may be due to the large number of rams used by matting, thereby creating an appropriate variation in flock.

Key words: **Awasi sheep, genetic diversity indications, genetic variation, secreted phosphoprotein**

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INTRODUCTION

The study of genetic variation within and among individuals has enabled researchers to obtain important information about any organism, or group of organisms, which may not be available in the light of traditional methods, as well as to help find and describe levels of genetic variation. And then get basic information on demographics (Eichler et al., 2019.(Genetic variation refers to the change or difference between members of the breed, strain, sample studied or between different species.Study of the relationship of the plasma Insulin-like growth factor-1 IGF-1 to the performance and characteristics of the body of the passive and cardiac pregnancies, there was a significant positive correlation between the concentrations of IGF-1 and both the average daily gains and the daily nutrition of the massacred Awasi carriers in 30. Also in the same field was studied Molecular analysis of FECGH gene in Hamdani sheep breed in sheep breed in Iraqi Kurdistan region Genetic variation refers to the change or difference between members of a clan, a strain, a specimen studied or between different species and much of this observed variation in animal species is due to genetic variation that reflects diversity in clan translated into diversity in DNA genetic tape, Researchers in animal production have tended to study genes that affect the productive qualities of sheep in order to develop strategies for improving passive sheep (Al-Maamory & Al-Anbari, 2023). refers to the relationship of multiple forms of the FASN gene in growth and the performance of wool recipes for local passionate sheep. further studies the impact of leptin and erythropoietin concentration levels on productive qualities on a sample of local passionate sheep (Abd-ALHusain et al., 2023a; Abd-ALHusain et al., 2023b; Eichler et al., 2019) . In the same field was Study the effects of the ND5 gene on the characteristics of growth and milk production in local Awasi sheep (Khalaf et al., 2022). Other genetic variability is the result of the genetic mutation with no effect such as point mutation, which

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resulted in no differences in amino acids due to non-coding but the effect was in the regulatory region of Exon 12 for Jin BMPR-1B showing a significant correlation with increased ovulation, number of births, and pregnancy rate in ewe (Yosef et al., 2013). This mutation can be used as a molecular marker for the selection of ewes with good genetic patterns through measurements taken from ewes and linked to good genotype, so we can use molecular information directed at reproduction against long-term natural selective reproduction to accelerate improvement programs, especially for low genetic features such as low births, increased loss and low fertility (Essa et al., 2023.(The effect on the distribution of the different genotype of Awasi ewe (Individual and twin ewes) had significant differences that allowed us to study and learn the possibility of studying the inheritance of productive qualities, calculating how clan history, clan structure, influential clan size and gene selection work, such as migration and recent expansion, genetic diversity is a prerequisite for animal husbandry, and genetic difference allows for a need for animal husbandry (Askari et al., 2011). The genetic variation in the clan or specimen studied is measured by the number and rates of alleles (Number of alleles and ranges) and the ratio of allelic mixing Heterozygosity (Hepsibha et al., 2013). The number of alleles acts in different positions as a measure of genetic change in sheep that has a direct impact on the differentiation of strains within species (Vajed Ebrahimi et al., 2017). and is one of the data required in the study of genetic variation within each clan, as the greater the number of alleles (Crispim et al., 2014).Heterozygosity is a statistical measure that reflects genetic variation within a particular strain, and includes both Observed heterozygosity Ho and Expected heterozygosity He. The allelic mixing ratio can be used to assess genetic diversity and determine the extent of variation in the strain. The percentage of mixing is an important indicator in genetics for understanding the composition and genetic diversity of strains (Musthafa et al., 2012).Fixation indexes can be used to assess the degree of genetic differentiation among subpopulations, a common measure of genetic stability is the F coefficient developed by Wright in a series of studies (1951, 1965, 1978, 1984), representing the hierarchical metrics of the F:1 coefficient. Fis (pyramid insides). 2. Fst (pyramid exterior stability). 3. Fit (hierarchical aggregate persistence): measures the overall genetic persistence of the strain as a whole, including internal and external differentiation. If Fit's value is near zero, it indicates that there is no significant differentiation in the strain as a whole, and when Fit's value is positive, it indicates an increase in total genetic differentiation.F coefficient is used to assess differentiation (Dashab et al., 2011). SPP1 is a 6.55 kB gene that encodes a protein consisting of 279 amino acids, SPP1 in the first half of the chromosome OAR6 in the sheep (Ovis aries) (36.651-36.658 MB), an area for milk recipes perpendicular to the region on the BTA6 (Bus Taurus 6 chromosome).The most prominent signatures of the selection were found in the proximity of genes associated with body size NCAPG, LCORL, LAP3, SPP1, PLAG1, ALOX12, TP53 and associated with the production of milk ABCG2, SPP1 the relevant filtered genes responsible for the strains (Signer-Hasler et al., 2019).Genes ABCG2, PKD2, LAP3, NCAPG, SPP1, FAM184B genes have been associated with the production of milk and meat in dual purpose cattle and sheep (Mastrangelo et al., 2018; Signer-Hasler et al., 2019; Yurchenko et al., 2019). The importance of the SPP1 gene is due to its role in fibroblast and bone cell activities in promoting bone tissue growth (Prince et al., 1987). because it's a glycoprotein protein with a severely degraded negative charge that lacks a large-scale secondary structure, an intrinsically disturbed protein (Kalmar et al., 2012.(The objective of this study was to estimate of genetic diversity indicators (heterozygosity, Shannon's index, number of influential alleles and gene flow) in flock of local Awassi ewe's.

MATERIALS AND METHODS

This study was carried out at the animal's field of the Barakat Abu Fadl al-Abbas sheep Breeding Station /Kafeel Public Investment

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Company/Al-Abasiyah in Karbala governorate (15 kg south of Karbala city) on a sample of local Awasi sheep consisting of 48 ewes from 1/12/2022 to 1/7/2023 .The Laboratory work was carried out at the Laboratory of Postgraduate Studies of Biotechnology and Molecular Genetic Analysis, University of Baghdad college of Agricultural Engineering Sciences for the purpose of detecting the genetic manifestations of the gene of SPP1 in samples ewes .5ml blood from jugular vein was collected from each ewe. The tubes were transported in a refrigerated container to the laboratory for freezing at a temperature of -4C, until the DNA attract process was initiated.

Estimate of genetic diversity indicators :

Estimate of allelic mixing ratio (H0)

MMR estimated by equation: H0 = Nij/n

H0 = (Nij) Number of individuals Mixture\(n) Total number of individuals according to..... (21).

The average allelic proliferation (na)

Measurements of Evidence of Stability

Fst = 1 (Hs/Ht) (Fst)

Hs: The mixing ratio is part of the breed.

Ht: is the overall mixing ratio.

Fis = 1 (H0/Hs) (Fis)=

H0: Mixing ratio within clan, Hs: Expected ratio.=

Statistical analysis

The indicators of genetic diversity, represented by both F-Statistics, Stability Guide, Expected Average Mixing and Scenes using the statistical programme Popgen (Yeh et al., 1999), and the following law for calculating iteration have been applied to Hardy Weinfoilg (Falconer & Mackay, 1996).

RESULTS AND DISCUSSION

Average allelic abundance (na), number of influential alleles (ne) and Shannon (I) guide for the gene SPP1 . The average abundance (na) adjusted for the SPP1 gene of the studied samples of the local sheep breed was 2.00, and the rate of the number of alleles affected (ne) was 1.0539, while the amended Shanon information index I was intended to mean the likelihood of uniformity to distinguish different individuals (19) is 0.1163, and the average allelic abundance was equal in all the changes studied (2.00), The number of affects was higher in the SNP2 and SNP3 variations. (1.0425) compared with SNP1 variations (1.0211) and SNP4 (1.1096), As for Shannon's evidence, it was higher in SNP4 variability (0.2046) than the rest of the changes SNP1, SNP2 and SNP3 (0.0579, 0.1013 and 0.1013, respectively). This may be attributed to different genetic patterns in changes obtained from molecular analysis of the SPP1 gene (table 1).

Table 1. Average allelic abundance (na), number of influential alleles (ne) and Shannon's Guide (I) to Changes in the SPP1 gene of the sample of local Awassi Sheep

Changes/Jane SPP1	sample volume	I	Ne	Na
SPP1/ SNP1: G>A	96	0.0597	1.0211	2.00
SPP1/ SNP2: C>T	96	0.1013	1.0425	2.00
SPP1/ SNP3: C>T	96	0.1013	1.0425	2.00
SPP1/ SNP4: G>A	96	0.2046	1.1096	2.00
average	96	0.1163	1.0539	2.00

na = Observed number of alleles.
ne = Effective number of alleles .
I = Shannon's Information index.

Heterozygosity

The values of the identical genetic structure of the total scenes Obs - Hom obtained in this study for all variations SNP1, SNP2, SNP3 and SNP4 variations of the local sheep sample's SPP1 gene are 0.9792, 0.9583, 0.9583 and 0.8958, respectively, at a rate of 0.9479, It is very close and sometimes identical to Exp-Hom's aggregate population genetic synthesis values of 0.9792, 0.9588, 0.9588 and 0.9002 at 0.9492. (Table 2), this may be due to a homogeneity in the composition of the studied clan being a herd of one breed and a limited sample size,It is also evident from the study that the values of the total viewer allelic mixing ratio Obs - Het in all changes obtained in the first primer SPP1 gene are at a total rate of 0.0521, which is an approach to the total projected allelic mixing rate Exp- Het of 0.0508, which shows a

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homogeneity (low variability) in the genetic pattern among the animals under consideration, and Nei values that mean the total projected allelic mixing value calculated according to Nei are 0.0502, the same as the average Ave-Het (0.0502).

Table 2. Synthesis Ratio, Genetic Composition, Nei Values and Average SPP1 Gene Mixing in local Awasi sheep

Changes/Jane SPP1	sample volume	Ave_Het	**Nei	*Exp_Het	Obs_Het	*Exp_Hom	Obs_Hom
SPP1/ SNP1: G>A	96	0.0206	0.0206	0.0208	0.0208	0.9792	0.9792
SPP1/ SNP2: C>T	96	0.0408	0.0408	0.0412	0.0417	0.9588	0.9583
SPP1/ SNP3: C>T	96	0.0408	0.0408	0.0412	0.0417	0.9588	0.9583
SPP1/ SNP4: G>A	96	0.0987	0.0987	0.0998	0.1042	0.9002	0.8958
average	96	0.0502	0.0502	0.0508	0.0521	0.9492	0.9479

Summary of F statistics and gene flow

To examine the genetic differentiation between Polymorphism sites of variations in the gene SPP1 F statistics were calculated and during the study it was shown that the summary of the individual's persistence guide for all changes within the Fis clan of G, A, C and T alleles of the local passive sheep sample is less than 0 -0.0367, This indicates that there is no severe or influential inbreed and upbringing of this group, and this is confirmed by the presence of the negative signal, and the summary of the Total Stability Guide Fit for all changes obtained according to the analysis of the SPP1 gene also (-0.0367) (Table 3), which confirms that there is no increase in the forgetfulness of the mixing, that the Fst genetic differentiation constant index is zero, and the Nm gene flow summary that refers to the movement of alleles from one herd or clan to another is also known as gene migration or compositions of all changes within the herd according to the gene SPP1 it was zero ,These values provide a picture of the absence of genetic or genetic drift, which causes genetic differentiation of the local passive sheep sample and may be due to the large number of rams being vaccinated in the herd under study.The most important causes and factors leading to this result are the decrease in the size of the effective herd. Basically, there are no large herds of aromatic sheep in Iraq, but rather limited genetic totals distributed in limited geographical areas as well. (so-called geographical isolation), so it has limited ability to migrate and decrease the size of an effective herd, and different geographical areas have no obstacle to the herd's genetic composition (Moradi et al., 2012), and genetic drift and delinquency and natural selection are key factors resulting in the emergence of genetic differentiation between herds (Qing et al., 2009).

Table 3. Summary of F and flow statistics of changes in the SPP1 gene of local Awasi sheep

gene SPP1	sample volume	Nm*	Fst	Fit	Fis
SPP1/ SNP1: G>A	96	****	0.00	-0.0105	-0.0105
SPP1/ SNP2: C>T	96	****	0.00	-0.0213	-0.0213
SPP1/ SNP3: C>T	96	****	0.00	-0.0213	-0.0213
SPP1/ SNP4: G>A	96	****	0.00	-0.0549	-0.0549
average	96	1000	0.00	-0.0367	-0.0367

* Nm = Gene flow estimated from Fst = 0.25(1 – Fst)/Fst.

CONCLUSION

The summary of the Perseverance Guide for all changes within the Fis clan for GIA, A, C and T for the local ewes sample is less than zero (-0.0367), and the summary of the total stability manual Fit for all changes obtained according to the analysis of the SPP1 gene as well (-0.0367). We can conclude that there is no genetic drift that causes genetic differentiation of the local ewe's sample and this may be due to the large number of rams that are vaccinated in the herd under study or the ewes may have come from different sources.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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مؤشرات التنوع الوراثي لجين SPP1 في النعاج العواسي المحلية في وسط العراق

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المستخلص

هدفت هذه الدراسة الى تحديد مؤشرات التنوع الوراثي لجين Secreted phosphoprotein 1 (SPP1) في قطيع من النعاج العواسي المحلية في وسط العراق . تكوين العينة من48 نعجة .وكانت مدة الدراسة ستة اشهر. تم تحديد المظاهر الوراثية والحصول على أربعة طفرات SNP(4). بلغ متوسط الغزارة الأليلية (Na) 2.00، ومعدل عدد الأليلات المؤثرة (Ne) 1.0539، دليل شانون(I) 0.1163 . ان متوسط الغزارة الاليلية كان متساوياً في جميع التغايرات المدروسة (2.00) وان عدد الاليلات المؤثرة كان أعلى في التغايرين SNP2 و SNP3إذ بلغ في كليهما (1.0425) مقارنةً مع التغاير SNP1 (1.0211) و SNP4 (1.1096). إنَ قيم التركيب الوراثي المتماثل المشاهد الكلي Obs–Hom وبمعدل(0.9479)وهي مقاربة جدا لتركيب الوراثي المتماثل متوقع الكلي Exp– Hom وبمعدل (0.9492).انَ دليل ثبات الفرد داخل العشيرة(Fis) للأليلات G و A و C و T لعينة الاغنام العواسي كان (−0.0367).كما ان دليل الثبات الكلي Fit المتحصل عليها وفق تحليل جين SPP1 ايضا (−0.0367).لذلك يمكن ان نستنتج عدم وجود انجراف وراثي والذي يسبب تمايز وراثي لعينة الاغنام العواسي المحلية وقد يرجع ذلك الى كثرة عدد الكباش التي تستخدم بالتلقيح مما يحقق وجود تباين مناسب في القطيع.

* الكلمات المفتاحية: جين SPP1, مؤشرات التنوع الجيني, أغنام العواسي, التباين الجيني

*البحث مستل من اطروحة الدكتوراه للباحث الاول.