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ASSOCIATION OF CYP17 GENE POLYMORPHISME IN PRODUCTIVE AND REPRODUCTIVE PERFORMANCE IN GOAT

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ABSTRACT

This study was conducted in the Ruminants Research Station, Office of Agricultural Research/ Ministry of Agriculture- Iraq to detect CYP17 gene polymorphisms in Local and Shami goat, as well as to establish if SNPs could be associated with some productive and reproductive traits such as total milk yield (TMY) and its components, in addition to reproductive traits which included litter size and fertility. An important single nucleotide polymorphisms (SNPs) (G2276C) were found in the studying fragment (804 bp), which include Exon2, intron2, and partial of Exon3 by using DNA sequencing technique, two genotypes were produced wild GG and GC in both breeds. Results proposed a highly differences among distribution of genotypes percentage and allele frequencies in both breeds, Results stated a significant relationship between polymorphisms of this SNPs and milk fat percentage and litter size, where hetero genotype GC exceeded GG genotype in milk fat percentage and litter size in Local goat, whereas wild genotype GG distinguished in milk fat percentage in Shami goat.

Keywords: CYP17, SNP, TMY,Litter size, Fertility.

علاقة التراكيب الوراثية لجين CYP17 (G2276C) بالأداء الإنتاجي والتناسلي للماعز

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الخلاصة

اجريت الدراسة في محطة بحوث المجترات التابعة للهيأة العامة للبحوث الزراعية/ وزارة الزراعة على 53 معزة من سلالتين (26 معزة شامي و 27 معزة محلي)، فضلا عن مختبر التقدم العلمي المتخصص بالتقانات الاحيائية وتحليل المادة الوراثية. وذلك لتحديد التراكيب الجينية والتكرار الأليلي في جين CYP17 للماعز لتحديد علاقته بالأداء الانتاجي والتناسلي، بعد الأنتهاء من تفاعل البوليميراز المتسلسل PCR ارسلت النتائج الى شركة Humainzing Genomics Macrogen Company في كوريا الجنوبية لغرض الكشف عن التراكيب الوراثية للمنطقة المدروسة بأستخدام تقنية الحمض النووي، تم الحصول على التركيبين الوراثيين GG و GC للقطعة (G2276C) لجين CYP17، وكانت النسبة المئوية للتركيب الوراثي GG هي 30.77% للماعز الشامي، اما النسبة المئوية للتركيب الوراثي GC كانت 69.23%، وكانت العلاقة بينهما معنوية ($P \geq 0.05$)، وكان التكرار الاليلي للأليل G وC 0.65 و0.30 على التوالي، حيث أظهرت النتائج وجود علاقة معنوية ($P \geq 0.05$) بين التراكيب الوراثية ونسبة الدهن للماعز الشامي حيث سجل التركيب الوراثي GG نسبة دهن 3.24 ±0.30 وسجل التركيب الوراثي GC نسبة دهن 3.83 ±0.81. اما الماعز المحلي قد سجلت النسبة المئوية للتركيب الوراثي GG 40.74%، والنسبة المئوية للتركيب الوراثي GC كانت 59.26%، وكانت العلاقة بينهما معنوية ($P \geq 0.05$)، وكان التكرار الاليلي للأليل G و C 0.70 و 0.30 على التوالي، وأظهرت النتائج وجود علاقة معنوية ($P \geq 0.05$) بين التراكيب الوراثية ونسبة الدهن للماعز المحلي حيث سجل التركيب الوراثي GG نسبة دهن 2.58 ±0.46 وسجل التركيب الوراثي GC نسبة دهن 2.01 ±0.40. وبذلك يمكن استخدام جين CYP17 في برامج التحسين الوراثي.

الكلمات المفتاحية: الماعز، جين CYP17 ، التركيب الوراثي، إنتاج الحليب، الاداء التناسلي.

INTRODUCTION

progress of molecular genetic in last decades allowed to study genes that effect in economic traits in farm animals **(Naeemah & Abraheem, 2016; Ali & Al-Samarai,2018)** CYPs genes play an important role in synthesis of steroids in ovary, Steroidogenesis is a sequential process that converts cholesterol to bioactive compounds in steroidogenic-specific tissues under the control of certain steroidogenic enzymes. The adrenal cortex and the ovary are two examples of steroidogenic specialized organs that produce hormones that govern a variety of reproductive, endocrine, and metabolic activities in females, as well as fertility maintenance. As a result, a disruption in steroidogenesis has been linked to a variety of disorders, including polycystic ovarian syndrome **(Roozbeh, 2021)** The process of synthesis of steroid hormones, including mineralocorticoids, progesterone, androgens, estrogen and glucocorticoid hormones, is carried out by enzymes known as steroidal enzymes such as steroid reductases hydrogeneses (HSDS) and cytochrome P450 enzymes (CYPS **(Zeng *et al*., 2020)** It begins with the conversion of cholesterol and the sequential processes that take place on progesterone, androgens, and estrogens (De Leo et al., 2016). Cholesterol is converted to Pregnenolone in the ovary under the influence of the ovulatory hormone LH, mediated by the CYP11 gene in Theca Cells **(Dumesic *et al*., 2020)** The CYP17 gene also converts pregnenolone (a steroid hormone that plays an important role in the synthesis of steroid hormones between species) into 17-hydroxy pregnenolone and then turns into Dehydroepiandrosterone (DHEA) and produces androgens **(Jaeger *et al*., 2017)** The CYP17 gene encodes for the enzymes 17-α hydroxylase and 17-20 hydroxylase, and the conversion of pregnenolone to 17-hydroxypregnenolone, which is converted to dehydroepiandrosterone and 4-androstenedione through 17,20-layse activity, it affects fertility **(Bestas *et al*., 2021)** Considering CYP17 gene pathway contribute critically with productive and reproductive traits in goats. The aim of the research is to determine whether variants of this gene are associated with economic traits in goats.

MATERIALS AND METHODS

Animals and management

The research was conducted on total 53 does belonging to two breeds Local goats and Shami goats, under same condition of management and feeding, Animals used were 2–5 years old, multiparous, lactating and in their first to fourth lactation.

Sampling, data collection and DNA extraction

A jugular vein blood sample was collected from each female goat using vacuum tubes with EDTA k2 as an anticoagulant. Total milk yield was calculated according to equation mentioned below, from each sample fat, protein, lactose, and solids- non- fat (SNF) values were analyzed by using (Julie-7 scop electric) device.

Total milk production= (one milking / d* 2)* No. of milking days

In addition to, Fertility and Fecundity were calculated from equations mentioned below according to **Amnate *et al*. (2016).**

$$\text{Fertility}=\frac{\text{No.of female give birth}}{\text{Total no.of female inseminated by male}}\times 100$$

DNA extraction

Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood gDNA Miniprep System, Promega.

Primers designed

Based on the goat CYP17 genomic reference sequence one pair of primers
forward:5`-AAGCAGGGAGCTCTACAA-3`
And Reverse: 5`- GGAGTGAACTGTAAGAGGAAAG-3
were designed to amplify 804 bp product covering partial of exon 2, intron2 and partial of exon3 (NCBI).

Statistical analysis

The data was analyzed by used Statistical Analysis System **(SAS, 2012)** to study of CYP17 gene polymorphisms (G2276C G/C; GG and GC) according to the mathematical model, significant differences was compared by used least square means method.

$Y_{ijkl} = \mu + G_i + A_j+S_k+T_l+ e_{ijkl}$

Y_{ijkl}: Observed value n., μ: Overall means, Gi: Effect of CYP17 gene polymorphism (GG, GA, AA). A_j:effect of age (2-5) years; $S_{k:}$ effect of sex; T_l: effect of type of birth; e_{ijkl}: Random error which distributed normally with mean = 0 and variation σ^2e.

Chi-square- $\chi 2$ test were used to compare between the percentages of CYP17 gene polymorphisms.

RESULTS AND DISCUSSION

Identification of CYP17 Gene Polymorphisms

In the current study, one SNP of CYP17 gene was detected in fragment 804bp of CYP17 gene in Local and Shami goat by direct DNA sequencing (Figure, 1). G2276C loci of CYP17 had two genotypes in these populations, which were GG and GC, where noticed absent mutant genotype CC (figures 2-4).

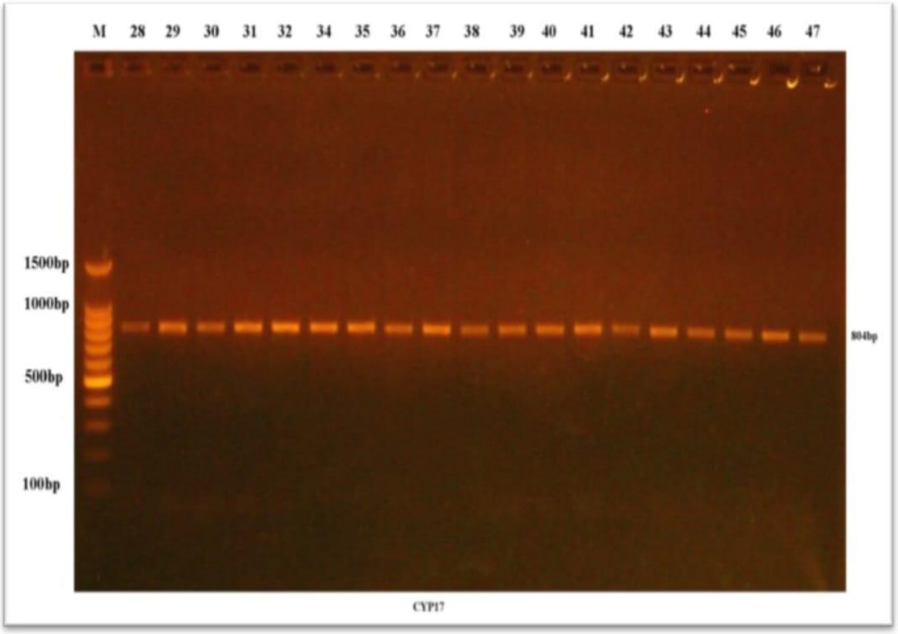

Figure (1): result of the amplification of CYP17 gene fragment of goats samples were fractioned on 1% agarose gel electrophoresis stained with Eth. Br. M: 100bp ladder marker.

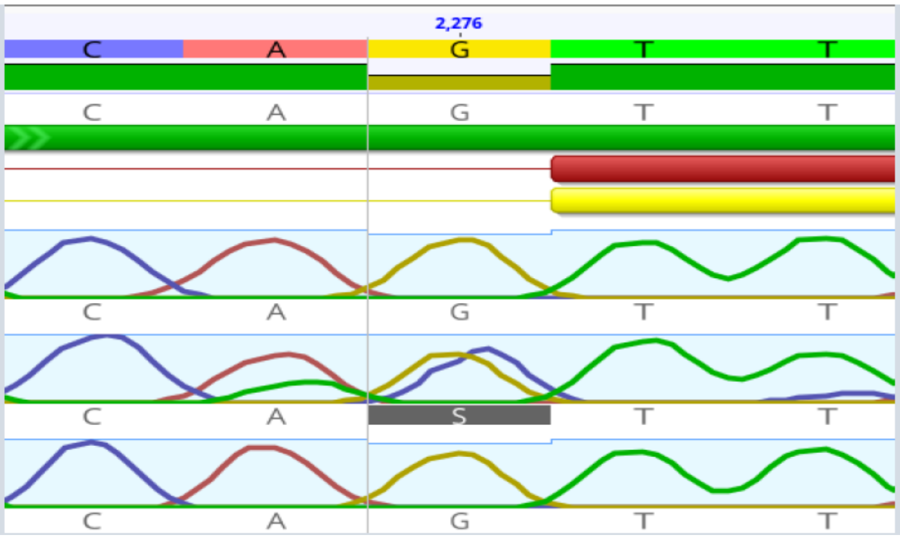

Figure (2): sequence chromatograph of (G2276C) SNP of CYP17 goat gene.

Genetic Parameter of (G2276C) SNP of CYP17 Gene Polymorphisms

The genotypes and allelic frequencies of SNP was calculated and is showed in (Table 1). That reported a highly differences between percentages of genotypes distribution of this SNP in Local and Shami goat, also highly differences in allele frequency of G2276C in both breeds.

Table (1): Distribution and allele frequency of (G2276C) SNP of CYP17 gene in Shami and Local goat.

Breed	Genotype	No.	Percentage (%)	Allele	Frequency
Shami	GG	8	30.77	G	0.65
	GC	18	69.23		
	CC	0	0.00	C	0.35
	Total	26	100%		
	χ2	-	** 8.278	-	-
Local	GG	11	40.74	G	0.70
	GC	16	59.26		
	CC	0	0.00	C	0.30
	Total	27	%100		
	χ2	--	** 9.865	-	-
P<0.01**					

Association between G2276C SNP of CYP17 and milk production traits

Results in (Table, 2) stated there was a significant relationship between polymorphisms of this variant with milk fat percentage in both breeds, where significant increase ($p<0.05$) in milk fat percentage was noticed in individuals that carried hetero GC genotype (3.83%) compared to others with wild genotypes (3.24%) in Shami goat, while results proposed individuals with wild genotype GG distinguished in the same trait (2.58%), whereas there was no significant relationship between this variant and other milk productive traits.

Table (2): relationship between G2276C with milk and its components traits.

Breed	Traits	Unit/No	Genotypes		p-value
			GG	GC	
Local	TMP	Kg	72.06±548.15	47.94±350.10	NS
	FAT	(%)	0.46±2.58a	0.40±2.01b	*
	PROTEIN	(%)	0.02±3.07	0.09±2.87	NS
	LACTOSE	(%)	0.04±4.57	1.05±4.75	NS
	SNF	(%)	0.06±8.35	1.74±9.85	NS
	No.				
Cyprus	TMP	Kg	61.08±503.53	52.12±446.45	NS
	FAT	(%)	0.30±3.24b	0.81±3.83a	*
	PROTEIN	(%)	0.11±3.02	0.05±3.02	NS
	LACTOSE	(%)	0.20±4.28	0.10±4.31	NS
	SNF	(%)	2.82±11.86	1.76±10.02	NS

N.S (non-significant), * ($p<0.05$) TMP (total milk production), SNF (solds-non-fat),
Values in rows with different letters differ significantly**.**

Association between G2276CSNP of CYP17 and reproductive traits

Results demonstrated that polymorphisms of this variant had a significant effect on litter size traits in Local goat, where individuals that carrying hetero GC genotypes were better (1.37 kids/L) than others with wild genotypes GG (1.27 kids/L), while there was no significant relationship between polymorphisms and litter size in Shami goat, also didn't notice effect of this variant and fertility (Table, 3).

Table (3): relationship between G2276C with reproductive traits.

Breed	Traits	Units/No.	Genotypes		p-value
			GG	GC	
Shami	Litter size	Kids/L	0.37±1.81	0.28±1.65	N.S
	Fertility	(%)	3.79±93.48	4.74±92.84	N.S
No.					
Local	Litter size	Kids/L	0.18±1.27b	0.30±1.37a	*
	Fertility	(%)	3.81±92.73	5.62±92.56	N.S

N.S (non-significant), * ($p<0.05$)
Values in rows with different letters differ significantly

CONCLUSION

longer gene CYP17one of the important genes in the genetic improvement programs for livestock, as this enzyme encodes a group of enzymes sub-family belonging to the major cytochrome family.P450Its expression is restricted to the endoplasmic reticulum and plays an important role in steroid hormone synthesis reactions. The genetic heterogeneity detected in the studied area (G2276C) had a clear effect on the percentage of fat, and due to the small size of the sample available during our study, there was no clear effect of the gene on the amount of total milk production and other milk components for the percentage of protein, the percentage of lactose and the percentage of non-solid fat components, so it is recommended to analyze a larger number of goat samples. In the light of this study, we note that there are no significant differences for most of the studied traits, with the exception of the fertility rate and the percentage of fat.$P\leq 0.05$This study confirms that the station's animals have been selected for productive and reproductive traits for a long period of time, so we note the lack of discrepancy between Shami and local goats, in addition to the fact that the number of animals used in the experiment is few 53 heads divided into Shami and local, in addition to obtaining two genetic structures in the nitrogen bases, As well genetic installation in each changes in Netrochemia and the mainstream (land) and alcohol, which indicates the sovereignty of the land of the land on the variable, so the genetic structures showed the same effect The edged qualities.

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