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Association of Growth Hormone Gene Polymorphism with Birth and Weaning Weight of Nuimi and Awassi Sheep at Kerbala Province

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Abstract

A study was conducted at Barakat Abul- Fadhul Station located in Kerbala province for Sheep Production on a total of 63 pregnant ewes from two local breeds (36 Nuimi and 27 Awassi) to find out the association of growth hormone gene polymorphism with the body weight performance of lambs at two stages of life, birth and weaning stages. Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used to detect polymorphism of growth hormone gene by using restriction enzyme (Hae III) to determine fragment of 422 base pair from growth hormone gene. Lamb weights at birth and at weaning age were measured. The results revealed that 3 genotypes of growth hormone gene were detected in both breeds (AA, Aa and aa) with significant effect of genotypes on mean birth and weaning weights in both breeds. The results also indicated that AA genotype in Nuimi breed recorded the highest value (6.35±0.70 kg) in the mean birth weight and (21.50±3.50 kg) in mean weaning weight with a significant effect (P<0.05). However, the results of Awassi breed reported that aa genotype had highest values in the mean birth weight (6.82±0.68 kg) with a significant effect (p<0.05) and mean weaning weight was (23.09±1.50 kg).

Keywords: Growth Hormone Gene, Polymorphism, Nuimi Sheep, Awassi Sheep, Birth Weight, Weaning Weight, Kerbala.

Introduction

Growth hormone possesses large physiological roles like appetite control, ageing, body composition, growth, reproduction (Cobra et al., 2013), and immune responsiveness (Kelley and Felton, 1995). However, growth hormone gene plays role in wool quality and quantity (Beh et al., 2001; Yousefi et al., 2012). The structure of sheep growth hormone gene is similar to other growth hormone genes but more homologous from cattle growth hormone gene (Cobra et al., 2013). Growth hormone gene in sheep was found in chromosome 3 (Allain et al., 1998). The length of growth hormone gene in sheep is about 1.8kb (Hajihosseinlo et al., 2013). However, the development of technologies of molecular genetic has made it easy to identify differences among individuals on the level of DNA (Tamer et al, 2017). Lately, genetic polymorphism in candidate genes has affecting role in production traits, have encouraged considerable research interest due to their ability for utilization as an aid to genetic determination and to demarcate evolutionary relationships in different animals' farm breeds (Sodhi et al., 2014; Afifi et al., 2014). Polymerase Chain Reaction" (PCR) and Restriction Fragment LengthPolymorphism" (RFLP), assist to study the specified genes and to amplify them in vitro (Lui and cordes, 2004). Many researchers studied an association of polymorphism of growth hormone gene with body weight and growth like Tamer et al. (2016) who reported that the sheep with homozygote mutant alleles had highest body weight and the daily live-weight gain (DLWG). Malewa et al. (2014) recorded that Donggala and East Java sheep had significantly highest weaning weight with genotype AA compared with BB genotype, but AB genotype did not show significant differences in weaning weight in both mentioned breeds. Depsion et al. (2017) worked on thin-tailed sheep in Jambi province and reported that the genotype (+/+) had highest body weight and body weight gain in mentioned breed. The aim of this study was to find out the relationship of growth hormone gene polymorphism with body weight performance in Nuimi and Awassi sheep in Kerbala province.

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Materials and Methods

Experiment animals: The experiment was conducted on 63 pregnant ewes from two local breeds Nuimi (36 ewes) and Awassi (27 ewes) reared at "Barakat Abul-Fadhl station" for sheep production located in Kerbala Governorate during the period of 20/9/2017 to 1/7/2018. The ages of pregnant ewes varied from 2-5 years.

Flock management: Animals were raised in semi-open barns (35% covered and 65% opened) designated for sheep production. Ear tags were used to identify sheep under study. The flock was managed according to a program that includes special feeding program for mothers during pregnancy and special nutrition for lambs after parturition as in tables (1) and (2). The flock was provided with the required veterinary services including vaccination programs against endemic diseases and dipping schedule for eradication of external parasites by using insecticides.

Table 1- diet of pregnant ewes

Substances of diet	Amount of substances
Barely	60% per ton
Bran	39% per ton
Alfalfa	(0.5 kg) to each ewe
Coarse feed	(0.5 kg) to each ewe
Salt	1% per ton

Table 2- diet of lambs (15) weeks of age (weaning age)

Substance of diet	Amount of substance
Barely	50% per ton
Barn	29% per ton
Crushed Corn	10% per ton
Soya	8% per ton
Limestone	1% per ton
Premix	1% per ton
Salt	1% per ton

Blood collection: Blood samples from pregnant ewes of two local breeds (36 samples of Nuimi and 27 samples of Awassi) were collected from the jugular vein of animals using a 5 ml syringe into "EDTA tube" after cleaning the jugular vein area and sterilizing the area with the alcohols. Then these tubes were numbered according to number of pregnant ewes, later on the lambs had the identical numbers of their mothers. The samples stored at -8 ◦C until further use.

DNA extraction: Laboratory work was carried out in The Research Laboratory at the College of Veterinary Medicine\Kerbala University. DNA of these samples was extracted by using DNA extraction kit (Geneaid extraction kit, Korea). The efficiency of the extraction process was detected by using 1% agarose gel electrophoreses (figure1).

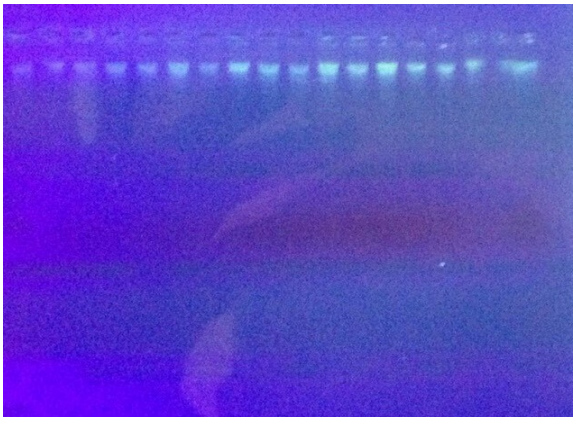

Figure 1: DNA extraction of blood samples of Nuimi and Awassi breeds**.**

Primer design and PCR amplification: Detection of growth hormone gene was indicated by selection of primer for amplification of this gene. A fragment of 422 bp of exon 2 was amplified by using forward primer: **CTCTGCCTGCCCTGGACT** and reverse primer: R: **GGAGAAGCAGAAGGCAAC** (Hue et al., 2009). The amplification reaction of PCR was carried in a total volume of 25 µl containing 5 µl Maxime PCR PreMix (INTRON Biotechnology, Korea), 1 µl of primer, and 5 µl of DNA template, then mixture was completed to the total volume of 5 µl with 13 µl of D.W. The programmed thermal cycler of PCR conditions were done as table (3).

Table 3- Molecular detection program using PCR technology

Sq.	Steps	Temperature C°	Time	Number of Cycles
1	Start Denaturation	94	5 min	1
2	Denaturation	95	30 sec.	13 cycles and decrease the temperature about 1C° per each cycle
	Annealing	65	30 sec.	
	Extension	72	45 sec.	
3	Denaturation	95	30 sec.	35 cycles
	Annealing	52	30 sec.	
	Extension	72	45 sec.	
4	End extension	72	7 min	
5	Finish	4	Unlimited	

The PCR products were separated by 2% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after ethidium bromide staining.

The restriction enzyme reaction: the restriction enzyme reaction was made by using 1.5 µl restriction enzyme Hae III (TAKARA) and the 5 µl of PCR product and 3.5 µl buffer for growth hormone. The mixture was incubated at 37 °C for 3 hrs. and visualized by using ethidium bromide staining in 2% of agarose gel.

Determination of lambs' weight: Birth weight was determined within 24 hrs. Weaning weight was also determined after 15 weeks by using balance modified for small ruminants.

Statistical analysis

The statistical analysis of data was performed by using SAS (statistical analysis system – version 9.1th ed.) significant differences were compared with the "Duncan Multidimensional Test" **(p<0.05).**

Results and Discussion

The PCR product was digested by RFLP technique using the Hae III restriction enzyme for this regions. The results showed three different sizes of genotypes in both studied breeds: AA with one undigested fragment at 422 bp; Aa with three digest fragments at 422 bp, 366 bp and 56 bp; aa with two digest fragments at 366 bp and 56 bp (Fig. 1 and 2). The results were partly consistent with those obtained by Zaid et al. (2018) who worked on three breeds of sheep in Iraq (Awassi, Hamadani and Karadi) and found 3 genotypes which were identical with those reported by our study.

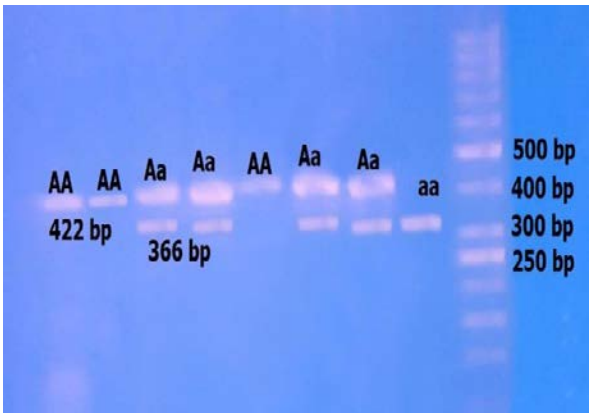

Figure 2: Enzymatic digestion of PCR products of the Nuimi pregnant ewes, 422 bp & 366 bp fragments were appeared, the fragments of 56 bp was not appeared.

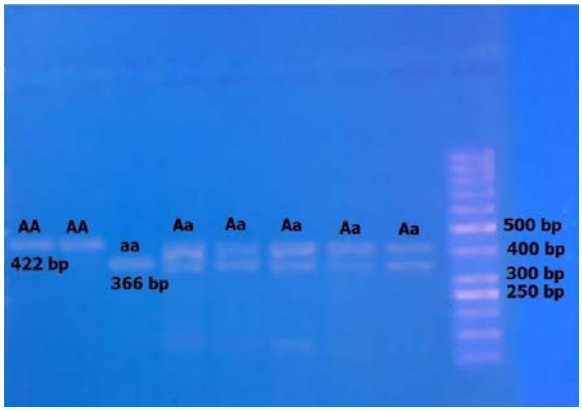

Figure 3: Enzymatic digestion of PCR products of the Awassi pregnant ewes, 422 bp & 366 bp fragments were appeared, the fragments of 56 bp was not appeared.

Table (4) showed that the AA genotype of Nuimi breed was excelled on Aa and aa genotypes in birth weight and weaning weight and genotype AA have significant effect ($p<0.05$) in weaning weights of lambs. The mean birth weights of lambs in genotypes AA, Aa and aa were 6.35, 4.50 and 5.05 kg, respectively. However, the weaning weights of lambs of genotypes AA, Aa and aa were 21.50, 19.09 and 20.09 kg, respectively. Whereas, genotype Aa has lower mean weights in both birth weight and weaning weight.

Table 4- Relationship of genotypes of growth hormone gene with birth and weaning weights in Nuimi sheep

Genotype	Number of ewes	Means ± Standard Error	
		Birth weight(kg)	Weaning weight (kg)
AA	14	6.35 ± 0.70 A	21.50 ± 3.50 A
Aa	20	4.50±0.0.50 A	19.09±0.26 B
aa	2	5.05 ± 0.36 A	20.09 ± 0.44 AB
Significant level		N.S	0.05

- Different letters in one column mean a significant difference ($p\leq0.05$).
-N.S. means non-significant.

The results of Awassi breed in table (5) revealed the means of lambs' birth weight in genotypes AA, Aa and aa were 6.25,4.94 and 6.82 kg, respectively, and the aa genotype has higher value in birth weight ($p<0.05$), while Aa genotype has lower mean value of birth weight. Lambs' weaning weights in genotypes AA, Aa and aa were 21.79, 19.65 and 23.50 kg, respectively; and aa genotype also has higher value in weaning weight and significant effect ($p<0.5$) while Aa genotype has lower value of mean weaning weight.

Table 5- Relationship of genotype of growth hormone gene with birth and weaning weights of Awassi sheep**.**

Genotype	Number of ewes	Mean ± standard Error	
		Birth weight(kg)	Weaning weight (kg)
AA	12	6.25 ± 0.23 A	21.71 ± 0.73 AB
Aa	13	4.94 ± 0.22 B	19.65 ± 0.40 B
aa	2	6.82 ± 0.68 A	23.50 ± 1.50 A
Significant level		0.05	0.05

- Different letters (A and B) in every column mean a significant difference $p\leq0.05$

The results in table (4) revealed the impact of genotypes on birth and weaning weights in Nuimi sheep. Our results recorded that AA genotype had higher values in birth and weaning weights (6.35 and 21.5 kg, respectively), while aa genotype had 5.05 kg in birth weight and 20.09 kg in weaning weight. Nevertheless, Aa genotype had 4.50 kg in birth weight and 19.09 kg in weaning weight. our results of the impact of genotypes on Nuimi breeds were concurrence with Malewa (2014) who performed a study on Donggala and East Java sheep in Jambi province and revealed that AA genotype had significantly higher weaning weight than BB genotype both in Donggala (11.6 kg vs 9.68 kg) and East Java (10.83 kg vs 9.37 kg) sheep. AB Genotype did not show significant differences in weaning weight compared to AA genotypes both in Donggala and East Java sheep. Depison (2017) dealt with Thin–tailed sheep in Jambi found that (+/+) genotype had higher body weight. The results in table (5) revealed the impact of genotypes on birth and weaning weights in Awaasi breeds. Our results indicated aa genotype had the higher value in birth weight (6.82 kg) and in weaning weight (23.5 kg). AA genotype had 6.25 kg birth weight and 21.79 kg weaning weight. Aa genotype has lower values in birth weight (4.94 kg) and in weaning weight (19.65 kg). These results were concurrent with Al-Salihi (2017) who worked on Awassi sheep and his study yielded 3 genotypes (AA, AG, and GG); GG genotype had a higher value in birth weight (4.53 kg) and weaning weight (21.26 kg). AA genotype had 3.92 kg birth weight and 20.32 kg weaning weight. However, AG genotype had lower values in birth weight (3.59 kg) and weaning weight (18.26 kg). Our results were in concurrence with Tamer (2016) who dealt with a Harri sheep and recognized three genotypes (GG, GA, AA) and recorded that the AA genotype had a higher value in birth weight (2.2 kg), while genotype GG had 1.5 kg and AG genotype had 1.8 kg birth weight. The results were concurrence with Cauveri (2016) who worked on Nilagiri sheep and revealed three genotypes (GG, AG, AA), and showed AA genotype had significantly ($p<0.01$) higher weaning weight (13.49 kg).

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