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Exon 2 polymorphism of the ß-lactoglobulin gene by restriction enzyme in three Iraqi sheep breeds

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Abstract

Beta-lactoglobulin (BLG) is an essential protein in whey. This experiment aimed to study the genetic variation in exon 2 of the beta-lactoglobulin gene in three local Iraqi sheep breeds (30 Al-Hamdani, 30 Al-Karadi, and 30 Al-Awassi). 5 ml of blood were collected from animals of each breed. Results of the migration of DNA fragments digested with the restriction enzyme (RsaI) revealed three genotypes: aa, Aa, and AA. It was found that the distribution ratios of the genetic structures of the three breeds, Al-Hamdani (0.47, 0.4, 0.13), Al-Karadi (0.47, 0.34, 0.10) and Al-Awassi (0.40, 0.50, 0.10), and the distribution ratios of the alleles (A) and (a) were for Al-Hamdani (0.67, 0.33) Al-Karadi (0.68, 0.32) and Al-Awassi (0.65, 0.35), the Chi-square values were significantly different for the three breeds. The electrophoresis data were analysed to calculate the observed and expected homozygous and heterozygous allele numbers, the influential allele numbers, and Shannon's index (I) for each sheep breed. The values of the observed and expected heterozygote alleles for the Al-Hamdani breed were (0.4000, 0.4250), Al-Karadi (0.4333, 0.4401) and Al-Awassi (0.5000, 0.4627). The number of influential alleles was for the Al-Hamdani breed (1.800), Al-Karadi (1.736, and Al-Awassi 1.8439. Shannon's index values showed a decrease in the three breeds: Al-Hamdani (0.6365), Al-Karadi (0.6243), and Al-Awassi (0.6474), with Al-Awassi having the highest estimate among the three breeds. The genetic affinity tree diagram shows greater genetic affinity between the Al-Hamdani and Al-Karadi breeds than between the Al-Awassi breed. The results show a higher prevalence of dominant and heterozygote genotypes, a higher proportion of the (A) allele over the (a) allele, higher observed heterozygote allele values for the Al-Hamdani and Al-Karadi breeds, and higher expected heterozygote allele values for the Al-Awassi breed.

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Introduction

Studying the genetic genotype of important genes in farm animals is essential for determining the extent of the relationship between this genotype and farm animal productivity (1-3). Understanding a species' genetic genotype and the genetic diversity within a species (across different breeds) or within a breed (among individuals of the same breed) is a necessary aspect of studying genetic diversity (4-6). The levels of selection that can be used to obtain animals with distinct genetic makeups are an essential matter in this field (7-9). One of the genes that plays a significant role in genetic diversity studies is the beta-lactoglobulin gene (BLG), which encodes the beta-lactoglobulin (BLG) protein, the main whey protein in ruminants and present in other animal species, but absent in camel milk (10). The beta-lactoglobulin protein consists of 162 amino acids. The beta-lactoglobulin gene is located on chromosome 3 in sheep and is characterised by its diversity, with three genetic forms identified: A, B, and C (11). The presence of one of these genetic forms of the gene depends on the milk components and the production amount (12). Due to the vital role of the beta-lactoglobulin (BLG) gene in one of the critical economic traits that include milk formation and production and the differentiation of individuals in terms of productive capacity and quality of milk, several studies have been conducted on various types of farm animals: cows (13,14), buffaloes (15,16), goats (17-19), sheep (20,21). Studies conducted on sheep breeds around the world regarding the beta-lactoglobulin gene have varied by studying the formation or genetic diversity of these breeds to compare individuals of the same breed (22) or between individuals of several breeds (23,24) or conducting studies linking the genetic diversity of this gene with milk production and components (24,25).

Sheep are raised in Iraq primarily for their meat, but they are also used for milk, wool, and leather production. Their meat is the most sought-after and consumed meat in Iraq (26). A group of major breeds is widespread in Iraq and geographically distributed. The Al-Awassi breed is widespread across most regions, while the Al-Karadi breed is found in the north. The Arabi breed appears in the south. Many breeds are also present, which are genetic breeds formed from existing breeds. The Naimi breed is a separate group from the Al-Awassi breed, but it is distinguished by its Due to its small size, the Al-Hamdani breed is a genetic group that traces its lineage back to the Al-Karady breed (27-30).

Therefore, the study aimed to identify genetic diversity in the beta-lactoglobulin gene second exon (RsaI) region of some Iraqi sheep breeds.

Materials and methods

Ethical approve

The data collection permit was issued by the Department of Animal Production, College of Agriculture, University of Diyala, No. 72, on 18/5/2023.

Animals' management

The work was conducted by studying three genetic groups of Iraqi sheep (Al-Hamdani, Al-Karadi, and Al-Awassi breeds), each comprising 30 animals. The animals were female sheep with an age ranging from (2.5 - 4 years). Blood samples were drawn from each animal 5 ml of which was preserved in EDTA tubes as an anticoagulant and then transported to the laboratory in a cold box. The DNA from these samples was extracted using a Geneaid extraction kit (Korea). To obtain the required region (exon 2 region) a polymerase chain reaction (PCR) was performed using a primer to locate the desired location in the beta-lactoglobulin gene (31): (forward 5'-AAA AGC CCT GGG TGG GCA GC-3') (reverse 5'-TTG GGT TCA GTG TGA GTC TGG -3'), materials for the PCR reaction were prepared in a sterile cabinet. The volume of the PCR reaction mixture was (25 µl) according to the volumes of each component as mentioned: Master Mix 5 µl, primer 1.0 µl for each of the forward and reverse primers, DNA template 5 µl, and D.W 13 µl. The PCR product was electrophoresed on a 2% agarose gel to ensure that the desired fragment was obtained 452 bp (Figure 1).

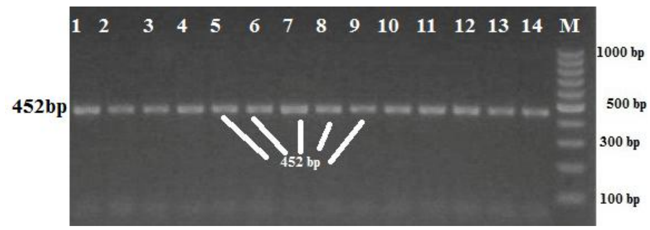

Figure 1: Agarose gel electrophoresis showing PCR-amplified exon 2 region of the BLG gene (452 bp). Lanes 1-10 represent individual sheep samples. M: 100 bp DNA ladder.

The RsaI restriction enzyme from (Promega company/USA) was used to determine the genotype of each animal. The company's instructions regarding reaction volumes and times were followed, with the following modifications: the final digestion reaction volume was 20 µl, including 2 µl of restriction enzyme, 5 µl of PCR reaction product, and 4 µl of buffer solution. The solution volume was completed using deionized water. The solution was incubated at 37°C for 16 hours. Electrophoresis of the digested samples was performed using 10 µL of enzyme-digested products and 10 µL of a DNA ladder on a 3% agarose gel. The power supply was maintained at 50 V and 40 A for 30 minutes, then the voltage was increased to 70 V and 60 A for 1.5 hours. After the electrophoresis of the samples was completed, the gel was immersed in ethidium bromide stain for 15 minutes and imaged using a photo documentation system.

Statistical analysis

genotypes, alleles frequency, chi-square test (χ^2), observed Homozygote, observed heterozygote, expected Homozygote, expected heterozygote, the total expected heterozygote allelic, the average of heterozygote, The Observed alleles number (na.), the Effective alleles number (ne.), the Shannon's Information index (I.) and the genetic affinity tree diagram were calculated using Pop-Gene, 1.31 software (32). A chi-square (χ^2) test was performed to test the goodness of fit to Hardy-Weinberg equilibrium expectations for the distribution of genotypes.

Results

This study was conducted to determine the genetic diversity of the beta-lactoglobulin gene in three local Iraqi sheep breeds. Electrophoresis of the PCR-digested DNA samples using the restriction enzyme (RsaI) showed that the AA allele produced bands with lengths of 175, 170, 66, and 41 bp, while the Aa allele had bands with lengths of 236, 175, 170, 66, and 41 bp, and the aa allele had bands of 236, 175, and 41bp (Figure 2). The results are shown as proportions and numbers of genotypes, allele ratios, and Chi-square values for each of the three breeds. The values of the genotypes AA, Aa, and aa were 14, 12, 4 for the Al-Hamdani breed 14, 13, 3 for the Al-Karadi breed and 12, 15, 3 for the Al-Awassi breed. The proportions of the genotypes AA, Aa, and aa were 0.47, 0.4, 0.13 for the Al-Hamdani breed, 0.47, 0.43, 0.10 for the Al-Karadi breed, and 0.40, 0.5, 0.1 for the Al-Awassi breed. The distribution ratios of the alleles A and a were for the Al-Hamdani breed. 0.33, 0.67, Al-Karady (0.32, 0.68 and Al-Awassi 0.35, 0.65, the Chi-square values were high and significant, which indicates that the three breeds were not in equilibrium according to the Hardy-Weinberg rule (Table 1).

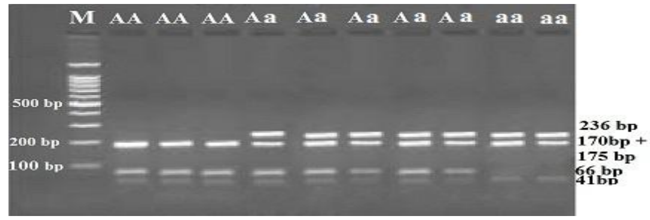

Figure 2: deportation PCR product of β-Lactoglobulin (β-LGg) digested by (RsaI) restriction enzyme. 1 - DNA ladder 100 bp, (2 - 4) genotype AA ((175 bp, 170 bp, 66 bp, 41 bp), (5 - 9) genotype Aa (236 bp, 175 bp, 170 bp, 66 bp, 41 bp), (10 - 11) genotype aa (236 bp, 175 bp, 41 bp).

Table 2 shows the observed and expected values of homozygous and heterozygous alleles and Nei values, which represent the total expected allelic mixing based on Nei values and the average of heterozygote alleles. The observed and expected heterozygote allele values for the Al-Hamdani breed were 0.4000, 0.4250 Al-Karadi 0.4333, 0.4401 and Al-Awassi 0.5000, 0.4627. The Nei values decreased, with 0.444, 0.4238, and 0.4550 for the three breeds Al-Hamdani, Al-Karadi, and Al-Awassi, respectively.

Table 1: Number and Distribution of genotypes, alleles frequency and chi-square test (χ2) In three sheep breeds

Breed	No.	Genotype			allele frequency		X^2
		AA	aa	Aa	A	a	
Hamadaani	30	14 (0.47)	12 (0.4)	4 (0.13)	0.67	0.33	7.866 *
Karadi	30	14 (0.47)	13 (0.43)	3 (0.10)	0.68	0.32	8.600 *
Awassi	30	12 (0.40)	15 (0.50)	3 (0.1)	0.65	0.35	5.400 *

Table 2: Value of Al-Hamdani, Al-Karadi and Al-Awassi sheep

BLGN	O. Hom.	O. Het.	E. Hom.	E. Het.	N.T.	Av. Het.
Al-Hamdani	0.6000	0.4000	0.5480	0.4520	0.4444	0.4441
Al-Karadi	0.5667	0.4333	0.5599	0.4401	0.4328	0.4441
Al-Awassei	0.5000	0.5000	0.5373	0.4627	0.4550	0.4441

O. Hom.: observed Homozygote, O. Het.: observed heterozygote, E. Hom.: expected Homozygote, E. Het.: expected heterozygote, N.T.: the total expected heterozygote allelic, Av. Het.: the average of heterozygote.

Table 3 indicates the values of the observed and the effective alleles number, and the index of Shannon's coefficient (I) for the three breeds. It was found that the observed allele numbers for each sheep group were 2, while the number of effective alleles was 1.800 for the Al-Hamdani breed, 1.736 for the Al-Karadi breed, and 1.8439 for the Al-Awassi breed. Shannon's index values showed a decrease in the three breeds: Al-Hamdani 0.6365, Al-Karadi 0.6243, and Al-Awassi 0.6474 which was the highest estimate among the three breeds. The genetic affinity tree diagram between the three breeds showed that there is genetic affinity between the Al-Hamdani and Al-Karadi breeds, whereas the percentage of affinity of the Al-Awassi breed to these two breeds was lower than that between them (Figure 3).

Table 3: The Observed alleles number (na.), the Effective alleles number (ne.) and the Shannon's Information index (I.)

BLGN	na. *	ne. *	I. *
Al-Hamdani	2.0000	1.8000	0.6365
Al-Karadia	2.0000	1.7630	0.6243
Al-Awassei	2.0000	1.8349	0.6474

Discussion

The results were similar to those reported by Mohammadi *et al.* (20), who studied the genetic variation of the beta-lactoglobulin gene (BLG) in some Iranian and Russian breeds, and they obtained identical genotypes with bands corresponding to the length values in this study. Yousefi *et al.* (23) also showed that the effect of the beta-lactoglobulin gene on milk components in the Iranian local Zel sheep breed resulted in the emergence of three genotypes with band lengths similar to those of the genotypes in the present study.

Figure 3: UPGMA the tree shows the extent of similarity between the three genetic groups of sheep. Al-Hammdani=1, Al-Kradi=2, Al-Awassie=3.

This study found that the percentages of the genotypes were similar to what was reached by Othman *et al.* (33) when they studied the genetic diversity of the B-LG gene in three breeds of Egyptian sheep, as the percentage of the genotype AA was the highest compared to the genotype Aa, aa, while Kawecka and Radko (34) when they studied the effect of the B-LG gene on milk production of four Polish sheep breeds (Friesian, Polish Merino, Bergschaf and Polish Mountain sheep) found that the Aa genotype was the highest compared to other genotypes. Triantaphyllopoulos *et al.* (35), when studying the relationship between the B-LG gene and milk characteristics in two Greek sheep breeds, found that the Aa genotype was dominant over the AA and aa genotypes in these breeds. Miluchová *et al.* (36), in a study of the diversity and genetic composition of the B-LG gene in the Valachian sheep breed in Slovakia, found that the aa genotype was the most frequent.

This study showed that the distribution ratios of alleles A and a in the three breeds were similar to the study Triantaphyllopoulos *et al.* (35) on the Chios and Karagouniko sheep breeds in Greece and the relationship of genetic diversity of the B-LG gene in milk production, as they found that the A allele is the allele with a higher percentage in these breeds than an allele. This is also what Rustempašić *et al.* (37) found when working on the Pramenka sheep breed in Bosnia to determine the genetic diversity of the B-LG gene in this breed, as it was noted that the A allele is superior in its presence rate over an allele, while in the study by Miluchová *et al.* (36) for a Valachian sheep breed in Slovakia to determine the composition and genetic diversity of the B-LG gene for this breed, an allele was more present than an allele in the animals of this experiment. Also, Iovenko *et al.* (38) found, in the Ascanian Fine-Fleeced and Ascanian Karakul sheep breeds in Ukraine, when studying the relationship between the genetic diversity of the B-LG gene and milk production and its components, that the A allele was higher than the B allele in both breeds. As for Elyasi *et al.* (24) they measured the genetic variation of the B-LG gene in a group of Iranian sheep (Ghezel, Afshary, Moghani, Makoii and Arkharmerino), they found that the proportions of the A and a alleles were different among the different breeds of sheep, as the proportion of the A allele was different in some of them, and the a allele was distinguished by a higher proportion in other breeds from the same study.

The results of the study showed the state of equilibrium for these breeds based on the X^2 values and comparisons with the Hardy-Weinberg rule; the Chi-square values were significantly high, indicating that these animals and the three breeds were unbalanced under the Hardy-Weinberg rule. This is due to interference from one of the factors affecting population balance (selection, chance, migration), as specific mating is dominant in the breeding processes of these animals under study. These results are similar to what was found by Triantaphyllopoulos *et al.* (35) they have studied the genetic diversity of the Beta-Lactoglobulin gene (BLGg) in two breeds of sheep, as they noticed a high value of the Chi-square X^2 in the Chios breed, which indicated the unbalance of the experimental animals according to the Hardy-Weinberg rule, while the value of X^2 was low in the Karagouniko breed, as it was balanced based on the Hardy-Weinberg rule. As for the study by Dokic *et al.* (39), it indicated that when they examined the diversity of the BLG gene and its relationship with milk production in the Sora sheep breed, the X2 value was low and the animals were in Hardy-Weinberg equilibrium. Also, a study by Jawasreh *et al.* (40) on some genes, including the BLG gene in Jordanian Al-Awassi sheep and the relationship of genetic diversity of these genes with milk production and components of Al-Awassi ewes indicated that the X^2 value was low and insignificant for the BLG gene, so the animals are considered balanced according to the Hardy-Weinberg rule. While (21) reported in their study on the genetic diversity of the BLG gene in the second exon region in Turkish Norduz sheep that the X2 value was high for this breed and significantly (15.955), indicating that the animals were unbalanced according to the Hardy-Weinberg rule.

Table 2 showed that the observed and expected heterozygosity values. These low values indicated weak genetic heterozygosity in the experimental animals. These results were similar to those reported by Miluchová *et al.* (36) in their study of the genetic diversity of the BLG gene in Slovakian Valachian sheep, as they found a decrease in observed and expected heterozygosity in this study. Yousefi *et al.* (23) reported in their study of the genetic variation of the BLG gene in Zel sheep that Iranian Low study animals had low Observed and Expected heterozygosity, indicating that these animals were not in Hardy-Weinberg equilibrium. Observed and Expected heterozygosity and Nei (total expected allelic mixing) in his study of the genetic diversity of the growth hormone gene in three breeds of Iraqi sheep, which showed a decrease in the genetic diversity present in this sample of the three breeds (30). Two breeds of Ukrainian sheep, Ascanian Fine-Fleeced (AFF) and Ascanian Karakul (AK), to determine the genetic diversity of the BLG gene for these two breeds, he showed a high value of Observed and Expected heterozygosity, which provided evidence of high genetic diversity within these flocks (39).

Abubakar *et al.* (41) explained in their study of four Nigerian sheep breeds: Balami, Yankasa, Ouda and West African Dwarf to determine the genetic diversity of the BLG gene for these breeds that they found the presence of two alleles among these breeds for the BLG gene and that the values of the effective alleles for these breeds ranged between 1.600 in the Yankasa breed to 1.992 in the Balami breed and that the Shannon index values ranged Between 0.562 in the Yankasa breed and 0.691 in the Balami breed, which indicated that low ne values lead to low Shannon index values and thus a decrease in the genetic diversity of this breed, unlike the breed with the highest values. Ukrainian sheep breeds, the presence of two alleles for the BLG gene in this study and that the ne values (number of effective alleles) were high in these animals, which provided evidence of the presence of high genetic diversity in these flocks for the BLG gene under study (38). Genetic diversity of the BLG gene in the Turkish Norduz sheep breed the presence of two alleles within this breed (21).

The genetic affinity tree diagram between the three breeds showed that there is genetic affinity between the Al-Hamdani and Al-Karadi breeds, while the percentage of affinity of the Al-Awassi breed to these two breeds was lower than that between them. Mnati AA (30) reported that, when studying the genetic diversity of the growth hormone gene in the Iraqi Al-Hamdani, Al-Karadi, and Al-Awassi breeds, there is a greater genetic affinity between the Al-Hamdani and Al-Karadi breeds than between the Al-Hamdani and Al-Awassi breeds. Al-Sabea *et al.* (42) reported, in their study of some Iraqi sheep breeds, a similarity between the characteristics of the Al-Hamdani and Al-Karadi breeds, indicating that the Al-Hamdani is a sub-breed within the Al-Karadi.

Conclusion

This study found a decrease in genetic diversity among the three Iraqi sheep breeds in this experiment, as measured by the BLG gene. This decrease in genetic diversity is due to the breeding and mating methods used in these animals, as well as the small flock size, which led to this result. This is despite numerous studies indicating the significant potential of genetic diversity in the BLG gene in farm animals. Therefore, it is preferable to use breeding methods or techniques that increase genetic diversity within farmed animal herds to leverage this phenomenon when evaluating and selecting the best animals.

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Nothing to say.

Conflict of interest

No conflict-of-interest authors declares.

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تعدد أشكال الإكسون ٢ لجين بيتا لاكتوجلوبولين بواسطة إنزيم القطع في ثلاث سلالات من الأغنام العراقية

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الخلاصة

يعد بروتين بيتا لاكتوكلوبيولين بروتين أساسي في مصل اللبن. هدفت التجربة الى دراسة التباين الوراثي لجين البيتا لاكتوكلوبيولين في منطقة الاكسون ٢ لثلاث سلالات من الأغنام المحلية العراقية (٣٠حمداني، ٣٠كرادي، ٣٠عواسي). تم سحب ٥ مل دم من ٣٠ حيوان لكل سلالة. أظهرت نتائج ترحيل قطع الدنا المهضوم بالأنزيم القاطع وجود ثلاث تراكيب وراثية AA , Aa , aa . وجد ان نسب توزيع التراكيب الوراثية للسلالات الثلاث الحمداني (٠,٤٧، ٠,٤، ٠,١٣)، الكرادي (٠,٤٧، ٠,٣٤، ٠,١٠) والعواسي (٠,٤٠، ٠,٥٠، ٠,١٠) ونسب توزيع الاليـلات A، a كانت للحمداني (٠,٦٧، ٠,٣٣)، الكرادي (٠,٦٨،

٠,٣٢) والعواسي (٠,٦٥، ٠,٣٥)، كانت قيم مربع كأي ذات فرق معنوي بالنسبة للسلالات الثلاث. حللت البيانات الخاصة بالترحيل الكهربائي لأجل حساب كل من الاليلات المتجانسة والهجينة المشاهدة والمتوقعة، عدد الأليلات المشاهدة، عدد الاليلات المؤثرة ومعامل شانون (I) لكل من السلالات الثلاثة، إذ كانت قيم الاليلات الهجينة المشاهدة والمتوقعة لسلالة الحمداني (٠,٤٠٠٠، ٠,٤٢٥٠) ، الكرادي (٠,٤٣٣٣ ، ٠,٤٤٠١) و العواسي (٠,٥٠٠٠، ٠,٤٦٢٧) ، وجد أن عدد الاليلات المشاهدة وللثلاث سلالات كانت (٢) بينما عدد الاليلات المؤثرة فكانت لسلالة الحمداني (١,٨٠٠) ، الكرادي (١,٧٣٦) و العواسي (١,٨٤٣٩)، اظهرت قيم دليل شانون انخفاضا في السلالات الثلاث الحمداني (٠,٦٣٦٥) ، الكرادي (٠,٦٢٤٣) و العواسي (٠,٦٤٧٤) الذي كان اعلى التقديرات فيما بين السلالات الثلاث، بين مخطط شجرة التقارب الوراثي بين السلالات الثلاث إن هناك تقارب وراثي اعلى فيما بين سلالة الحمداني و الكرادي اكثر مما للعواسي. من النتائج يمكن ملاحظة وجود ارتفاع في التراكيب الوراثية السائدة والهجينة وارتفاع نسبة الاليل A على الاليل a، وارتفاع قيم الاليلات الهجينة المشاهدة لسلالة الحمداني والكرادي وارتفاع الاليلات الهجينة المتوقعة بالنسبة لسلالة العواسي.