



Using the pop-gene program to study two genetic sites of the growth hormone gene in Iraqi local sheep

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Abstract

The study was conducted on three breeds of local Iraqi ewes sheep (20 Karadi, 20 Hamdani, and 20 Awassi). An analysis of the data from the study of two genetic sites for the growth hormone gene was carried out based on the PopGene program to determine some criteria for the genetic diversity of these breeds. A calculation was made for each of the observed and expected allelic heterozygotes, the Observed number of alleles, adequate number of alleles (n_e), Shannon's Information index (I) for each of the three breeds, and the inbreeding coefficient for the three breeds together depending on the values of (Fis, Fit, Fst). It was found that the expected heterozygote allelic for the Karadi (0.26, 0.18), Hamdani (0.50, 0.09), and Awassi (0.26, 0.09) for the two genetic loci, respectively. It was found that the Observed number of alleles (n_a) for both sites was (2.00) for all three breeds, while the adequate number of alleles (n_e) for the Karadi (1.34, 1.21), Hamdani (1.98, 1.90) and Awassi (1.34, 1.10) for the two genetic sites, respectively. Its height in the first than the second site. The value of Shannon's index (I) for the three breeds was higher in the first site than in the second site for all breeds Karadi (0.42, 0.32), Hamdani (0.68, 0.19), and Awassi (0.42, 0.19). The results show that the rate of homozygous alleles observed and expected was higher than the observed and expected allelic heterozygote for the three breeds.

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Introduction

Sheep are among the most widespread agricultural animals in Iraq in terms of breeding and possession because they are highly desirable to the Iraqi consumer, as their meat is considered one of their main dishes (1); sheep are divided into several breeds, as well as sub-genetic lines within the same breed these breeds are spread in different parts of Iraq according to their geographical distribution and their presence may overlap in their regions as a result of the transfer of herds for breeding (2). The number of sheep in Iraq reached 6723866 heads, and their production of meat is 46483 tons (3); the goal of raising these animals varies according to their production, which is either to benefit from their meat, milk, or wool (4). The breed of Awassi sheep is the most widespread in Iraq and is found in various regions of Iraq; as for the Karadi and Hamdani breeds, their presence

is in the northern parts of Iraq, and the Arabiya and Naimiya sheep breeds are found in the central and southern regions of Iraq (5). Several studies were conducted on the relationship of genes with some traits in farm animals in Iraq, which examined the relationship between the genotypes of these genes and the studied traits (6-10). The growth hormone gene is one of the primary genes affecting several characteristics in the animal's body, as it controls the action and secretion of growth hormone, which is responsible for several vital activities, particularly growth traits (6,7,11). The growth hormone gene is located on chromosome 19 (12). It consists of 4 introns and five exons (13). It is a polypeptide hormone consisting of 190 -191 amino acids, with a molecular weight of 22 kDa (14). Thus, a growth hormone gene is essential for animal growth (15), as well as affecting milk production characteristics and components (16-18) and wool production (19). Genetic diversity is an

essential source of knowledge of individual differences between animals within a breed or between breeds within a single species, as it represents the sum of actual or potential genetic information, the differences involved in the genetic material, and the ability of genetic differences to differentiate in production (20). There are many ways to study and measure the genetic diversity of individuals. The analysis of genotype data using the Pop Gen program is one of the methods that provide many measures that can explain individual differences within the same breed and between different breeds within the same species, which are determined by the genotype of these individuals (21). The Pop Gene program provides several results by which genetic variation can be studied, which include the observed and expected allelic heterozygote, the observed number of alleles (na), the adequate number of alleles (ne), Shannon's information index (I), the inbreeding coefficient for the three breeds together depending on the values of (Fis, Fit, Fst), and the gene flow (22).

Therefore, this study found several of these determinants that can be measured in the Iraqi sheep breeds (Kradi, Hamdani, and Awassi) to determine the extent of genetic diversity in these breeds based on two genetic sites for the growth hormone gene.

Materials and methods

Ethical approve

The ethical approval was under the Collage of Science, Al-Karkh University No:89 in 23/1/2023.

Sample collection and preparation

The experiment was conducted on three breeds of Iraqi local sheep (20 Karadi, 20 Hamdani, and 20 Awassi) raised in the Department of Animal Production, College of Agriculture, University of Diyala. The ages of the animals ranged between 2-5 years, and all of them were females. 5 ml of blood was drawn through the jugular vein of each

animal, and the blood samples were refrigerated until they were transported to the laboratory specialized in molecular genetic tests. A multiplication process was performed for the target pieces after extracting the DNA using the required primers (23), and all the required tests were conducted to obtain the number and type of alleles for each breed and each genetic site (24).

Statistical analysis

Statistical analysis of the number and type of alleles obtained using the Pop Gene program to get the observed and expected allelic heterozygotes. The observed number of alleles (na), the adequate number of alleles (ne), Shannon's information index (I), the inbreeding coefficient for the three breeds together depending on the values of Fis, Fit, Fst, Gene flow, and the Genetic distance, according to the following model: Test Data Set II: Diploid Data of 3 populations, each with 20 records (genotypes) and 2 loci. Where number of populations = 3, number of loci = 2, locus name: GrH-1 GrH-2 (25).

Results

Table 1 shows the values of the observed and expected alleles, the total expected heterozygote allelic 25, and the average of heterozygote alleles for the three breeds' first and second genetic loci. The value of heterozygote alleles was lower than the value of homozygote alleles for the first and second genetic loci of the three breeds, the expected values of heterozygote alleles for the Karadi 0.26, 0.18, Hamdani 0.5, 0.09 and Awasi 0.25, 0.09 for the first and second sites, respectively, while the expected homozygote allele values for Karadi 0.73, 0.81, Hamdani 0.49, 0.90 and Awassi 0.73, 0.9 for the first and second site, respectively. The total expected heterozygote allelic values were close to the predicted heterozygote allele values as it was Karadi 0.18, 0.25, Hamdani 0.09, 0.49, and Awasi 0.25, 0.09.

Table 1: Observed homozygote, observed heterozygote, expected homozygote, expected heterozygote

		Obs._Hom.	Obs._Het.	Exp._Hom.	Exp._Het.*	Nei **	Ave._Het.
Karadi	<i>GRHP1</i>	0.7000	0.3000	0.7385	0.2615	0.2550	0.3350
	<i>GRHP2</i>	0.8000	0.2000	0.8154	0.1846	0.1800	0.1233
	<i>Mean</i>	0.7500	0.2500	0.7769	0.2231	0.2175	0.2292
Hamdani	<i>GRHP1</i>	0.9000	0.1000	0.4923	0.5077	0.4950	0.3350
	<i>GRHP2</i>	0.9000	0.1000	0.9026	0.0974	0.0950	0.1233
	<i>Mean</i>	0.9000	0.1000	0.6974	0.3026	0.2950	0.2292
Awassei	<i>GRHP1</i>	0.7000	0.3000	0.7385	0.2615	0.255	0.3350
	<i>GRHP2</i>	0.9000	0.1000	0.9026	0.0974	0.095	0.1233
	<i>Mean</i>	0.8000	0.2000	0.8205	0.1795	0.1750	0.2292

Obs._Hom.: observed Homozygote, Obs._Het.: observed heterozygote, Exp._Hom.: expected Homozygote, Exp._Het. *: expected heterozygote, Nei **: the total expected heterozygote allelic (Nei's, 1973), Ave._Het.: the average heterozygote. GRHP1: First site of the growth hormone gene, GRHP2: Second site of the growth hormone gene.

Table 2 found that the Observed number of alleles (na) for the three breeds was two. The value of the Effective number of alleles (ne) for the first and second genetic loci to the three breeds Karadi 1.34, 1.21, Hamdani 1.98, 1.10, and Awassi 1.34, 1.10, The value of the first site for all three breeds were superior to the second site. The values of the Shannon's Information index (I) for the first and second genetic loci of the three breeds showed the superiority of the first site over the second site (Table 2), as the values of the Karadi 0.42, 0.32, Hamdani 0.68, 0.19 and Awasi 0.42, 0.19, the highest value of the Shannon's Information index (I) for the first site it belonged to the Hamdani, in the second site the Karadi breed is the higher.

Table 2: The Observed number of alleles (na), the Effective number of alleles (ne), and the Shannon's Information index (I)

		na *	ne*	I *
Karadi	GRHP1	2.0000	1.3423	0.4227
	GRHP2	2.0000	1.2195	0.3251
	Mean	2.0000	1.2809	0.3739
Hamdani	GRHP1	2.0000	1.9802	0.6881
	GRHP2	2.0000	1.1050	0.1985
	Mean	2.0000	1.5426	0.4433
Awassei	GRHP1	2.0000	1.3423	0.4227
	GRHP2	2.0000	1.1050	0.1985
	Mean	2.0000	1.2236	0.3106

GRHP1: First site of the growth hormone gene, GRHP2: Second site of the growth hormone gene.

Table 3 represents the data of the three flocks for the inbreeding (F-Statistics) and gene flow values. The values of inbreeding were estimated based on Fis (estimation of inbreeding within each herd), Fit (estimation of total inbreeding for the herd), and Fst (measurement of differentiation (difference) of the herd); it was observed that the Fis values were higher for the first site than for the second site, while the Fit, Fst values were higher in the second site than the first. The value of gene flow for the first site was superior to the second site (Table 3). Figure 1 shows the genetic distance between these three breeds. It was found that the Karadi and Hamdani breeds have the highest level of gene affinity between them, which supports that the origin of the Hamdani breed is the Karadi breed.

Table 3: Shows sheep groups' inbreeding and gene flow values

	FIS	FIT	FST	NM*
GRHP1	0.3035	0.3778	0.1067	2.0938
GRHP2	-0.0811	0.7129	0.7344	0.0904
Mean	0.2000	0.5632	0.4540	0.3007

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



Figure 1: The UPGMA tree shows the affinity between sheep breeds. Kradi=1, Hammdani=2, Awassie=3.

Discussion

Al-Akilli (26), In his study of Saudi Najd and Hari sheep, found That the values of the observed heterozygote alleles were higher than the expected heterozygote alleles as they were for Najd sheep 0.46, 0.42 and the Hari 0.26, 0.20, that these results indicated the absence of genetic diversity and the appearance of a specific genetic structure and the reason for this was mentioned due to the breeding method (mating) used by the breeders of these herds in Saudi Arabia. Ayied and Hana (27) said that the values of expected heterozygote alleles were higher than the values of heterozygote alleles observed in their study of Arabi sheep in southern Iraq as the expected values of heterozygote alleles were 45.02, while the observed values of heterozygote alleles were 28.57, stated that the reason for this is due to the mating method that depends on mating from Outside the herd (external breeding) which led to an increase in heterogeneous genotypes within these herds.

The results in Table 2 are similar to those David *et al.* (28) when they studied the genetic diversity in one of the Brazilian sheep breeds using microsatellites. They found that the values of the Effective number of alleles (ne) were low, which indicated a decrease in the heterozygous alleles in the experimental animals, while the condoled for this was attributed to the decrease in the number of experimental animals and the type of breeding (inbreeding). While Ayied and Hana (29) indicated when they studied the genetic diversity of three breeds of local Egyptian sheep using microsatellites, an increase in the Effective number of alleles (ne) in one of the microsatellites reached 14.4 for the Ossimi breed. The researcher stated that these high values indicate genetic diversity within Experimental animal subjects.

The values obtained in current study indicated that the low number of the Effective number of alleles (ne) indicates that the number of different genotypes within a single genetic site is a few, which is confirmed by the low Shannon's Information index (I) values that indicate too low genetic diversity within experimental animals. Shannon's Information Index (I), which represents the genetic morphology information of experimental animals, showed a decrease for both sites and the three breeds. These results indicate a decrease in the genetic diversity (27).

when they studied Arabian sheep in southern Iraq to find genetic diversity based on the lactoferrin gene, where they

obtained low Shannon Information Index (I) values of 0.64 that led to a decrease in heterozygote alleles in the herd. Likewise, Hussain *et al.* (30), when they studied two breeds of Pakistani sheep to determine genetic diversity using microsatellites, found a decrease in Shannon Information Index (I) values. This is due to the breeding methods and the type of mating (inbreeding) used in the flocks, which led to a decrease in heterozygous alleles. In the study conducted by Musthafa *et al.* (31) of Najdi sheep in Saudi Arabia, using several microsatellites to test the genetic diversity of this breed, a number of them showed high values of Shannon's Information index (I) 2.28, 2.23, 2.22, with a high Observed number of alleles (na) and higher values of observed and expected allelic heterozygote for each marker compared to the microsatellites with Low values of the Shannon's Information index (I) that indicate a decrease in the Observed number of alleles (na).

The results mentioned by Ayied and Hana (29). They studied three breeds of Egyptian sheep using microsatellites. They found that if the values of Fit and Fst are low and close to zero, it indicates a decrease in genetic diversity among the herd. In the study of Maitra *et al.* (32) of six genetic sites within three genes in seven breeds of Indian goats, they noticed a decrease in the values of Fis, Fit, and Fst, as the values were low and close to zero, which was reflected in a reduction in the level of genetic differentiation between these seven different breeds. Gene flow values measure the number of migratory genes entering the population that lead to increased genetic diversity (33-35). The value of gene flow study in Dashaba (36) reported that they obtained a high positive value when they studied the genetic diversity of the Iranian Baluchi sheep breed using microsatellites as the average value of the gene flow for some microsatellites was 10.14 and considered it as a guide to the internal breeding that occurs within the herd.

Figure 1 shows that the origin of the Hamdani breed is the Karadi breed, as the Hamdani breed is considered a sub-breed of the Karadi breed (37). These two strains are found in northern Iraq and southern regions of Turkey (38,39), While Bayraktar and Shoshin (40) indicated that the Hamdani breed is a non-migratory sub-breed that goes back to the Karadi breed.

Conclusion

In this work, two genetic sites of growth hormone were studied based on the Pop Gene program to determine some criteria for the genetic diversity of three breeds of Iraqi local sheep (20 Karadi, 20 Hamdani, and 20 Awassi). These low values for each of the expected heterozygote alleles and the total expected heterozygote allelic values indicate a decrease in the genetic diversity within this group of animals belonging to the three breeds. Therefore, external intermarriage must be conducted for such herds to increase genetic diversity by adding new genotypes.

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No comment.

Conflicts of interest

The authors declare no conflict of interest.

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استخدام برنامج بوب جين لدراسة مواقع وراثيين لجين هرمون النمو في الأغنام العراقية المحلية

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قسم الأحياء المجهرية، كلية العلوم، جامعة الكرخ للعلوم، بغداد، العراق

الخلاصة

أجريت الدراسة على ثلاث سلالات من الأغنام المحلية العراقية (٢٠ كردي، ٢٠ حمداني، ٢٠ عواسي). تم إجراء تحليل للبيانات الناتجة عن دراسة مواقع وراثيين لجين هرمون النمو بناء على برنامج بوب جين لتحديد بعض معايير التنوع الجيني لهذه السلالات. اجري حساب لكل من الاليليات الخليطة المشاهدة والمتوقعة، عدد الاليليات الفعال ومعامل شانون (I) لكل من السلالات الثلاثة، ومعامل التربية الداخلية حسب قيم F_{is} و F_{it} و F_{st} للثلاث سلالات معا. وجد أن الاليليات الخليطة المتوقعة للكردي (٠,٢٦، ٠,١٨)، حمداني (٠,٥٠، ٠,٠٩) والعواسي (٠,٢٦، ٠,٠٩)، للموضعين الوراثيين على التوالي. وجد أن عدد الاليليات المشاهدة لكلا الموقعين كان (٢,٠٠) للسلالات الثلاثة، بينما عدد الاليليات الفعال (ne) للكردي (١,٣٤، ١,٢١)، حمداني (١,٩٨، ١,٩٠) والعواسي (١,٣٤، ١,٠١) للموقعين الجينيين على التوالي، إذ لوحظ ارتفاع قيمه في الموقع الأول من الموقع الثاني. كانت قيمة مؤشر شانون (I) للسلالات الثلاثة أعلى في الموقع الأول منها في الموقع الثاني لجميع سلالات الكردي (٠,٤٢، ٠,٣٢) الحمداني (٠,٦٨، ٠,١٩) والعواسي (٠,٤٢، ٠,١٩) من النتائج يمكن ملاحظة أن معدل الاليليات المتماثلة المشاهدة والمتوقعة كانت أعلى من الاليليات الخليطة المشاهدة والمتوقعة للسلالات الثلاثة.