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Almaamory & Al-Anbari

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ASSOCIATION OF ANXA9 GENE POLYMORPHISM WITH SOME ECONOMIC TRAITS OF AWASSI SHEEP

Yasseen A. Almaamory[1], Al-Anbari, N .N[2].
[1]Assist Lecturer, Department of Animal Production, College of Agriculture Engineering Sciences, University of Baghdad, Iraq. yasseen.a@coagri.uobaghdad.edu.iq
[2]Professor, Department of Animal Production, College of Agriculture Engineering Sciences, University of Baghdad, Iraq. nassr.n@coagri.uobaghdad.edu.iq

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ABSTRACT

The study was conducted in the sheep farm of the Al-Fayhaa station in the Jableh sub-district/ Al-Musaib project (55 km south of Baghdad), as well as the Biotechnology Laboratory in the College of Agricultural Engineering Sciences/ University of Baghdad for the period from 5/1/2022 to 30/10/2022. With the aim of detection the ANXA9 gene polymorphism and its relationship with body weight, weight gain of lambs, Litter size and wool traits ,as well as the polymorphism distribution and allele frequency in 52 Awassi sheep and its lambs, three polymorphism appeared in this variant (T>G SNP) which are TT, TG and GG, and their percentage were 51.92, 40.38 and 7.69%, and differences between them were highly significant (P≤0.01), with a frequency of 0.72 and 0.28 for the T and G alleles, respectively. there was a significant discrepancy (P≤0.05) in the weight at weaning and at the age of 6 months for the GG genotype, as the rates for lambs with ewes carrying the GG genotype in this study were 19.25 and 26.37 kg, respectively. The results showed that was significant (P≤0.05) and superiority for mothers with the TG heterozygous compared to those with the GG in Litter size , at a rate of 1.19 and 1.00 births / litter, respectively. It turned out that there was a significant variation (P≤0.05) in the fiber diameter of wool according to the ANXA9 gene polymorphism in Awassi ewes. We can conclude by studying the ANXA9 gene polymorphism that they can be adopted in developing strategies for genetic improvement of sheep, and the application of the study to a larger sample and to several sites and extracting the interaction between two SNPs would give more accurate results and determine the best method for managing and improving sheep flocks.

Keywords: Awassi sheep, ANXA9 gene, body weight of lambs, Litter size , wool traits.

علاقة تعدد المظاهر الوراثية لجين ANXA9 ببعض الصفات الاقتصادية للأغنام العواسي

ياسين عبد السلام حسين[1]، نصر نوري الأنباري[2]
[1] مدرس مساعد ، قسم الإنتاج الحيواني، كلية علوم الهندسة الزراعية ، جامعة بغداد، بغداد، العراق. yasseen.a@coagri.uobaghdad.edu.iq
[2] استاذ ، قسم الإنتاج الحيواني، كلية علوم الهندسة الزراعية ، جامعة بغداد، بغداد، العراق. nassr.n@coagri.uobaghdad.edu.iq

الخلاصة:

أجريت الدراسة في الفترة من 2022/1/5 إلى 2022/10/30 في مزرعة الأغنام التابعة لمحطة الفيحاء بناحية جبلة/ مشروع المسيب (55 كم جنوب بغداد) بالإضافة إلى مختبر التكنولوجيا الحيوية في كلية علوم الهندسة الزراعية/ جامعة بغداد. بهدف الكشف عن تعدد الأشكال لجين ANXA9 وعلاقته بوزن الجسم، والزيادة الوزنية الحملان، وحجم البطن الواحدة وصفات الصوف، فضلاً عن نسب توزيع المظاهر الوراثية والتكرار الاليلي للجين في 52 من النعاج العواسي وحملانها، إذ ظهرت ثلاثة مظاهر وراثية في التغاير (T>G SNP) وهي TT و TG و GG ونسبتها المئوية كانت 51.92 و 40.38 و 7.69٪، وكانت الفروق بينها عالية المعنوية (P≤0.01)، وبتكرار 0.72 و 0.28 لكل من الاليلين T و G على التوالي، لوحظ وجود فرق معنوي (P≤0.05) في الوزن عند الفطام وعند عمر 6 أشهر بالنسبة للتركيب GG، حيث كانت معدلات الحملان لتابعة للنعاج الحاملة للتركيب الوراثي GG في هذه الدراسة 19.25 و 26.37 كجم على التوالي. كما أظهرت النتائج اختلافاً معنويا (P≤0.05) وبتفوق للأمهات ذات التركيب الهجين TG موازنة

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بمثيلاتها ذات التركيب الطافر GG في حجم البطن وبمعدل 1.19 و 1.00 مولود/ بطن على التوالي، اتضح أن هناك تباينًا معنويًا (P≤0.05)في قطر ألياف الصوف وفقًا لتعدد المظاهر الوراثية لجين ANXA9 في النعاج العواسي، يمكننا أن نستنتج من خلال دراسة تعدد الممظاهر الوراثية لجين ANXA9 أنه يمكن اعتمادها في تطوير استراتيجيات للتحسين الوراثي للأغنام، وتطبيق الدراسة على عينة أكبر وعلى عدة مواقع وكذلك استخراج التداخل بين التغايرين سوف يعطي نتائج أكثر دقة ويحدد أفضل طريقة لإدارة وتحسين قطعان الأغنام.

الكلمات المفتاحية: الاغنام العواسي، جين ***ANXA9***، وزن جسم الحملان، حجم البطن، صفات الصوف.

INTRODUCTION

The scarcity of pedigree and management becomes an important, which is required for genetic evaluation and reliable selection decisions, is a major barrier to improving the genetic traits of small farmers' flocks in developing livestock systems **(AL-Zaiadi & AL-Shekdhaher, 2016; Gizaw *et al.*, 2022; Salam *et al.*, 2022).** Genetic selection has evolved into an important approach in genetic improvement**(Al-Anbari *et al.*, 2006; Al-Anbari & Al-Samarai, 2007; Nasir *et al.*, 2018; Al-Sarai & Al-Anabri, 2019; Naeemah & Al-Anbari, 2023).** Annex ins have been suggested as membrane-membrane or membrane-cytoskeleton linkers, and have been linked to Ca^{2+} regulated exocytosis, and plasma membrane domains, Furthermore, annex ins are assumed to function as Ca^{2+} channels, but how calcium solubility is accomplished remains unknown **(Golczak *et al.*, 2001)**. Other possible activities, however, have been proposed in light of the fact that some of these proteins may migrate to the nucleus or be released into the extracellular space, functioning as essential regulators of numerous physiological processes such as coagulation or inflammatory **(Lizarbe *et al.*, 2013) .** The cellular process by which various annex ins are secreted is still debated. There is no hydrophobic signal peptide in annex ins, and it has been proposed that annex in A1 is secreted via ATP-binding-cassette (ABC) carriers **(Wein *et al.*, 2004)**. Various components of the annex in group have been shown to interact with the cytoskeleton, most notably actin. The first annex in to be demonstrated to bind to actin filament in a Ca^{2+} dependent way was Annex in A2. Furthermore, this annex in has the capacity to bundle F-actin filaments and is involved in their reaction**(Morel *et al.*, 2009)**. Annex in A5 in the plasma, on the contrary side, may be caused by endothelium or blastocyst injury **(Wang *et al.*, 2001)**. In this section, we will quickly review many tasks that are closely connected to annex ins' capacity to interact indirectly or directly with phospholipid membranes within the cell or in the extracellular environment**(Lizarbe *et al.*, 2013)**. The aim of this study to determination of the ANXA9 gene polymorphism and its relationship with body weight, weight gain of lambs, Litter size and wool traits.

MATERIALS AND METHODS

In the current study, 52 female sheep (ewes) and their lambs are used. During the period 5-1-2022 to 25-6-2022, samples were collected in a sheep farm/ Al-Fayha station in Jableh sub-district/ Al-Musaib project (55 km south of Baghdad city). The goal of this study was to separate the genetic information and determine the genotype of ANXA9 gene polymorphism and its relationship to body weight, weight gain of lambs, Litter size and wool traits., as well as to study the percentages of the distribution of their constructions in the herd and the intensity of alleles obtained. Five milliliters of blood were drawn from the jugular vein **(Hassan & Almaamory, 2019; Abass *et al.*, 2020)** for each sheep in a collection tube supplemented with a Jordanian company's (AFCO) K_2 EDTA anticoagulant, and transmitted in a cool box to the lab (the Biotechnology Laboratory in the College of Agricultural Engineering Sciences/ University of Baghdad) for cryopreservation at -4 °C, then DNA extraction from blood using the kit of

Extraction of DNA according to the instructions in the attached leaflet supplied by Geneaid Company. The genomic DNA's integrity was assessed using electrophoresis on agarose gels. The ANXA9 gene polymerase chain reaction (PCR) technique is focused on the major polymorphism observed in Intron 4 of the ANXA9 gene. The 675 bp segment Gene Bank accession number AY785286.1**(Pecka- Kiełb *et al.*, 2021)** observed in sheep genomic DNA was amplified using the matching primer pairs and the Annealing temperature 55.5°C at 30 cycle for 30 sec, according to the size of the fragments and kind of primers employed (forward and reverse). The details of the primer sequences are as follows:

F:5' CATTCCTGTGTGTCCGGTAC 3'

R: 5' TCATCTCAGACCTAACCACCA 3'

After the end of the polymerase reaction, the ANXA9 gene polymorphism was detected in sheep blood samples using a sequencing technique using the NCBI Blast software (by Nappo Corporation) and data program. In the genius program, the genotypes of ANXA9 were discovered by comparing the different sequences in nitrogen bases for the studied sheep to the wild sequence of the gene. The ewes are sheared during May of each year using an electric shearing machine. As for the wool samples that were carried out by laboratory (Al-Nabaa Laboratory) measurements, they were taken with an area of 10 * 10 cm from the upper left thigh (hip bone) for all experimental animals. The diameter of the fiber was measured using an optical microscope and an ocular micrometer, after cutting the fiber with a length of no more than 0.8 mm from the lower end of the wool tuft. the length of the tuft in the wool sample was measured using the usual ruler and without stretching or pulling the tuft from the base of the tuft until the density of the fibers decreases and from the same sample. Growth efficiency of lambs was measured according to **(Kesbi & Tari, 2015)** the data was analyzed by used Statistical analysis system **(SAS., 2018)** program was used in the analysis of data to study the effect of the ANXA9 gene polymorphism on the studied traits by applying the general linear model (GLM), according to the below mentioned equation. The significant differences were compared between averages by **Duncan (1955)** multiple range test with the application of least square means.

Statistical model: (Traits on ewes).

$Y_{ijk} = \mu + G_i + A_j + e_{ijk}$

Y_{ijk} = The observed value , μ = The Overall mean of trait ,G_i = The effect of gene polymorphism , A_j = Adjusted to effect of ewe age , e_{ijk}= The random error.

Statistical model: (Traits on lambs).

$Y_{ijklm} = \mu + G_i + A_j + S_k + T_l + e_{ijklm}$

Y_{ijklm} = The observed value , μ = The Overall mean of trait, G_i = The effect of gene polymorphism of dam , A_j = Adjusted to effect of Age of dam at birth , S_k = The effect of Sex , T_l = The effect of type of birth , e_{ijklm}= The random error.

Chi-square (χ^2) test was used to significantly compare between the percentage (0.05 and 0.01 probability) in this study. Calculator of allele frequency of ANXA9 gene according of Hardy Weinberg's equilibrium **(Falconer & C.Mackay, 1996)**.

RESULTS AND DISCUSSION

The nitrogenous bases sequences of the ANXA9 gene

The number of polymorphism, their percentages, and the allelic frequency of the ANXA9 gene were shown in (Table, 1), three polymorphism appeared, and the percentage of TT, TG, and GG polymorphism were 51.92, 40.38 and 7.69 %, respectively, while the allelic frequency of the T and G alleles was 0.72 and 0.28, respectively.

Table (1): Distribution and allele frequency of ANXA9 gene /T>G SNP Polymorphism in sample study of Awassi sheep.

ANXA9 gene /T>G SNP Polymorphism	Number	Percentage (%)
TT	27	51.92
TG	21	40.38
GG	4	7.69
Total	52	100%
Chi-Square (χ^2)	---	22.269 **
Allele	Frequency	
T	0.72	---
G	0.28	---
** (P≤0.01).		

Relationship of ANXA9 gene with body weight of lambs of SNP (T>G) Polymorphism

The results showed that there were significant differences (P≤0.05) in Weaning weight and Body weight at 6 months according to the different polymorphism, as it was noted that a significant superiority for both the GG and TG polymorphism (19.25 ±0.62 and 18.83 ±0.41 Kg) for Weaning weight, respectively, and (26.37 ±0.48, 25.78 ±0.52 kg) for weight at 6 months, respectively compared to the TT polymorphism (18.37 ±0.33 kg) for Weaning weight and (24.89 ±0.36 kg) for weight at 6 months. While no significant differences were observed between the different polymorphism of birth weight (Table, 2).

Table (2): Relationship of ANXA9 gene /T>G SNP Polymorphism with body weight of lambs**.**

ANXA9 gene /T>G SNP Polymorphism	Mean ± SE (kg)		
	Birth weight	Weaning weight	Body weight/ 6 month
TT	4.17 ±0.06	18.37 ±0.33 b	24.89 ±0.36 b
TG	4.26 ±0.12	18.83 ±0.41 ab	25.78 ±0.52 ab
GG	4.25 ±0.10	19.25 ±0.62 a	26.37 ±0.48 a
Level of Sig.	NS	*	*
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.			

Grewal *et al.* (2019) reported that several studies indicate that A powerful pro-resolving mediator that influences the control of body weight and metabolic health is annex in A1 (AnxA1) and AnxA2 participates in coordinating glucose transporter type 4 (GLUT4) translocation and interacts with the fatty acid transporter CD36, both of which are important for glucose metabolism and fatty acid absorption in adipose tissue, while AnxA6 has been connected to the regulation of adipocyte lipolysis and adiponectin release, on the other hand , many additional annex ins are also expressed in fat tissues, although little is known about their

functions in adipocytes. **Gerke *et al.* (2005)** explained that the initial interactions between membrane-associated proteins, annex ins bind negative charges phospholipids and cholesterol in a Calcium ions and oxidative way. This results in dynamic modifications in the functional and structural organization of membrane domains.

Relationship of ANXA9 gene with weight gain of lambs of SNP (T>G) Polymorphism

The results showed that no significant differences (P≤0.05) observed between the different polymorphism of gain: birth to weaning, gain: birth to 6 months, gain: weaning to 6 months, and growth efficiency birth and weaning (Table, 3).

Table (3): Relationship of ANXA9 gene /T>G SNP Polymorphism and weight gain**.**

ANXA9 gene /T>G SNP Polymorphism	Mean ± SE			
	Gain: birth to weaning (gm)	Gain: birth to 6 month (gm)	Gain: weaning to 6 month (gm)	Gain efficiency birth and weaning
TT	14.20 ±0.19	20.72 ±0.27	6.52 ±0.18	0.772 ±0.002
TG	14.57 ±0.24	21.52 ±0.38	6.95 ±0.15	0.773 ±0.002
GG	15.00 ±0.20	22.12 ±0.25	7.12 ±0.20	0.779 ±0.005
Level of Sig.	NS	NS	NS	NS
NS: Non-Significant.				

Relationship of ANXA9 gene with Litter size of SNP (T>G) Polymorphism

The results explained that there were significant differences (P≤0.05) in Litter size for TT, TG, and GG Polymorphism (1.07 ±0.05, 1.19 ±0.08, and 1.00 ± 0.00), respectively (Table 4)

Table (4): Relationship of ANXA9 gene /T>G SNP Polymorphism and Litter size**.**

ANXA9 gene /T>G SNP Polymorphism	No of ewes	No of lambs	Mean ± SE
TT	27	29	1.07 ±0.05 ab
TG	21	25	1.19 ±0.08 a
GG	4	4	1.00 ±0.00 b
Level of Sig.	---	---	*
Means having with the different letters in same column differed significantly. * (P≤0.05).			

Drouilhet *et al.* (2009) Explained that, in the complicated process of reproduction, numerous small genes as well as certain big genes known as fecundity (Fec) genes have an impact on features including ovulation rate and litter size. **Abdoli *et al.* (2016)** Reported that high variance in ovulation rate and litter size mixed with strong repetition are traits of a key gene influencing prolificacy in a population. With the use of genomic mapping and scanning, significant genes in highly productive sheep may be found.

Relationship of ANXA9 gene with wool traits of lambs of SNP (T>G) Polymorphism

The results of the statistical analysis explained that there were significant differences (P≤0.05) in wool fiber diameter according to the different polymorphism, as it was noted that there was a significant superiority of the TT polymorphism (30.70 ±0.07 **µm**) over the GG polymorphism (30.25 ±0.10 **µm**), while it did not differ significantly from TG polymorphism (30.65 ±0.09 **µm**) Through the results of the statistical analysis, no significant differences were observed between the different Polymorphism of weight of raw wool, weight of clean wool, and length of the wool tuft (Table, 5).

Snyman *et al.* (1995) ascribed their high heredity to the high variation in the coefficient for clean fleece weight in dual-purpose breeds, which is also reflected in the wide range in clean fleece weight because of the absence of selection in the Afrino flock. Mean heritability's for fiber length (0.46±0.04), yield (0.56±0.03), and the variation coefficient of fiber diameter (0.52±0.04) in wool breeds were all extremely high and comparable to those in dual-purpose breeds. In both wool and dual-purpose breeds, the mean heritability's for fiber diameter were marginally greater than those noted by **Fogarty (1996)** (0.51±0.03 and 0.52±0.03, respectively).

Table (5): Relationship of ANXA9 gene /T>G SNP Polymorphism and wool traits**.**

ANXA9 gene /T>G SNP Polymorphism	Mean ± SE			
	Weight of raw wool (gm)	Weight of clean wool (gm)	Length of the wool tuft (cm)	Wool fiber diameter (micron)
TT	1964.81 ±20.46	1842.59 ±21.95	11.72 ±0.10	30.70 ±0.07 a
TG	1961.90 ±29.66	1845.24 ±28.84	11.19 ±0.17	30.65 ±0.09 a
GG	1975.01 ±25.02	1850.13 ±20.41	11.75 ±0.32	30.25 ±0.10 b
Level of Sig.	NS	NS	NS	*
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.				

CONCLUSION

In light of the aforementioned results included in the current study on the relationship of target coding regions in the ANXA9 gene, the target coding region in the ANXA9 gene (T>G SNP) has a role in causing a significant variation in the body height, weight at weaning and at the age of 6 months for lambs, the prolificacy rate, as well as the fiber diameter of the wool of Awassi ewes.

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