

The Relationship between Genetic Markers (HSC, BM1818 and MAF035) and the Production of Milk and Its Components in Awassi Sheep

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Abstract: This study was carried out at the Research Station / College of Agriculture /Al-Muthanna University and Laboratories specializing in molecular genetics for the period from 1/11/2015 until 30/7/2016 , to determine the relationship between the genetic markers HSC , MAF035 and BM1818 with loci 2, 3, 4, 5 alleles , 2,3,4 alleles and 3,4,5 alleles respectively, and milk production and its components. The results showed a significant differences ($P \leq 0.05$) for HSC marker in ewes carrying genetic locus 2 allele in the total milk production, length of the milking season and the milk fat ratio when compared with ewes carrying genetic locus 3, 4 and 5 alleles .Also it was found high significant difference ($P \leq 0.01$) for MAF035 marker in ewes carrying genetic locus 4 alleles in the total milk production, length of the milking season when compared with ewes carrying genetic locus 2 and 3 alleles, While significantly differed ($P \leq 0.05$) in the ratio of milk fat for ewes carrying genetic locus 3 alleles on the ewes carrying genetic locus 2 and 4 alleles which differed among themselves. The referred to a significant differences ($P \leq 0.05$) for BM1818 marker in the ewes carrying genetic locus 5 allele in the total milk production, length of the milking season on ewes carrying genetic locus 3 and 4 alleles , while the ewes carrying genetic locus 4 alleles significantly differed in the ratio of milk fat. The results obtained significant differences ($P \leq 0.05$) for BM1818 marker in ewes carrying genetic locus 4 alleles in the proportion of non fatty solids and protein ratio which amounted 6.60% as compared with ewes carrying genetic locus 3 and 5 alleles which have protein ratio 5.76 % and 5.06% for both respectively.

Keywords: Sheep, Microsatellite, Milk production, HSC, MAF035, BM1818

1. Introduction

Maintaining of the local diversity is considered essential to meet the future needs of meat and milk, therefore precautions should be take to Predict of genetic aspects capable to rapid response to various Iraqi environments and variable food conditions, which requires diversity in breed strains, therefore the storage of locations should be determined to know the breeds and animals which have the ability to resist different circumstances and know the animals production on the genetic basis (14). Estimating the amount of milk produced by the sheep provide sufficient information to implement management strategies and optimal nutrition of ewes and lambs (1) , the survival rate of births and the weight gain before weaning reflect the ability of the ewes in milk production (19). Ewes differs in milk production and its components due to the differences between breeds, also the content and the level of the main and secondary components in the milk are affected by many factors such as lactation stage, season, type of birth, type of diet and other factors (15). In order to know its genetic ability , many studies were conducted on local sheep to know the milk production, it was noticed a variation in the milk production between the breeds and between the individuals within the same breed which could be due to two parts are the genetic and non-genetic factors, and the milk production is considered a main guide for the mothers ability to persevere the production (4). There are several environmental factors that profoundly affect in the milk production in sheep were outlined by many studies carried out by some researchers (18, 5).

HSC marker is exist in sheep on chromosome 20 and consists of a 22 bp according to the following order:
CTG CCA ATG CAG AGA CAC AAG A F R-
GTC TGT CTC CTG TCT TGT CAT C

Studies are differ about the size of the marker , the degree of annealing and the number of alleles, it was found by (13), that the marker size was 263-297 bp with annealing degree 560 ° C and the number of alleles was 13. (6) was found that the number of alleles was 15 in on Pantaneiro sheep. BM1818 Marker is exist on chromosome No. 20 in sheep, and consists of 20 bp and a single base according to the following order:

F: AGC TGG GAA TAT AAC CAA AGG
R: AGT GCT TTC AAG GTC CAT GC

Studies were differed about the size of marker , numbers of alleles and the degree of annealing (2) was found that the size of the marker was 258-284 bp , the annealing temperature was 52-54 ° C and numbers of alleles was 5 in kail sheep using the Microsatellite technique. (20) was found that numbers of alleles for BM1818 marker was 17 in Kivircik sheep.

Marker MAF035 is exist on chromosome No. 23 in sheep, and consists of 23 bp and three single bases according to the following order:

F: TCA AGA ATT TTG GAG CAC AAT TCT GG
R: AGT TAC AAA TGC AAG CAT ACC TG

As in previous markers, studies were differed about the marker size , numbers alleles and the degree of annealing, a

study carried out by (12) were found that the marker size was 90-130 bp and the annealing degree was 55 ° C .

2. Materials and Methods

2.1 Experimental animals

This study was carried at the Research Station / College of Agriculture - Al-Muthanna University and Laboratories specializing in molecular genetics on group of Awassi ewes for the period from 1/11/2015 until 30/7/2016 for one productive season. Ewes age ranged from 2 to 8 years.

2.2 Studied traits

It has been evaluating the sheep performance for total milk production traits as well as genetically evaluation of sheep for studied traits through:

1-The total milk production of ewes included in this study was accounted depending on the measurement of daily milk production, which has been measured monthly for each ewe by manual milking and isolating lambs from their mothers at night for a period of 12 hours. The ewes were milked in the morning then the daily production was estimated (10) Depending on the following equation (9):

$$TMY = (T_1 - T_0) M_1 + \sum (T_r - T_{r-1}) (M_r + M_{r-1}) / 2$$

TMY = total milk production, T0 = date of birth, T1 = date of first measurement, M1 = the first measurement (the amount of milk / kg), Tr = date of measurement in the month, Tr-1 = measurement date for the previous month, Mr = measurement in that month (the amount of milk / kg), Mr-1 = measurement in the previous month (the amount of milk / kg). 2-Milk samples were collected during the morning , it was taking the milk sample and well mixed in order to be homogeneous with amount 50 ml and transported directly to the laboratory as well as the preservation of samples and did not exposure to sunlight or high temperatures and then the milk components of fat , protein and lactose were calculated every two weeks starting from the second week until the end of the production season using the Milk analyzers Julie Z7 .

2.3 Blood samples collection

Blood samples were collected from the jugular vein , 3 ml for each animal using a medical syringe with capacity of 10 ml after cleaning jugular vein area and sterilized with alcohol ethyl, blood samples were placed in tubes containing anti coagulation material (Ethylene Diamine Tetra Acetic Acid EDTA) then blood samples kept in freezing degree (- 4 °c) until the DNA extraction process .

2.4 DNA extraction

DNA was extracted from blood samples of ewes using Kit equipped by Geneaid company / Korea, according to the following steps:

- 1) 200 Micro liters were taken from blood and placed in Eppendorf tube with capacity 1.5 ml
- 2) added 20 micro liters of (Proteinase K) solution then flipping process to Eppendorf tube for vortexing . 3-

Incubation the tube for five minutes in a water bath at 60°c.

- 3) Shake the mixture with Vortex apparatus.
- 4) Added 200 micro liters of GSB solution then a simple shaken and placed in a water bath for 20 minutes and 60 degrees of heat.
- 5) Extraction the tube and added 200 Micro liters of absolute ethanol and then the mixture placed in a double tube for filtration, then a tube placed in a centrifuge at speeds of 1500 r / min for one minute .
- 6) Conducting washing process by adding 400 Micro liters from W1 to GS column and then placing the tube in a centrifuge at speeds of 3000 r / min for one minute duration. 8- Added 600 micro liters of washing buffer then placing the tube in a centrifuge at speeds of 3000 r / min for one minute duration.
- 7) 9-The empty tube was placed in the centrifuge at speeds of 14000 r / min for one minute duration.
- 8) 10- The clear material was transferred to a new Eppendorf tube (1.5 ml) and added Elution and left for three minutes then introduced into the tube to a centrifuge at speeds of 14000 r / min for one minute duration.

2.5 Agaros gel preparation

Before the starting of extraction process (Total-DNA) the extracted samples deportation on gel Agarose has been conducted with a concentration of 1% by dissolving 1 g of Agarose in 100 ml of diluted TBE solution (X1) then heated in the microwave for 5 minutes until they get a clear color then added 5 micro liters of Ethidium bromide pigment and left to cool slightly then pour the gel in the basin of deportation for sclerosis. After gel hardening and raise the comb, added 5 micro liters of DNA then connect the poles to power supply and demonstrate the power to 80 volts and 65 amperes for 30 minutes.

After the completion of the deportation, gel was examined using documentation Gel to confirm the presence of DNA (16).

2.6 Microsatellites technique

The special materials of PCR technique was prepared and placed in a container containing pieces of ice to protecting from the heat, the work was done in a sterile and clean place in special PCR Cabinet containing ultraviolet rays in order to sterilize the micro pipettes , tubes and other tools, a mixture of PCR was prepared in the Eppendorf tube 100 micro liters capacity and the final size of the components was 25 micro liters then placed in a centrifuge (Micro centrifuge) for 30 seconds to blend the reaction mixture.

2.7 Marker Microsatellite

Three markers (BM1818, HSC and MAF035) were selected to determine their relationship with some production traits in sheep, it has been starting the degree of correlation (Annealing) by sequentially complement in the DNA template for each marker using temperature grading process for each marker.

2.8 Electrophoresis technique of PCR product

To determine the success of the multiplication process or amplifying a piece of DNA to be determined by the used markers using the electrophoresis agarose gel, taking 5 micro liters of the PCR product and placed in the drill with the use of a ladder in the size of 25 nitrogen base (DNA marker 25 bp).

3. Statistical Analysis

Data were statistically analyzed using the (17) program (Statistical Analysis System) to study the effect of genetic polymorphism for markers HSC, MAF035 and BM1818 in milk production and its components. Significant differences between means were compared using Duncan test (7) Multinomial by applying the method of least square means.

The mathematical model to investigate the relationship of HSC marker in the production of milk and its components. In the same way for other marker.

$$Y_{ijkl} = \mu + G_i + P_j + T_k + e_{ijkl}$$

Y_{ijkl} = the value of viewing l belonging to the installation of the genetic sequence of i and j production cycle and the type of birth k .

μ = general mean of trait

G_i = The effect of alleles for HSC marker (2 to 5).

P_j = The effect of the sequence of the production cycle (from 1 to 4).

T_k = The effect of birth type (single, twin).

e_{ijkl} = Random error which is normally distributed with mean equal to 0 and variance of σ^2 .

It was also used the chi square test (Chi-square- χ^2) to compare the percentages for the presence of alleles for each genetic marker in the studied samples of sheep

4. Results and Discussion

Genetic polymorphism relationship for HSC marker in milk production

Table (1) the ewes carrying genetic polymorphism their numbers of alleles were 2, 3, 4 and 5 for HSC marker. It was found a significant differences ($P \leq 0.05$) for ewes carrying loci of alleles 2,3 and 4 on the ewes carrying loci consist of 5 alleles in total milk production which were 75.00, 76.80, 77.08 and 72.60 kg respectively. there were a significant superiority ($P \leq 0.05$) in the length of milk season for ewes carrying genetic polymorphism which have two numbers of alleles was 115.02 day on the rest of ewes that carrying genetic loci consist of 3,4 and 5 alleles which were 112.50, 110.92, 110.50 days respectively. the milk production was affected with this marker and their composition, these results are closer to the findings by (3) whom reported that there are effects for the genetic marker)BM6444, ILSTS087, MAF070, MAF209, OARFCB304 and SRCRSP15 in the total milk production in Hamdany sheep and also found that the Pvalue for total milk production were 0.017, 0.040, 0.050, 0.039 and 0.001 for the genetic markers BM6444, ILSTS087, MAF070, MAF209, OarFCB304 and SRCRSP15 respectively, and this indicate can infer from the results the superiority of ewes that carry genetic loci consist of 2 alleles in the total milk production and the length of milk season.

Table 1: Genetic polymorphism relationship for HSC Genetic polymorphism relationship for HSC marker in milk components

Polymorphism	Average \pm standard error	
	Total milk production (kg)	length of lactation (Day)
2	75.00 \pm 1.87 a	115.02 \pm 1.10 a
3	76.80 \pm 1.04 a	112.50 \pm 0.61 b
4	77.08 \pm 1.03 a	110.92 \pm 0.61 b
5	72.60 \pm 2.57 b	110.50 \pm 1.52 b
Level of significance	*	*
Averages that carry different letters within the same column significantly differ among themselves ($P \leq 0.05$) *		

Significant differences ($p \leq 0.05$) were noticed for ewes that carry genetic bands consist of two alleles on ewes that carry different genetic bands of their alleles in milk fat ratio which were 7.69 %, 6.33 %, 6.69 % and 6.36% for ewes carrying genetic bands consist of 2,3,4 and 5 alleles respectively, non significant differences were found in the rest of milk components (lactose, protein and non fatty solids) which were 4.56 %, 4.62 %, 4.42 % and 4.21% for lactose and 5.35 %, 5.78 %, 5.59 % and 5.29% for protein and 11.51 %, 10.97 %, 10.81 %, and 10.53% for non fatty solids for ewes carrying genetic bands consist of 2,3,4 and 5 alleles respectively. (8) when he studied microsatellites for CSN3 marker found a relationship between milk production and it is components and the size of marker, fat and protein ratio was 5.95% and 5.08% when the size of marker was 287bp and 6.03, 5.14% respectively when the size of marker was 295bp.

Table 2: Genetic polymorphism relationship for HSC marker in milk components

Poly morphism	Average \pm standard error			
	Fat %	Lactose %	Protein %	non-fat Solids %
2	12. \pm 7.26 a	..52 \pm 0.72 a	5.25 \pm 0.12 a	77.57 \pm 0.66 A
3	2.22 \pm 0.2. b	..26 \pm 0.0. a	5.1. \pm 0.62 a	70..1 \pm 0.66 A
4	2.2. \pm 0.2. b	...6 \pm 0.0. a	5.5. \pm 0.6. a	70..7 \pm 0.6. A
5	2.22 \pm 7.1. b	..67 \pm 0.72 a	5.6. \pm 0..0 a	70.52 \pm 0..7 a
Level of significance	*	NS	NS	NS
Averages that carry different letters within the same column significantly differ among themselves ($P \leq 0.05$) *, NS: Non significant				

Genetic polymorphism relationship for MAF035 marker in milk production

The results in (table 3) were indicated a high significant differences ($P \leq 0.01$) between ewes that carry different genetic bands for MAF035 marker, ewes with genetic bands that contain 4 alleles were superiority in total milk production on the ewes carrying the number of alleles 2, 3 and also higher than the sheep with genetic bands of two

alleles on ewes with bands containing 3 alleles, the total milk production were 78.50, 82.87, and 72.28 kg, respectively, and there was a significant superiority ($P \leq 0.05$) in the length of the milking season for ewes carrying bands consist of 4 alleles on the rest of ewes, also there was a superiority for ewes carrying bands consist of 2 alleles than those carrying band consist of 3 alleles in their length of season which were 116.00, 112.75 and 109.14 days respectively (3) indicated when studying this marker and its relationship with the milk production when sheep was divided into three groups (high, medium and low) depending on (Genetic- economic index), the milk production in the high group was 190.20 kg / ewe, which their genetic bands consisted of 5 alleles for MAF035 marker and in low group was 22.44 kg / ewe in those that carry genetic bands consisted of 4 alleles for MAF035 marker. (8) when they studied Microsatellites for CSN3 marker in lacaune sheep found a relationship between milk production and its components and the size of marker in milk production in season which were 359, 378 and 385 kg when the size of a piece marker was 287/287, 287/297 and 295/297 bp, respectively.

Table 3: Genetic polymorphism relationship for MAF035 marker in milk production

Poly morphism	Average \pm standard error	
	Total milk production (kg)	length of lactation (Day)
2	1.50 \pm 2.6 b	776.15 \pm 6.2 b
3	16.6. \pm 7.21 b	70.7. \pm 0.2 c
4	.6.1 \pm 7.5 a	772.00 \pm 7.01 a
Level of significance	**	*
Averages that carry different letters within the same column significantly differ among themselves ($P \leq 0.05$) *		

The relationship of Polymorphism MAF035 marker in milk components

Significant differences ($p \leq 0.05$) were obtained in milk fat ratio for ewes that carry genetic bands consist of 3 alleles on the rest of ewes that consist of different bands, and ewes that carry genetic bands consist of 2 alleles were superior than those carrying 4 alleles, fat ratio were 6.14, 7.16 and 5.85 % for ewes carrying genetic bands consist of 2, 3 and 4 alleles respectively. No significant differences were found in the milk components ratio (lactose, protein and non fatty solid) which were 4.42, 4.50, 4.52 % for lactose and 5.73, 5.70, 5.42 % for protein respectively. non fatty solid ratio were 11.28, 10.89 and 10.74 % for ewes carrying genetic bands consist of 2, 3 and 4 alleles respectively. (8) when he studied microsatellites for CSN3 marker in East Friesian Dairy sheep found a relationship between milk production and its components and the size of marker piece where milk and fat ratio were 5.68, 4.99, 6.12 and 5.30 % when the size of marker were 287/287 and 297/297 bp, respectively.

Table 4: The relationship of Polymorphism MAF035 marker in milk components

Poly morphism	Average \pm standard error			
	Fat %	Lactose %	Protein %	non-fat Solids %
2	2.7 \pm 7.67b	.6 \pm 0.01a	5.12 \pm 0.6 a	77.6. \pm 0.26a
3	1.72 \pm 0.52 a	.50 \pm 0.05 a	5.10 \pm 0.66a	\pm 0.6670.a
4	5.5 \pm 0.7 c	.56 \pm 0.05a	5.6 \pm 0.6.a	70.1. \pm 0.62 a
Level of significance	*	NS	NS	NS
Averages that carry different letters within the same column significantly differ among themselves ($P \leq 0.05$) *, NS: Non significant				

The relationship of Polymorphism BM1818 marker in milk production

The results indicated a significant differences ($P \leq 0.05$) (table 5) between ewes carrying different genetic bands for BM1818 where the ewes carrying genetic bands consist of 3 and 5 alleles were superior than those carrying genetic bands consist of 4 alleles in the total milk production which were 72.59, 76.02 and 76.18 kg for ewes carrying 4, 3 and 5 alleles respectively. results of the present study showed a significant differences ($P \leq 0.05$) between ewes carrying genetic bands consist of 5 alleles on the rest bands in the length of milk season which were 113.01, 107.04 and 106.73 days for ewes carrying 3, 4 and 5 alleles for BM1818 respectively.

(3) indicated a relationship for this marker and the milk production when sheep was divided into three groups (high, medium and low) depending on (Genetic-economic index), the milk production in the high group was 190.20 kg / ewe, which their genetic bands consisted of 6 alleles for BM1818 marker and in low group was 22.44 kg / ewe in those that carry genetic bands consisted of 7 alleles for BM1818 marker.

Table 5: The relationship of Polymorphism BM1818

Polymorphism	Average \pm standard error	
	Total milk production (kg)	length of lactation (Day)
3	12.06 \pm 7.62 a	701.0. \pm 0.2 b
4	16.5. \pm 7.5. b	702.12 \pm 7.0. b
5	76.18 \pm 7.22 a	772.07 \pm 7.0. a
Level of significance	*	*
Averages that carry different letters within the same column significantly differ among themselves ($P \leq 0.05$) *		

Significant differences ($p \leq 0.05$) were noticed in milk fat ratio for ewes that carry genetic bands consist of 4 alleles on ewes that carry genetic bands consist 5 alleles, on the other hand no significant differences were found in milk fat ratio between ewes that carry genetic bands consist of 3 alleles and the rest of ewes that carry different genetic bands of their alleles which was 6.78, 7.44 and 6.05 % for ewes that carry genetic bands consist of 3, 4 and 5 alleles respectively. There were a significant superiority ($p \leq 0.05$) in protein ratio for ewes that carry genetic bands consist of 4 alleles on the rest of ewes that carry different genetic bands of their alleles which was 5.76, 6.60 and 5.06%, and

Significantly differenced ($p \leq 0.05$) in non fatty solid for ewes that carry genetic bands consist of 4 alleles on ewes that carry genetic bands of consist 5 alleles, also, no significant differences were obtained between ewes that carry genetic bands consist of 3 alleles and the ewes that carry genetic bands of consist 4, 5 alleles was 11.04, 11.53, and 10.48% respectively. No significant differences were found in lactose in ewes milk for the rest of ewes that carry different genetic bands of their alleles. (8) when he studied Microsatellites for CSN3 marker in East Friesian Dairy and Lacaune sheep found no significant differences between marker, milk and fat ratio and in contrast there were high significant differences ($p \leq 0.01$) between marker and milk ratio in East Friesian Dairy sheep.

Table 6: Genetic relationship Polymorphism of genetic Marker BM1818 with milk components

Poly morphism	Average \pm standard error			
	Fat %	Lactose %	Protein %	non-fat Solids %
3	2.1. $\pm 0.25ab$.2 $\pm 0.0.a$	5.12 $\pm 0.62B$	77.0. $\pm 0.66 ab$
.	1... $\pm 7.66a$..1. $\pm 0.02 a$	2.20 $\pm 0.07A$	77.52 $\pm 0.7.a$
5	2.05 $\pm 0.21b$..7 $\pm 0.05 a$	5.02 $\pm 0.66 c$	70... $\pm 0.62b$
Level of significance	*	NS	*	*

Averages that carry different letters within the same column significantly differ among themselves ($P \leq 0.05$), *: Significant, NS: Non significant

- 1) Ewes that carry the genetic marker MAF035 same location the genetic component of the four alleles superiority over the rest of the ewes that carry the other in total milk production
- 2) The ewes that carry 2 allele for genetic marker HSC were superior than milk fat component on the rest of the ewes that carry other markers on milk fat ratio.

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