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To cite this article: Haider Malik Abd Ali *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **923** 012037

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Relationship of Genetic Polymorphism For CYP19 Gen of Exon 3 With Quantity and Milk Composition in Domestic Female Goats

Haider Malik Abd Ali¹, Razak Naser Abd² and Yousif Hamad Karim³

^{1,2}Al-Furat Al-Awsat Technical University, Iraq.

³Al-Qasim Green University, Iraq.

¹Email: JanabiHaider1984@atu.edu.iq

²Email: razzaq.naser@biotech.uoqasim.edu.iq

³Email: yousifhk@atu.edu.iq

Abstract

This study was carried out to investigate the effect of the genetic polymorphism of the third exon for CYP19 gene on the quantity and composition of milk in local female goats. Blood samples were collected from female goats in the fields of some breeder in Babil Governorate/ Al-Qasim City. Milk composition were examined in the Public Health Laboratory at Al-Qasim Green University/College of Veterinary Medicine, the genetic bundle of the third axon of the CYP19 gene was isolated, which was 140 bp in size, and its genotyping was determined according to the different nucleotide sequences and their association with the quantity and milk composition in the samples which tested in the Bayolat Laboratory in Al-Diwaniyah Governorate. The proportions of the distribution of the genotypes of the CYP19 gene in a sample of local female goats TT, GG and TG were 33.34%, 26.66 and 40%, respectively. The results showed prevalence of the TG genotype for CYP19 gene in the Iraqi local goats on the pure genotypes TT and GG, which was a highly significant superior ($P < 0.01$) in the percentages of fat, protein, lactose and CFPP, as well as the significant superiority of the mixed genotype significantly on the pure genotype in the percentages of solid non-fat and density. It could be concluded from the current study that the selection of females with TG genotype as source for future generations to obtain a generation selected for the desired traits.

Key words: Exon3, CYP19 gene, Local female goats, Milk composition.

1. Introduction

Goats are considered one of the animals that have not received attention in breeding in most Arab countries and are still bred on the margins of agriculture, and they have been efficiently exploited in many Asian and African countries due to their production of twins [1], source of meat as well as high productivity of milk compared to sheep, and the ability to benefit from poor quality feed sources from shrubs and plants more than cows [2], and tolerate different environments [3]. Goat milk contains a high quality of protein, minerals and vitamins, as well as it does not cause allergy which found in cow and sheep milk, in addition to containing protein that is easier to digest compared to cow milk, despite the similar chemical composition of milk between goats and cows [4], and the importance of goats in Iraq appears from the fact that these animals are adapted to hard environmental conditions and poor nutrition ,therefore, attention must be paid to improving these animals, since there are breeds that have a wide scope for improvement.

The genetic production of goat will contribute to filling a part of the deficit resulting from the lack of meat and milk and their high prices, the milk also considered one of the economic traits that are affected by genetic and non-genetic factors like any other economic trait [5]. Therefore, the productive traits depend largely on the environment in which the goat grows, and there are complications that obstruct the genetic improvement of milk production and the length of the milk season, as it is the result of the influence of several factors, some of which are genetic, therefore, it is necessary to calculate the non-genetic factors because they obscure the genetic effects and another is not genetic [6]. This study aimed to determine the CYP19 gene polymorphism in the third exon region in local goat breeds and to investigate its effect on milk components by diagnosing alleles and comparing the genotypes of CYP19 gene.



2. Materials and methods

Blood samples were collected from local goats from the jugular vein with a volume of 3 ml in EDTA Tube for DNA extracting, the study was conducted at the fields of one of the breeders in Babil Governorate / Al-Qasim District, (field side), while genetic analyzes were conducted in the Bayolat laboratory / AL- Qadisiyah (laboratory part), where the genetic material (DNA) was extracted and the CYP19 gene was detected by PCR technique, a sequencing of nitrogenous bases was conducted outside Iraq, and the composition of the milk were examined by the Lacto Flash device in the Public Health Laboratory at Al-Qasim Green University / College of Veterinary Medicine.

2.1 Nutrition

The goats were raised in semi-open barns to provide the appropriate climatic conditions and environment, especially during the breeding season, pregnancy and parturition, in addition to providing health, veterinary care and nutrition. Concentrated fodder was provided, as well as green fodder and rough fodder, this quantity increased during the breeding and pregnancy season.

2.2 Experimental animals

In the present study 30 local goat breed were used in this experiment, were born single and produced milk (not dry), with healthy condition and are given rations in the barn and grazing in the morning.

2.3 Blood sample collection

Blood samples was collected from the jugular vein of each animal in a collection tube containing anticoagulant material (EDTA tube) type K3 EDTA and transported in a cool box to the laboratory for freezing at -18°C until the time of DNA extraction.

2.4 DNA extraction

DNA was extracted from goat blood samples to perform a molecular assay for CYP19 gene as follows:

2.4.1 Protocol of DNA extraction

DNA was extracted from frozen blood according to the instructions of the kit supplied by USA Geneaid company and according to the manufacturer of the detection kit (Elabscience, China).

2.4.2 Measurement of DNA purity and its resulting concentration using a Nanodrop

The extracted genomic DNA from blood was assayed using a Nanodrop (THERMO USA).

2.4.3 DNA Electrophoresis

Electrophoresis was carried out to determine the DNA fragments post extraction process and to detect the presence of DNA to determine the size of the resulting bundles.

2.5 Detection of the CYP19 gene using PCR

2.5.1 Primer selection

The primer was selected as shown in table (1) to conduct molecular detection and study phenotypic polymorphism of the CYP19 gene.

Table 1. Primer sequence supplied from IDT Integrated DNA Technologies, Canada.

Abbreviated Gene	Sequence	Product Size	reference
	(F) 5-CCA GCT ACT TTC TGG GAA TT-3		Davari (7)
CYP19 EXON3	5-AAT AAG GGT TTC CTC TCC ACA-3 (R)	140bp	Varanlou (2017)

2.6 Polymerase chain reaction (PCR)

The materials in table (2) were used for molecular detection using the polymerase chain reaction of the CYP19 gene with a volume of 25 microliters. The samples were placed in the reaction apparatus according to the reaction conditions for each duplicated gene segment. After the reaction was completed, the polymerase reaction product was migrated to ensure that the required segment was doubled, then materials were mixed by the mixer device (Vortex), then the tubes were transported to the polymerase reaction device and the conditions of the polymerase chain reaction were set as shown in the table (2).

Table 2. Materials used in the polymerase chain reaction for the CYP19 gene.

Materials	The quantity in microliter
Master Mix 5	5
Extracted DNA	2
Primer	F : 1 R: 1
Distilled Water	16
final size	25

Table 3. Conditions for bundle duplication of CYP19 gene in PCR reaction.

No.	Steps	Temperatures	Time (minute)	Cycles
1	Initial denaturation	94 °C	5	1
2	denaturation	94 °C	30	
3	Annealing	55 °C	30	35
4	extension	72 °C	30	
5	Final extension stage	72 °C	5	1

2.6.1 Loading the product of the polymerase chain reaction and electrophoresis

10 µL of DNA ladder and 5 µl of PCR products were loaded in agarose gel with concentration of 1.5%. The migration was carried out at a voltage of 100 V/cm and a current of 65 mA for one hour, and the bundles were viewed by a UV (light transilluminator), and then photographed using a photographic documentation system (photo documentation system).

2.6.2 Sequencing of the nitrogen bases of the bundle

After DNA extracting and multiplying the target bundle by PCR technology, which is 140 base pairs, the bundles was sent to the Korean Macrogen Company to find out the sequence of nitrogenous bases for each experimental sample, and then the results were analyzed.

2.6.3 Analysis the results of the nitrogenous base sequence of the CYP19 gene

Sequencing results were analyzed based on the NCBI website. To perform the alignment sequencing, the Bio edit program and Mega7 program were used to detect the presence of the SNP and draw the evolutionary tree of the CYP19 gene (3).

3. Results and discussion

3.1 DNA extraction

The presence of DNA in the studied samples was confirmed by electrophoresis (Fig. 1), which is one of the common ways to confirm the presence of DNA using the electrophoresis technique (8).

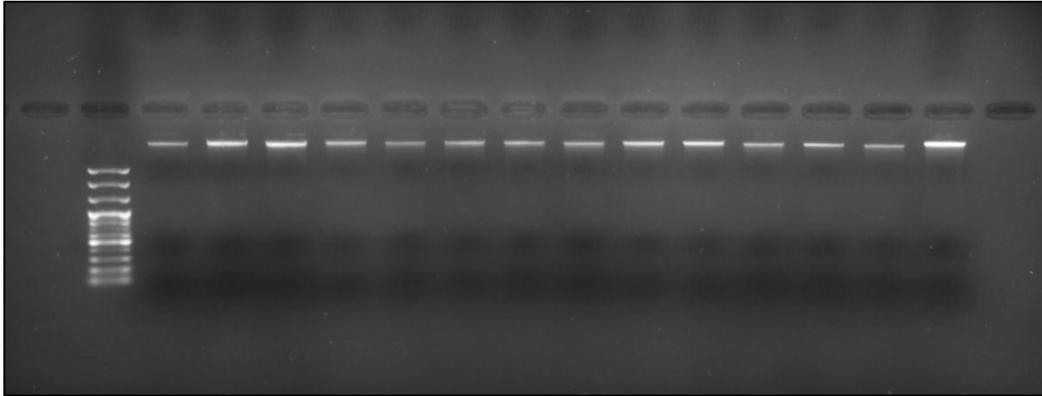


Figure 1. DNA packages extracted from local goat samples using electrophoresis technique.

3.2 Detection of CYP19 gene by PCR

The results of the detection of the CYP19 gene using the special primer (Exon3) showed that all study samples contain this exon and the results of the electrophoresis showed the presence of the bundle of this exon with a size of 140 base pairs (Fig. 2).

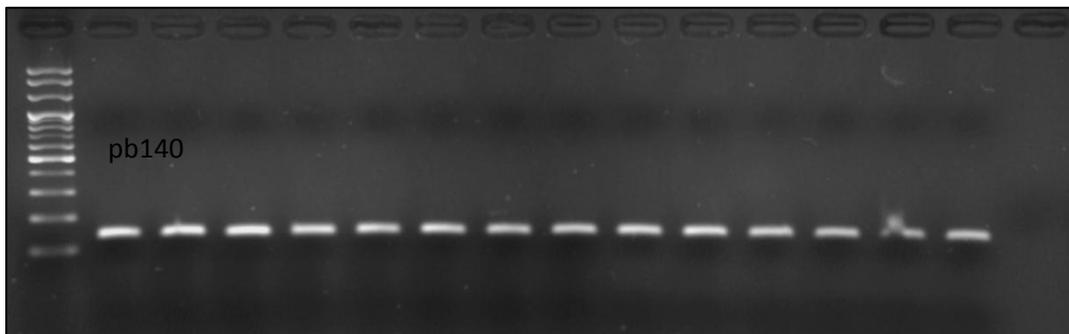


Figure 2. Electrophoresis of the CYP19 gene in the third exon region using the gene product amplified by PCR technique.

3.3 Detection of the genotypes of the CYP19(Exon3) gene

The technique of detecting the sequence of nitrogenous bases was used to find out the series of nitrogenous bases constituting the bundle (140 base pairs) of the CYP19 gene for the third exon site (Exon3). The results of the genetic analysis showed the presence of three genotypes for which are (TT, TG, and GG) using the (Bio edit) program, as well as the NCBI website.

TT: dominant genotype., TG: heterozygous mutant genotype, GG: homozygous mutant genotype

The results shown in table (4) showed the percentages of genotypes for CYP19 gene in the studied samples, with the highest percentage for genotype (TG) 40%, and the lowest percentage for genotype (GG) 26.66, while the percentage of genotype (TT) was 33.34.

Table 4. Percentages of CYP19 genotypes for local goat samples.

Genotype	Number	Percentage
TT	10	33.34%
GG	8	26.66%
TG	12	40%
Total	30	100%

3.4 Relationship of the genotypes with fat percentage

The results in Table (5) indicated a highly significant ($p < 0.01$) increase in the percentage of fat among female goats carrying the TG and TT genotypes than GG genotype, which amounted to 6.71%, 6.11% and 4.95%, respectively. The results are close to what was reached by (9) on Libyan goats in the Al-Bayda and Al-Durna region, the percentage of fat among them reached 3.54 % and 3.16 %, respectively, which means that there is a significant difference between the two breeds. While (10) found a significant difference between the local and Shami goat breeds, with the percentage of fat reaching 3.54 % and 3.16 %, respectively. The results of the present study were higher than what was found (11) when studying the Cypriot, local and battered goats between them, which amounted to 3.17%, 2.90% and 2.87%, respectively, with no significant difference between them.

3.5 Relationship of the genotypes with solids SNF percentage

Table (1) showed significant differences ($P < 0.05$) for females with TG and TT genotypes over their GG counterparts in the percentage of solids in their milk, which amounted to 8.87%, 8.05% and 7.97%, respectively. These results agree with (9) in a study on the Libyan goats in the Al-Bayda region, if they amounted to 12.60%, while they did not agree with what was reached in the goats of the Al-Durna region, which amounted to 15.49%.

3.6 Relationship of genotypes with density

The results in Table (5) showed a significant superiority ($P < 0.05$) in milk density of females with TG genotype than their counterparts TT and GG, which amounted to 1.026, 1.018 and 1.015, respectively. These results are similar to what was reached (9) in his study on Libyan goats in Al-Bayda and Al-Durna, where they reached 1.043 and 1.033, respectively.

Table 5. Genotypes for fat, solid non-fat, and density ratios.

Mean \pm standard error			No.	Genotype
Density	Solid non-fat	Fat		
1.026 a	8.875 a	6.716 a	12	TG
1.018 ab	8.050 ab	6.110 a	10	TT
1.015 b	7.970 b	4.959 b	8	GG
*	*	**	30	Level of Significance

3.7 Relationship of genotypes with protein percent

Table (6) shows high significant superiority ($P < 0.01$) in the percentage of protein of females with genotype TG than those of genotype TT and GG, which reached 4.404%, 3.13% and 3.02%, respectively. (8) Indicated a significant superiority ($P < 0.05$) in his study on the Libyan goats in the Al-Bayda and Al-Durna region where the protein percent in the milk of the studied breeds was 3.85 and 4.43%, respectively. While (10) found no significant difference between local and Shami goats in protein percentage, which reached 3.57% and 3.42%, respectively. (11) also showed no significant difference between the Cypriot and local goats and the striking goats, which were 3.00%, 2.96% and 2.99%, respectively.

3.8 Relationship of genotypes with lactose

The results in Table (6) showed a high significant superiority ($P < 0.01$) in the percentage of lactose in milk of females carrying the TG genotype than TT and GG genotypes which were to 5.98 %, 4.76 % and 4.58 %, respectively. These results are similar with (9) that there is a significant superiority ($P < 0.05$) in the percentage of lactose in his study on the Libyan goats in the white area which were 5.30 % and 4.09 %, respectively, while, (11) indicated no significant difference between studied breeds of Cypriot, local and striking goats, which were 4.41%, 4.43% and 4.49%, respectively.

3.9 Relationship of genotypes to CFPP

Table (6) indicated that there was a highly significant ($P < 0.01$) superiority of CFPP in the milk of females with TG genotype than those with TT and GG genotypes, which were 0.65%, 0.50% and 0.55%, respectively.

Table 6. Genotypes for protein, lactose and CFPP percentages.

Mean \pm standard error			No.	Genotype
Lactose	CFPP	Protein		
5.983 a	0.652 a	4.404 a	12	TG
4.762 b	0.500 b	3.135 b	10	TT
4.581 b	0.558 b	3.026 b	8	GG
**	**	**	30	Level of Significance

It could be concluded from the current study prevalence the TG genotype of CYP19 gene in the Iraqi local goats, which predominated than the pure genotypes (TT and GG), which was highly significant superior ($P < 0.01$) in the percentage of fat, protein, lactose and CFPP, as well as the significant superiority of the mixed genotype than the pure structures in the percentage of solids non-fat and density. With these results, we showed the selection of females with TG genotype as mothers for future generations to obtain a selected generation of desirable qualities.

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