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ASSOCIATION OF β-LACTOGLOBULIN GENE POLYMORPHISM WITH MILK PRODUCTION AND COMPOSITION IN LOCAL AWASSI SHEEP

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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, data were collected on a sample of 50 local Awassi sheep beside the genetic structures analysis which is done at AL-Takdom Laboratory in Baghdad–Iraq, to identifying the genetic structures of β-Lacto globulin gene and the relationship of those structures to a with productive characters, it was found two genetic structures A and Aa in studied sample , the distribution percentage were 30 and 70% respectively, while alleles distribution was were 65 for A and 35% for a. The results of the present study showed a significant differences (P<0.05) of total milk production which was 81.43 ± 1.78 kg for AA , and 73.22 ± 1.39 kg for Aa, while and it was non significant on lactation period, 113.86 ± 1.19 and 111.47 ± 0.8 day for AA and Aa respectively. For fat percentage in milk components had significant effects (P<0.05) for AA (4.26 ± 0.73), there was no significant differences of protein, lactose and solid materials of both genetic structures.

Keywords : Awassi sheep - β-lactoglobulin – Production traits

Introduction

Awassi sheep contribute 58.2% of the total sheep population in Iraq (AL-Barzinji and Othman, 2013). The Awassi is the most numerous and widespread breed of sheep in south west Asia .It's the dominant type in Iraq. Is dual purpose (mutton and wool) sheep breed (Falconer and Mackay, 1996). This breed was widespread because of its good characteristics in regards to meat price and quality, milk quality, validity of wool for the carpet industry and its ability to cope stress of high environmental temperature (AbiSaab and Sleiman, 1995 and Al-Salmarai, 2015). β-lactoglobulin is the main whey protein of cow, sheep, goat and horse milk; it is lacking in milk from human, rodents, rabbits and camels in which, instead, another major whey protein (whey acidic protein) is found (Perez and Calvo, 1995). The whey proteins showed a high nutritive value being a precious source of digestible proteins. Sheep milk whey proteins account for 17–22% of total proteins. Whey obtained from sheep milk is particularly rich in proteins with a high β-lactoglobulin and low α-lactalbumin content (Moatsou *et al*., 2005). β-lactoglobulin is a globular protein member of the lipocalin family, small proteins with many properties, such as the ability to bind small hydrophobic molecules. Although its biological function is still unclear, β-lactoglobulin provides amino acids to the offspring anda possible role in the transport of retinol and fatty acids has been suggested (Perez and Calvo, 1995). β-lactoglobulin was the first protein in which polymorphism was found, it consists of 162 amino acids and forms stable dimers in milk having a molecular weight of 18 kDa per monomer (Kontopidis *et al*., 2004). β -lactoglobulin, the major whey protein of ruminant milk, is controlled by the b-lactoglobulin locus, one of several specific genes that may affect economically important traits in sheep. This protein, which is found in several other species but is absent in humans and rodents, is expressed in the mammary glands during pregnancy and lactation (Ali and Clark 1988). Though b-LG is able to bind miscellaneous hydrophobic molecules, its biological role is largely unknown (Mercier and Vilotte 1993). The β-lactoglobulin coding gene is located on ovine chromosome 3. This protein is synthesized in the mammary glands during pregnancy and the lactation stages Apart from its ability to bind and transport small hydrophobic molecules in milk (e.g. retinol and small fatty acids) its biological function is still unclear. The aim of this paper is to determine the relationship of β-lactoglobulin polymorphism with milk production and its contents in Awassi ewes and consider the results as guidelines for the management strategies for ewes under the farming conditions for selecting and improving the performance of domestic animals depending on this indicators.

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 Jordanian company 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.

Download DNA and Electrical Relay

8 micro liters of DNA were mixed with 2 micro liters of dye loading (Bromophenol blue dye). The samples were carried in single holes from the gel. The specimens were

carried over 70 volts of electricity and 40 ml amp. for an hour. Use the (UV light transill minatory) to view the DNA packets, the colored bands of the Ethidium Bromide Fluorescence are photographed using a (photo documentation system) (Al-Salihi *et al.*, 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 globulin gene (Kuulasma, 2002).

F : 5'- AGGAAGCACCTCTACGCTC -3'	**Exon2**
R : 5'- CTTCAAGGCTTCAGCACC -3'	

Materials: Kits, Primers, and Instrument :

Kits

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

DNA Extraction

Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood DNA Miniprep System, Promega as the following steps:

1. Blood sample thoroughly mixed for 15 minutes in rotisserie shaker at room temperature.
2. For each 1.5ml micro centrifuge tube 20µl of Proteins K (PK) Solution was dispensed then 200µl of blood was added and briefly mixed.
3. For Cells lysis 200µl of Cell Lyses Buffer (CLD) was added to the tube and mixed by vortex for 10 seconds.
4. All mixes were incubated in water bath at 56°C for 30 minutes.
5. While the blood sample was incubated a ReliaPrep™ Binding Column placed into an empty collection tube.
6. After incubation the tube removed from water bath and 250µl of Binding Buffer (BBA) was added and mixed by vortex for 10 seconds.
7. All the tube contents was transferred to the ReliaPrep™ Binding Column and centrifuged for 3 minute at 12000 rpm.
8. The collection tube containing flow through removed and discarded.
9. The binding column placed into a fresh collection tube.
10. For column washing 500µl of Column Wash Solution (CWD) was added to the column and centrifuge for 3 minutes at maximum speed, the flow through discarded this step repeated three time.
11. After washing step, column placed in a clean 1.5ml micro centrifuge tube and 100µl of Nuclease-Free Water was added to the column.
12. After 5 minutes, the 1.5ml tube with column centrifuge for 5 minutes at 5000rpm.
13. After centrifuge the ReliaPrep™ Binding Column discarded and eluate saved.

Reaction Setup and Thermal Cycling Protocol

Gene β- lacto globulin

65	Annealing temperature of primers	Rxn	50	No. of Reaction
452	Length of PCR product (bp)	µl	20	Reaction Volume /run
30	No. of PCR Cycles	%	5	Safety Margin

Volume		**Unit**	**Final**	**Unit**	**Stock**	**Master mix Components**
50 Samples	1 Sample					
625	12.5	X	1	X	2	Master Mix
50	1	µM	1	µM	10	Forward primer
50	1	µM	1	µM	10	Reverse primer
425	8.50					Nuclease Free Water
	2	ng/µl	10	ng/µl	10	DNA
	25					Total volume
2 µl of Template 23 µl of Master mix per tube and add						Aliquot per single rxn

PCR Program :

Cycle	m:s	°C	Steps
1	05:00	95	Initial Denaturation
30	00:30	95	Denaturation
	00:30	65	Annealing
	00:30	72	Extension
1	07:00	72	Final extension
	10:00	10	Hold

Agarose Gel Electrophoresis:

After PCR amplification, 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.
- 1 gm (for 1%) agarose was added to the buffer.
- The solution was heated to boiling (using microwave) until all the gel particles were dissolved.
- 1µl of Ethidium Bromide (10mg/ml) was added to the agarose.
- The agarose was stirred in order to get mixed and to avoid bubbles.

The solution was allowed to cool down at 50-60C. *

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 minutes. The comb was carefully removed and the gel was placed in the gel electrophoresis ank. The tank was filled with 1X TAE-electrophoresis buffer until the buffer reached 3-5 mm over the surface of the gel.

PCR products loading:

For PCR product, 5µl was directly loaded to well. Electrical power was turned on at 100 vol. t/ 50 ml amp. for 90 min. 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 lacto globulin 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 of the relationship of the genetic aspects of LEP gene to the production of milk and its components:

$$Yijkl = \mu + Gi + Pj + Tk + eijkl$$

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 (1st to 4th). 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 isolation and Lacto globulin extraction

Its a first step, DNA was extracted from lacto globulin gene in PCR technology and using the diagnostic kit and the method of work referred to in the materials and methods of work. As shown in Fig.1.

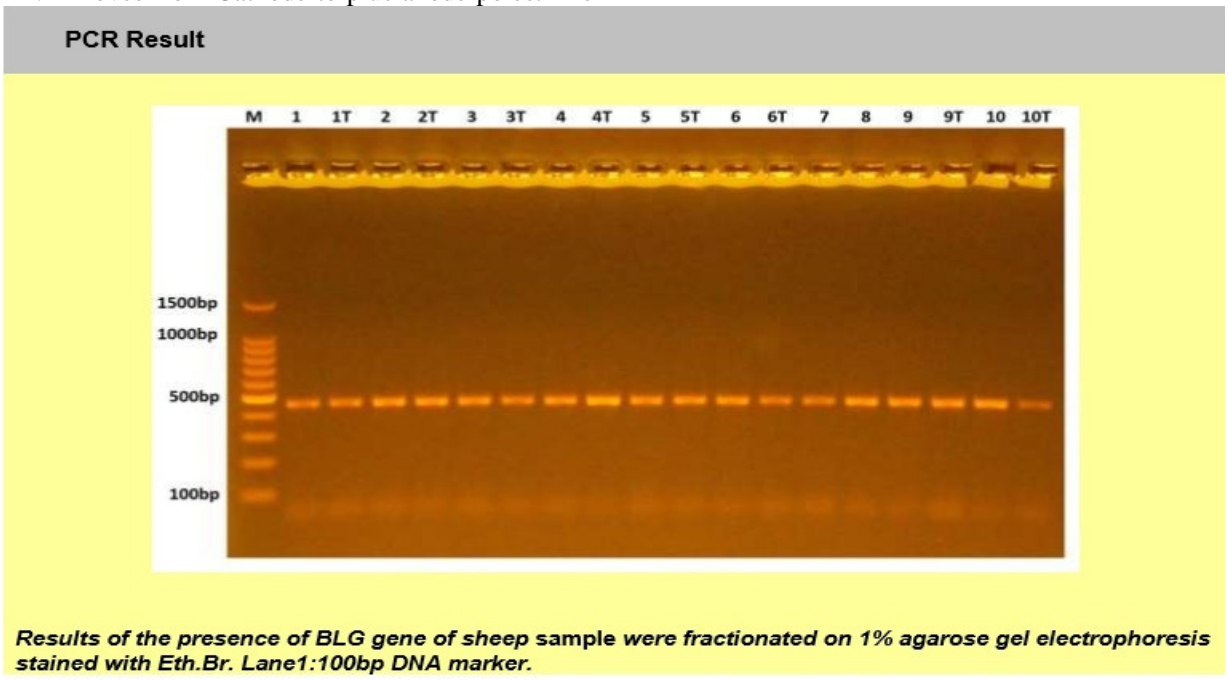

Fig. 1 : DNA extraction process

Using PCR- RFLP technology

Restriction enzyme was used to limit genetic structures of Lacto globulin piece.

The result of this study showed presence of two genetic structures AA and Aa as showing in Fig. 2.

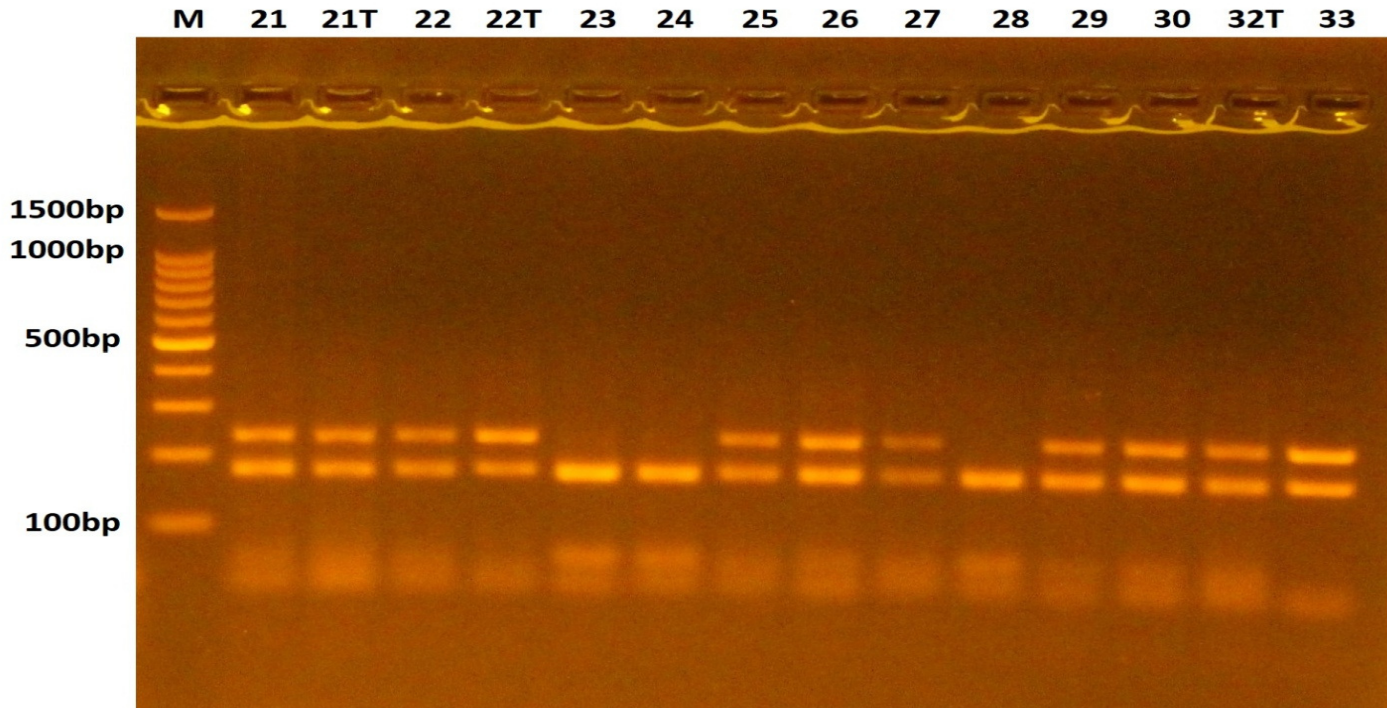

Fig. 2 : The genotypes identified in this study

Genetic structures distribution and ratios and allele of β-lacto globulin gean in local awassi sheep

Table (1) shows, numbers and percentages of lacto globulin gene, was highly significant differences (P<0.01) between genetic structures distribution and ratios it was 30 and 70 % for AA and A Hybrid individuals a respectively, that's mean a clear evidence that hybrid individuals excelled pure individuals, while transient genetic structures (Mutation) BB did not show in any of studied samples in this research. The repeat frequency of the lacto globulin gene in the studied sheep sample 0.65, while the recurrence of the BB gene 0.35 and this result showed the prevalence of the A allele of the gene (Table 2).

Table 1: Number and Percentage of genotypes in β-lacto globulin gean in local awassi sheep

Genotype	No.	Percentage (%)
AA	15	30.00
AB	35	70.00
Total	50	100
Kay square value (χ^2)	-----	1700 **

Table 2: Number of alelel frequencies in β-lacto globulin gene in local Awassi sheep

Allele	Repetition
A	0.65
B	0.35
** (P<0.01)	

Relation of genetic structures of lacto globulin gene on Production Performance

Table (3) shows a significant differences (P<0.05) in total milk production, the genetic structures AA were superior (81.43 ±1.78) on genetic structures AB (73.22 ±1.39) kg, while there were no significant differences lactation period between the genetic structures, it was (113.86 ± 1.19) and (111.47 ± 0.8) day for both genetic structures AA and AB respectively, for milk components it was significant differences (P<0.05) (4.66 ± 0.73) and (3.39 ± 0.32) % for both genetic structures AA and AB respectively for fat, lactose and protein and solid materials its was no significant differences between genetic structures it was (4.37 ± 0.05) and (4.47 ± 0.03) % for lactose in both of genetic structures AA and AB respectively, while it was (5.41 ± 0.026) and (5.67 ± 0.14) % for protein percentage respectively, and it was (1.74 ± 0.33) and (10.17 ± 0.18)% for solid materials for both genetic structures AA and AB respectively.

Table 3 : Least square means and standard errors of milk production in genotypes of % β-lacto globulin

Lactation period (days)	Total milk yield (kg)	Ewes No	Genotypes
113.86 ± 1.19	81.43 ±1.78	15 (45Sample)	AA
111.47 ± 0.8	73.22 ±1.39	35 (105Sample)	AB
NS	*	50 (150Sample)	Significant level

The averages with different letters within the same column vary significantly between them (P<0.01)*

Table 4 : Least square means and standard errors of milk composition traits in genotypes of β-lacto globulin

SNF %	Protein %	Lactose %	Fat %	Ewes No.	Genotypes
10.74± 0.33	5.41± 0.26	4.66 ± 0.73	4.26 ± 0.73	15 (45Sample)	AA
	5.67± 0.14	3.39 ± 0.32	3.39 ± 0.33	35 (105Sample)	AB
NS	NS	NS	*	50 (150Sample)	Significant level

The averages with different letters within the same column vary significantly between them (P<0.01)*

Discussion

The distribution of allele frequencies of β-lacto globulin gene showed That The frequency of allele A(0.65) was higher Than That of allele B (0.35) in Local Awassi sheep. Similar results were reported in Racka sheep by Baranyi *et al*. (2010). Also (Mohammadi *et al*., 2006; Mele *et al*., 2007; Dario *et al.,* 2008; Michalcova & Krupova 2009; Carral *et al.,* 2010) reported That allele A is dominants in different sheep brreds. Baranyi *et al* (2010). Reported to be at a high frequency of allele B Than That of allele A in Awassi breed Also The B allele was detected to be at a high frequency by several studies (Wesseis *et al.,* 2004; Mroczkowski *et al.,* 2004; Barrill *et al.,* 2005; Staiger *et al.,* 2010; Arora *et al.,* 2010).

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