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Original Research

Study of growth traits for local Awassi sheep for the genotype of β- lactoglobulin gene

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ABSTRACT:

This research was done at the first research station of the Animal Production Department, College of Agriculture, Al-Muthanna University, Muthanna Governorate, Iraq on a sample of 50 local Awassi sheep concerning practical part while genetic analysis part was done at AL-Takdom Laboratory in Baghdad, Iraq, to identify the genetic structures of β-lacto globulin gene and the relationship of those structures to a number of productive characters of local Awassi sheep. The genotype of β-lacto globulin differed according to the difference of the enzyme bundles resulting from enzymatic digestion, which had two structures, AA and AB, with a distribution rate of 30 and 70% respectively and their distribution ratios were 65 and 45 for A and B alleles respectively. The results of the present study showed that the effect of the gene on birth weight was not significant for pure ewes (AA) and hybrids (AB), for AA it was (4.98±0.29) kg and for AB (4.56±0.17) kg, while it was significant (P<0.05) on weaning weight, reaching in ewes with genetic genotype AA (16.81±0.53) kg and (15.68±0.25) kg for AB.

Keywords:

Awassi sheep, β-lactoglobulin, Production traits.

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INTRODUCTION

The Awassi sheep are most common breeds in Iraq and the middle East are high-quality meat, moderate reproductive capacity, fertility rate of 67 to 95%, and the percentage of twins with 1.05% (Salah *et al*., 2008). Data obtained from the replication of genes through the study of polymorphisms makes it possible not only to compare animal gene strains (potential effects of genes in economic characteristics or performance), but also to study genetic variability under different environmental conditions (Piccione *et al.*, 2009). Genetic competence is essential to animal behaviour using phenotypic values and comparison of individuals used for economically important traits including growth traits through birth weight and weight at weaning and subsequent weights according to the animal fields breeding system strategy. As a result of the development of molecular genetics, it has become possible to identify high correlation genes with one or more components DNA of genes that have a major effect on economic characteristics such as growth character and other factors (Imad, 2017). Recently gained more interested in β- lacto globulin factor as a key to biological control, which is associated with a number of economic characteristics in animal husbandry (Kevorkian *et al.*, 2008), beside this factor has an effect on regulating body weights, metabolism and fertility, improving health and increasing the vitality of individuals of different ages.

Economic characteristics, including growth traits, are controlled by a number of genetic sites known as quantities trait loci-QTL during positioning and identification of associated markers, the phenotypic variation of the traits to be improved, such as growth, can be predicted ,these markers are functional mutations in the genes affecting these traits (Arora *et al.*, 2010). The current study aimed at identifying the polymorphisms of β-lacto globulin in the local studied Awassi sheep sample (extracting the distribution ratios of these manifestations and the first frequency of the gene), and study of the effect of the gene on the growth character of multiple genetic manifestations in growth traits. β-lactoglobulin gene is a plasma protein along with albumin and is found in cow's milk and in the milk of several sheep (Soheil *et al.,* 2013), its the first protein discovered by polymorphism Maria *et al*. (2015). Through this general pattern of proteins consists of a heterogeneous series of protein families coupled with larger molecules and less water solubility in pure water compared to albumin ,this property makes the carbines migrated less rapidly than the albumin in the electrical relay (Imad, 2017).

MATERIALS AND METHODS

This research was done at the first research station of the Animal Production Department / College of Agriculture / Al-Muthanna University - Muthanna Governorate - Iraq on a sample of 50 local Awassi sheep in the practical part, while the genetic analysis (laboratories analysis part) was done at AL-Takdom Laboratory in Baghdad – Iraq, (β-lacto globulin) and the relationship of these structures to a number of productive properties of local Awassi sheep. 5 mg of blood from the jugular vein was collected for each ejaculation in a collection tube supplemented with the K2 EDTA coagulation inhibitor produced by the Aviation Facilities Company - Amman office / Jordanian (AFCO). In order to prevent blood clotting, the tube was pumped immediately after collecting the blood for one minute for the purpose of mixing blood with the substance, then they fixed the number of the animal on the tube and transferred to the laboratory for freezing at 4°C and directly to the DNA. DNA was extracted from blood samples of ewes for the purpose of molecular examination of LEP.

DNA and electrical relay

Eight micro liters of DNA were mixed with two micro liters of loading dye (Bromophenol blue dye). The samples were carried out in single holes from the

gel. The specimens were carried over 70 volts of electricity and 40 mL amp for an hour. UV light transilluminatory is used to view the DNA packets, the colored bands of the ethidium bromide fluorescence are photographed using a photo documentation system (Al-Salihi *et a.,* 2017).

Molecular characterization of the gene

The primers were selected for the purpose of conducting molecular detection and identifying the phenotypic diversity of the genes and mutations of the beta-lacto lobulin gene (Kuulasma, 2002).

Exon 2
F : 5'- AGGAAGCACCTCTACGCTC -3'
R : 5'- CTTCAAGGCTTCAGCACC -3'

DNA extraction

Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood DNA Miniprep System, Promega (Table 1) following the method of Sambrook and Russel (2001).

Agarose gel electrophoresis

After PCR amplification Table 4, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

Solutions

1 X TAE buffer, DNA ladder marker, ethidium bromide (10 mg / ml).

Preparation of agarose

100 ml of 1X TAE was taken in a beaker and 1 g (for 1%) agarose was added to the buffer, the solution was heated to boiling (using microwave) until all the gel particles were dissolved, then 1µl of Ethidium Bromide (10mg/ml) was added to the agarose, the solution was allowed to cool down at 50-60°C.

Casting of the horizontal agarose gel

The agarose solution was poured into the gel tray after both the edges were sealed with cellophane tapes and the agarose was allowed to solidify at room temperature for 30 min.

PCR products loading

Electrical power was on at 100 vol. t / 50 mL amp for 90 min and 5µl was directly loaded. DNA moves from cathode to plus anode poles. The ethidium bromide stained bands in gel were visualized using gel imaging system.

Statistical analysis

Statistical data were statistically analyzed using the Statistical Analysis System (SAS, 2012) to study the effect of the genetic features of the beat lactoglobulin gene (the mathematical model below). Morphological differences between the averages were measured using the Duncan (1955) test by applying the least squares mean method. Mathematical model for the investigation

Table 1. Kits, Primers and Instrument

Kits	Company/ Origin
ReliaPrep™ Blood g DNA Miniprep System, Agarose, Ethidium Bromide .Solution (10mg/ml), GoTag Green Master Mix, Nuclease Free Water, TAE 40X	Promega, USA
Primers	
Seq. Primer Name	
5`- 5`-TTGGGTTCAGTGTGAGTCTGG-3` BLG- R BLG -F AAAAGCCCTGGGTGGGCAGC-3	
Instrument	
Instrument	Company/ Origin
Centrifuge	Fisher Scientific, USA
Micro spin centrifuge	My Fugene, China
Thermo cycler	Bio-Rad, USA
OWL electrophoresis system	Thermo, USA
Gel imaging system	Major Science, Taiwan

Table 2. Reaction setup and thermal cycling protocol (Sambrook and Russel, 2001) of Gene β - lacto globulin

S. No	No. of reaction	50	Rxn	Annealing temperature of primers	65
1	Reaction volume / run	20	µl	Length of PCR product (bp)	452
2	Safety margin	5	%	No. of PCR Cycles	30

S. No	Master mix Components	Stock	Unit	Final	Unit	Volume	
						1 Sample	50 Samples
1	Master mix	2	X	1	X	12.5	625
2	Forward primer	10	µM	1	µM	1	50
3	Reverse primer	10	µM	1	µM	1	50
4	Nuclease free water					8.50	425
5	DNA	10	ng/µl	10	ng/µl	2	
6	Total volume					25	
7	Aliquot per single rxn	23 µl of Master mix per tube and add µl of Templat 2					

PCR Program (Sambrook and Russel, 2001)

S. No	Steps	°C	m:s	Cycle
1	Initial denaturation	95	05:00	1
2	Denaturation	95	00:30	
3	Annealing	65	00:30	30
4	Extension	72	00:30	
5	Final extension	72	07:00	1
6	Hold	10	10:00	

of the relationship of the genetic aspects of LEP gene to the production of milk and its components:

Yijkl = µ + Gi + Pj + Tk + eijkl

Yijkl: the value of the observation l belonging to the genotype; I: the sequence of the production cycle j and the type of birth k; µ: The overall mean of the characteristic; Gi: Effect of genetic manifestations of the LEP gene (AA and AB); Pj: Effect of sequence of production cycle (1^{st} to 4^{th}); Tk: Effect of birth type (individual, twin); eijkl: random error which is distributed naturally at an average of zero and a variation of 2eσ. The Chi-square- test was used to compare the percentages of gene distribution of the gene in the studied sheep sample.

RESULTS AND DISCUSSION

DNA was extracted as a first step in PCR technology using the diagnostic kit and the method of work referred to in the materials and methods of work. After that, 5µL samples of DNA and 2µL of loading dye were carried in 2% agarose gel containing ethidium bromide dye added 5µL to 100 ml of the regulator solution for 1X TBE relay and adjust voltages at 70 V and 40 m ampere current for an hour and depict the relay output to ensure successful extraction as in Figure 1.

The aim was to determine genetic genotype structures of β-lacto globulin gene and the relationship of those structures to a number of productive traits of local Awassi sheep. The genotype of β-lacto globulin differed according to the difference of the enzyme bundles resulting from enzymatic digestion, which had two structures, AA and AB, with a distribution rate of 30 and 70% respectively, and the alleles repetition was 65 and 45 for both A and B respectively Table 3. The results of the present study showed that the effect of gene structure on weight at birth was none significant, it

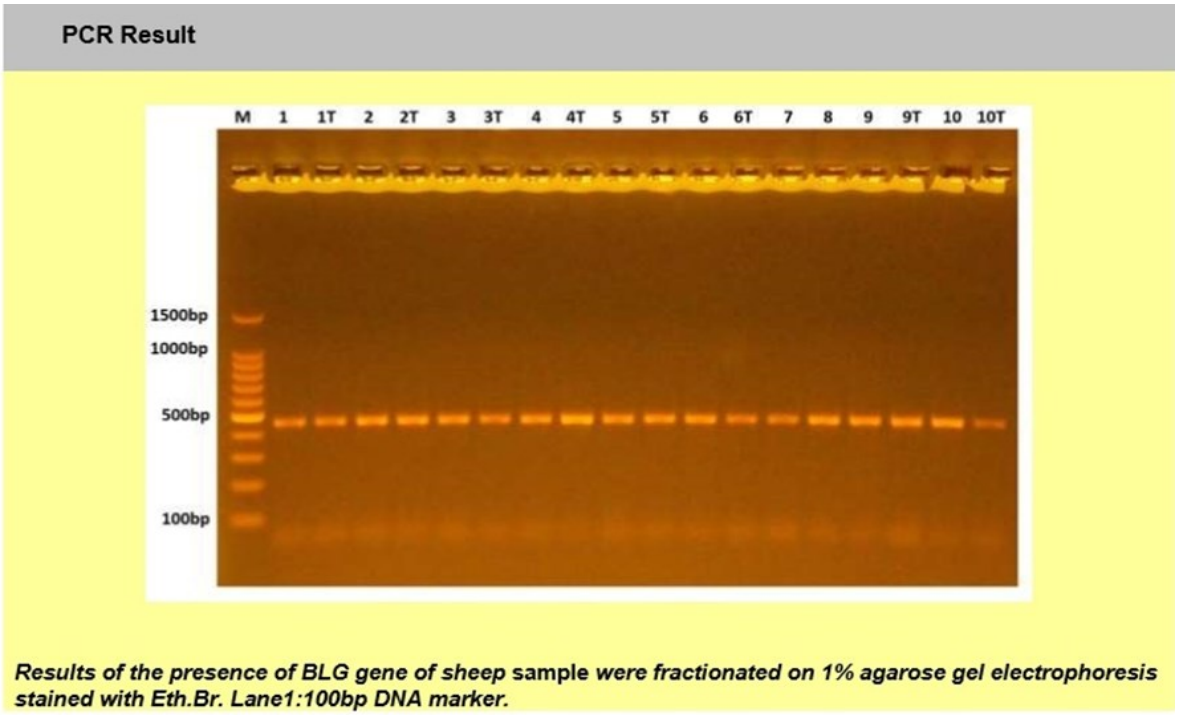

Figure 1. DNA extraction process

was in ewes with the genetic structure of AA (4.98 ± 0.29) and AB (4.56±0.17) kg, while it was significantly (P<0.05) on weighing weights, reaching AA (16.81 ± 0.53) and AB (15.68 ± 0.25) kg (Table 4).

As shown in Table 3, the number and percentages of the genotypes of β-lacto globulin have shown significant differences (P<0.01) between the distribution ratios of the different genotypes, there is a clear prevalence of the hybrid species AB and thus the pure (AA). These results are evidenced that the genome β-lacto globulin used in the research is already present in the genus of the Awassi sheep. It differed from the geographical location. The results of the previous studies indicated that there are significant differences (P<0.01) in the distribution of the genotypes of sheep in Suffolk and Dorset sheep (Boucher *et al.*, 2006). Zhou *et al.* (2009) explained that there are four different genetic structures (P<0.01). The differences between the distribution ratios of these structures for each strain were highly significant (P<0.01). The prevalence of allele A in this study may be due to the adaptation of the first allele to the environmental conditions in which Iraqi sheep live from high temperatures for most months of the year, scarcity of rainfall and acute lack of nutrition or dependence of animals on poor coarse feed. It was also found that the proportion of the distribution of the

Table 3. Number and percentages of genotypes and baseline replication of β-lacto globulin

Genotype	No.	Percentage (%)
AA	15	30.00
AB	35	70.00
Total	50	100
Kay square value (χ^2)	-----	1700 **
Allele		Repetition
A		0.65
B		0.45
	**(P<0.01)	

Table 4. Relation of genotype of β-lacto globulin gene in growth characteristics

Genotype	Ewes No	Mean ± Standard Error (kg)		
		Birth wt. (No. 60)	Weaning wt. (No. 60)	Average daily gain (No. 60)
AA	15	0.29 ± 4.98	0.53± 16.81	0.62 ±11.85
AB	35	0.17 ± 4.56	0.25± 15.68	0.56 ±11.12
Significant level	50	NS	*	NS

The averages with different letters within the same column vary significantly between them (P<0.05)*

genetic structure AB is higher than that of the genetic genotype AA of the Awassi sample. Both the AA and AB genotypes have the authority to live and adapt to local environmental conditions. The genetic genotype of AB is in the fore front because it is higher than the AA. However, the pure genetic genotype of B is low, which may reflect its lack of validity under such conditions. A repeat allele A of the genus in the Awassi sample of the studied intramuscular sheep was 0.65 while the allele frequency B was 0.45, and this result reflected the prevalence of the allele A of the gene in the local Awassi sheep Table 3.

Table 2 shows that the β-lactoglobulin gene had a none significant effect on the ewes with the genetic genotype (AA) and (AB) on the birth weight of 4.98 ± 0.29 and 4.56 ± 0.17 kg, respectively, while it was significant (P<0.05) on weaning weights for ewes (AA and AB), reaching 16.81 ± 0.53 and 15.68 ± 0.25 kg, respectively. There were no significant differences between the two groups (AA and AB) in the average daily gains. The current study recorded the highest weight at weaning, which was found by (Al-Douri, 2001), as well as higher than that found by (Al-Anbari, 1998; AL-Azzawi *et a*l., 1997). These results were consistent with Korkmaz *et al*. (2016) when they studied the Turkish Romanov sheep, where they found significant differences in lambs weights at birth and in weaning. The results of this study strongly support the possibility of genetic analysis of this gene in the programs of the selection, if the goal is to improve the growth characteristics on lambs in sheep herds, especially for the birth and weaning weights of lambs and the rate to increase in daily gains weight between them, taking in consideration that the characteristics of early growth have a positive and highly significant in genetic link with weights and subsequent body measurements, which supports the possibility of adaption of these results in accelerating the programs of improvement to maximize the economic return of the herd, as the attributes of growth is one of the most important economic attributes in sheep breeding projects.

Table 5 shows that the difference was significantly (P<0.05) for the ewes with the AA genetic genotypes in the length of the body than the ewes with the AB genotypes, which were 40.13±1.06 and 37.68±0.58 cm respectively, while it was non significant on the chest circumference, the height at the front and the height at the back, (42.00±1.33, 43.08±64.64), (37.33±0.92, 37.68±0.73) and (40.20±0.94, 39.02±0.61) cm for ewes with genotypes AA and AB respectively.

CONCLUSION

It can be concluded through the study of the genotype of β-lacto globulin gene and its future adoption to set the genetic rehabilitation strategies for sheep to maximize their economic income of sheep genotype selection and interaction breeding projects which already achieved best economic parameters. Additionally, the application of this study on larger sample for many production seasons can give more accurate results to adopt exclusion and replacement strategy.

Table 5. Relation Genotype of β-lacto globulin in body dimensions at birth

			Genotype	**Ewes No.**	**Mean ± Standard Error (kg)**
		Body length (No. 60) cm	Chest circumference cm (No. 60)	Height at the front (No. 60) cm	Height at the rear (No. 60) cm
AA	15	40.13 ± 1.06	42.00± 1.33	37.33 ± 0.92	40.20± 0.94
AB	35	37.68 ± 0.58	43.08± 64.64	37.68 ± 0.73	39.02 ± 0.61
Significant level	Total No. 50	*	NS	NS	NS
					No significant NS, (P<0.05)*

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