Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Comparison of Growth Hormone Genetic Polymorphism in Awassi Ewes and Their Newborns Using PCR-RFLP Technology

Haytham M.S. Hadi, Omar D. Mohamed and Falah H. Ahmed

Department of Animal Production, College of Agriculture & Forestry, University of Mosul

THIS study was conducted at the University of Mosul / College of Agriculture and Forestry / Department of Animal Production to determine the genetic structures (Genotype) of the Growth hormone gene for Awassi ewes and their progeny and compares between them, For conducting this work, forty Awassi ewes of age (3-5) years and 20 lambs aged between birth and weaning (1-3) months were used, The DNA was separated from collected blood samples, growth hormone gene was amplified using specific primers and genotyping was performed using RFLP technique. The distribution ratios of the genetic structures varied in the ewes, as only two genetic structures appeared, namely AA and AG, with a frequency of 0.24 for the AA and 0.16 for the AG genetic structure, and with a allele frequency of (0.8) for the A allele and (0.2) for the G allele, while three genotypes appeared for the lambs, namely AA, AG and GG, with the distribution ratios of the genetic structures (0.75, 0.1 and 0.15) respectively and with the same allelic frequency (0.8, 0.2) for each of the allele (A and G) respected . We conclude from this study that it is possible to consider the multiple genetic form of the growth hormone gene and its dependence on genetic improvement in ewes and their newborns to achieve a higher economic return than selection methods.

Keywords: Growth hormone, PCR-RFLP technology, Float sheep, Genotypes.

Introduction

Awassi sheep constitute 65% of Iraqi sheep and are considered the most desired local breed by sheep breeders in Iraq under traditional breeding conditions, as they are distinguished by the quality and palatability of their meat, in addition to the consumer's preference for them. They are also distinguished by the quality of their milk and their resistance to diseases, as they are adapted to environmental conditions and their ability to Walking long distances to graze. Improving productive and reproductive performance faces many difficulties that led to a decline in its reproductive performance, and this is what prompted researchers and breeders to find alternative methods to traditional methods of selection, and the recent discovery of genetic maps and molecular genetics led to the identification of many methods and programs that would Improving the performance of productive animals by improving their genetic composition [1].

The growth hormone (GH) gene plays an important role in postpartum growth, milk production, tissue growth, and reproduction, such as metabolism of proteins, carbohydrates, and fats [2,3]. Growth hormone gene in sheep is about 1.8 kilobytes long and contains five exons and four introns [4,5]. Mentioned that growth hormone is a anabolic hormone that is synthesized and secreted by the somatic cells of the anterior pituitary gland in a daily and pulsating manner. With the development of molecular genetic technologies, it has made it possible to identify differences between individuals at the genetic level and determine the polymorphism in candidate genes that affect economic traits

*Corresponding author: Haytham M. S. Hadi, E-mail: haytham-mohammed@uomosul.edu.iq, Tel.: 09647716026614
(Received 21/09/2023, accepted 11/12/2023)
DOI: 10.21608/EJVS.2023.237664.1623

consequently act as a catalyst in genetic selection and open new horizons for the development of livestock of different breeds [6,7]. Through the use of modern programs in genetic selection and the development of new techniques to increase the number of animals and their production to carry the desired genes within breeding flocks [8,9]. Using polymerase chain reaction (PCR) and restriction fraction length polymorphism, several researchers have also studied the association of growth hormone gene polymorphism with body weight and growth, some authors[9] reported that sheep with homozygous alleles had the highest body weight and daily weight gain. Sheep farming has become of great economic importance in the Middle East in this way and the sheep industry has become the main source of income for breeders in these areas. However, the growth of lambs is influenced by many factors, including genetic factors, birth season, age of birth, sequence of birth, sex of lambs and weaning period [10]. Improving sheep growth traits is the basis of concern for many researchers, and growth traits is the most important economic factors in animals controlled by several genes [11,12].

The most prevalent sheep breed in Iraq is Awassi with 58.2% more than other breeds such as Al-arabi (21.8%) and Al-Karadi (20%) [13]. In addition, Awassi sheep have the ability to survive and breed in conditions of drought and extreme climatic. fluctuations [14]. This study is designed to investigate the multiplicity of forms of growth hormone between Awassi ewes and their newborns using PCR-RFLP technique

Material and Methods

Experimental animals

This study was conducted at the University of Mosul - College of Agriculture and Forestry - Animal Production Fields during the period from (3/4/2022) to (3/2/2023) (40 Awassi ewes) whose ages ranged from (3-5) years were selected from the field flock, in addition to the Awassi and Hamdani males. The DNA was extracted and their genetic profiles with regard to female growth hormone were determined in the DNA laboratory. Two genetic structures emerged: AA 25 ewes and AG 15 ewes. They were The number of ewes born from the two combinations was 24, with the number of ewes of the genotype (AA) being 17 and the number of ewes of the genotype (AG) being 7 ewes. Treatments and vaccines were given according to the approved vaccine schedule in the field of animal production/College of Agriculture and Forestry/University of Mosul. The ewes were fed a standardized diet to eliminate the nutritional factor, a protein content of 14.79% and a capacity of 2435 kilograms. Calorie/kg feed. through the study all animals were fed standards diet contain 14.79% protein and 2435 kcal / kg of metabolic energy.

Blood samples

Blood samples were collected (5 ml) from the jugular vein of ewes and lambs by a sterile medical syringe of 5 ml capacity , 1 ml of blood was placed in a sterile tube containing the anticoagulant (EDTA) at a concentration of (1 mg / 1 ml) to prevent blood clotting then shake the tube for one minute blood sample were kept at a (- 20℃) degrees Celsius until DNA extraction .

DNA extraction

The DNA was extracted from sheep blood Promega wizard genomic DNA (purification kit USA) according the manufacturer's instructions, The degree of purity and concentration of extracted DNA were examined using Nano Drop. The extracted DNA samples were kept at -20°C until further testing.

Polymerase chain reaction (PCR)

Amplification of specific fragment (950 bp) of Awassi ewes growth hormone gene was performed by PCR-RFLP using specific primer (table 1) as described by (put reference of primer sequence).

TABLE 1. Primers sequence of sheep growth hormone gene.

Primer	Sequence
GH-F	GGAGGCAGGAAGGGATGAA
GH-R	CCAAGGGAGGGAGAGACAGA

For conducting of PCR reaction, 4 microliters (100 nanograms) of template DNA and 1 µl (10 picomole) of each gene initiator were added to the contents of the master mix in the reaction tubes and these tubes were inserted into the thermocycler device to perform a replication reaction for the growth hormone gene in sheep and newborns The PCR condition was described in Table (2).

TABLE 2. PCR condition for amplification of sheep growth hormone gene.

No.	Stage	Temperature	Time	Cycle number
1	Initial denaturation	95	6 min.	1
2	Denaturation	95	45 sec.	
3	Annealing	59	1 min.	35
4	Extension	72	1 min.	
5	Final extension	72	5 min.	1

After extracting the DNA , using the initiator, then inserting the reaction tubes into the thermocycler device in order to perform the multiplicative reaction, the PCR samples were migrated on the 2% agarose gel electrophoresis and the voltage was set to 70 volts and a current of 40 mA for two hours and the results were photographed after the migration to ensure the success of the PCR amplification,, as the required fragment was obtained with a size of 950 base pairs. DNA fragments were used for volume information (100) and a base pair

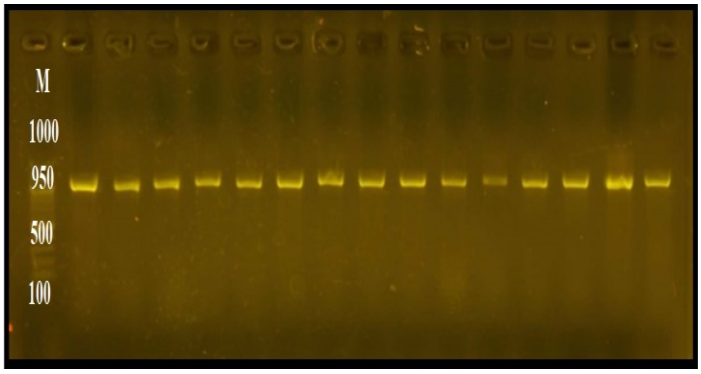

Fig. 1. Agarose gel electrophoresis of PCR product of sheep growth hormone gene represented by clear band of molecular weight 950bp

Using polymerase chain reaction (PCR) technology, the variation in the growth hormone (GH) gene was detected, in which the reaction kit for PCR technology, primers for the gene, and extracted DNA samples were used. The results of the PCR reaction were carried forward with the presence of the volumetric guide, DNA Ladder. It was confirmed that the amplification of the required piece was successful. Treatment with the cutting enzyme HaeIII resulted in reaction products of 296 bp, 202 bp, and 150 bp in the blood samples of sheep (mothers), as it was shown that the genotypes AA and AG appeared and the mutant GG gene disappeared in the mothers. As for the blood samples of the lambs (births), there were three genotypes: the homologous (AA), the hybrid (AG), and the homozygous mutant (GG), and the required fragment was amplified by treatment with the HaeIII cutting enzyme, with reaction products of 296 bp, 202 bp, and 150 bp in base pairs.

Results and Discussion

Genotyping of growth hormone gene:

Treatment of PCR products with restriction enzyme (HaeIII) using RFLP technique and electrophoresis of the rection product, the results showed different genetic structures of the coding region (exon 2) of the growth hormone gene and the reaction product represented by three bands with different molecular weight of 296 , 202 and 150 bp in Awassi ewes as shown in Figure 2 Two genetic structures AA and AG appeared, and through Table (3), we note the number of genetic structures, their percentages, and the nocturnal frequency of the growth hormone (GH) gene in ewes and that the distribution ratios of genetic structures were 24 for the AA genotype and 16 for the AG genetic structure, that is, the percentage of individuals with homologous genetic makeup AA (60) % was the highest and that the individuals with the mixture AG was the lowest (40)%. The night frequency of allele A was (0.8) while the frequency of allele G was (0.2) with a sign difference between the two alleles of 0.6. In newborns, three genotypes of the growth hormone gene GH were appeared, resulting from the

enzymatic digestion of the PCR products by categorical enzyme (HaeIII) and the reaction products were 296 , 202 and 150 bp in births as shown in Figure (3), namely AA, AG and GG, and the distribution ratios of genetic structures were 0.75 for the AA genotype and 0.1 for the AG genotype and 0.15 for the GG genotype, and the percentage of pure individuals with a genetic structure AA was also the highest and that hybrid individuals with shows the genetic makeup of AG genotype was lowest (0.1)%and the third genotype GG was 0.15%. The allele frequency of allele A was (0.8) while the frequency of allele G was (0.2). These results were similar to what (15) pointed out of the superiority of individuals with a genetic makeup AA, as it was 65% compared to the genetic type AB, which amounted to 35% in local Awassi ewes, as well as (16) in their study of the effect of growth hormone on Awassi ewes obtaining the genetic structures AA, Aa and aa, as the nocturnal frequency of allele A was 0.69, while the frequency of allele a was 0.31, as the AA genotype was better.

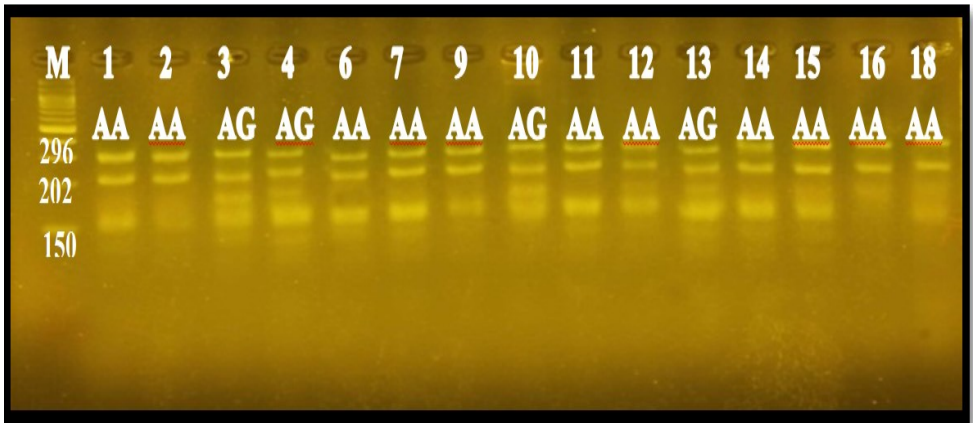

Fig. 2. RFLP of PCR products of growth hormone gene in Awassi sheep.

The PCR reaction product of the growth hormone gene in the blood of Awassi ewes treated with HaeIII cutter enzyme with a reaction product of 296, 202 and 150 bp, in which lane 1,2,6,7,9,11,12,14,16 and 18 represent genotype AA. While lane 3,4,10 and 13 represent genotype AG.

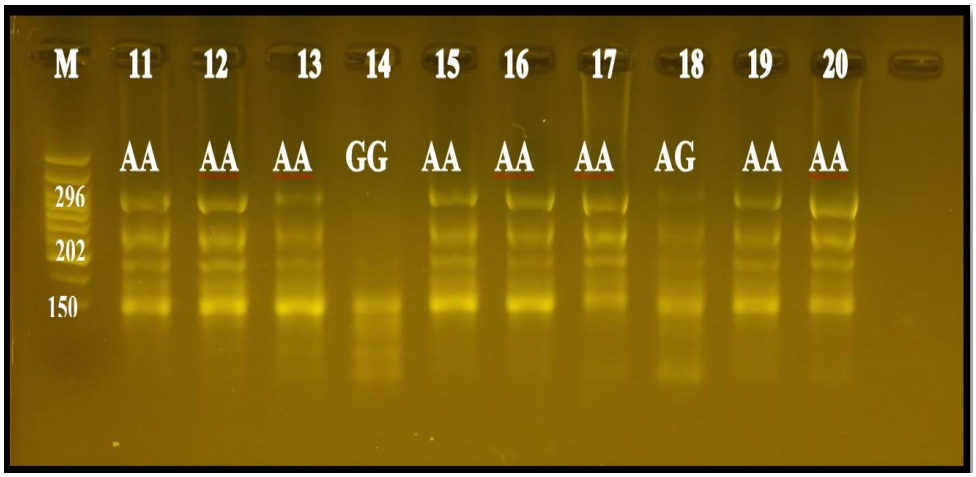

Fig. 3. RFLP of PCR products of growth hormone gene in blood of newborn lambs treated with HaeIII and a reaction product of 296 bp, 202 bp and 150 bp

The results also agreed with some reports[17] To the emergence of three genetic structures of the growth hormone gene in Awassi sheep, namely AA, Aa and aa, where the frequency of the genetic structure AA was (0.44) the structure Aa was (0.49) and the genetic structure aa was (0.07) where the allele repetition was for A (0.615) while for a (0.315), i.e. superior to allele A. The results were consistent with what they found [18] when they studied the genetic polymorphism of growth hormone in Indonesian sheep, they observed the appearance of three genotypes (AA, BB and AB) on

two types of Indonesian sheep and the replication was in the genetic structures of Donggala sheep (0.357, 0.286 and 0.357) for each of the genotypes (AA, BB, AB) respectively and with a nocturnal frequency (0.536, 0.464) for each of the allele A and B respectively, while East Java sheep amounted to (0.464 , 0.286 and 0.250) for each of the genotypes (AA, BB, AB) respectively and with a nocturnal frequency (0.589, 0.411) for allele A and B respectively, as allele A had higher genetic frequency. Some authors [19] reported genetic polymorphism of the growth hormone gene in two locales, A1575G and A781G in 9 breeds of Indian sheep and observed the presence of two genotypes AA and AB and allele A 0.602 and allele B 0.398.Others [20] observed three genotypes in the growth hormone gene in Harry's sheep, namely AA was 0.48 and AG was at least 0.22, while the frequency of the similar genotype GG (0.30) and the detection of alleles A and G were (0.59 and 0.41) respectively, as the frequency of allele A was higher than the frequency of allele G. While the results of this study were contrary to (21,22), as they stated that the gene frequency of G is higher than the allele A.

TABLE 3. Shows the genetic makeup, percentages and nocturnal frequency of the GH hormone gene for Awassi ewes.

Genetic structures	Number of ewes	Percentage (%)
AA	24	60
AG	16	40
Total	40	100%
Significance level (P<0.001)		
Allele	**Frequency**	
A	0.8	
G	0.2	

Table (3) shows the genetic makeup, percentages and nocturnal frequency of the GH hormone gene for Awassi ewes AA 60, AG 40 100% Moral level (P< 0.01) Allele Repetition A 0.8 , G 0.2 .

TABLE 4. Shows the genetic structures, percentages and nocturnal frequency of the GH hormone gene for the newborns (lambs)

Genetic structures	Number of lambs	Percentage (%)
AA	15	0.75
AG	2	0.1
GG	3	0.15
Total	20	100%
Significance level N.S		
Allele	**Frequency**	
A	0.8	
G	0.2	

Conclusion

The molecular genetics and gene detection programs related to productive performance have given important opportunities for the improvement of local animals, including the use of PCR-RFLP technique to determine and compare the genotype of the growth hormone gene of ewes and their newborns. The results of this study showed that the AA genotype of growth hormone in ewes and their newborns is higher than the rest of the genetic structures and with a nocturnal frequency (0.8, 0.2) for both allele (A and G) and for each of the ewes and their newborns, so the AA genotype of the GH gene was most common in Awassi sheep and their newborns, while the lowest genetic structure was AG.

Acknowledgment

The authors are very grateful to the College of the College of Agriculture and Forestry and the Presidency of the University of Mosul for providing most of the requirements for supporting this research.

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مقارنة تعدد الشكل الجيني لهرمون النمو في النعاج العواسية ومواليدها باستخدام تقنية PCR–RFLP

هيثم محمد صبيح هادي وعمر ضياء الملاح و. فالح حسن الحسن

قسم الانتاج الحيواني - كلية الزراعة والغابات - جامعة الموصل - العراق.

اجريت هذه الدراسة في جامعة الموصل/ كلية الزراعة والغابات / قسم الانتاج الحيواني لتحديد التراكيب الوراثية (Genotype) لجين هرمون النمو للنعاج ومواليدها والمقارنة بينهما اذ استخدمت 40 نعجة عواسية بأعمار تراوحت بين (3-5) سنوات وعدد حملان 20 حمل تراوحت اعمارها بين الميلاد فطام (1-3) اشهر ، فصلت المادة الوراثية وتباينت نسب توزيع التراكيب الوراثية في الامهات اذ ظهر فقط تركيبين وراثيين هما AA وAG وبتكرار قدره 0.24 للتركيب الوراثي AA و0.16 للتركيب الوراثي AG وبتكرار اليلي بلغ (0.8) للاليل A و(0.2) للاليل G بينما ظهرت ثلاثة انماط جينية للولادات (الحملان) وهي AA وAG و GG وكانت نسب توزيع التراكيب الوراثية (0.75 ، 0.1 و0.15) على التوالي وبنفس التكرار الاليلي (0.8 ، 0.2) لكل من الاليل (A وG) . نستنتج من هذه الدراسة ان بالامكان اعتبار تعدد الشكل الجيني لجين هرمون النمو واعتماده في التحسين الوراثي لدى النعاج ومواليدها لتحقيق عائد اقتصادي اعلى من طرق الانتخاب.

الكلمات الدالة : هرمون النمو تقنية PCR-RFLP ، النعاج ،صفات مظهرية.