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Relationship of genetic polymorphism of β-lactoglobulin gene with some milk production traits and its components in local goats

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ABSTRACT

This study aims to detection polymorphism of β-lactoglobulin (β-*LG*) and its impact the yield of milk and chemical composition of Iraqi goats. in this project 75 local goats were taken with age (1.5-5.5 years), period of this study obtaining from 31-1-2023 for 28-5-2024, genotyping of this group by PCR-SSCP then sequencing for diagnostic the variation, genotyping and allelic frequency by PopGene32 software, milk samples taken in morning, analyzing by milk analyzer. Result show we have two allele T and A were 0.81, 0.19 respectively, whereas for genotypes frequency, TT and TA were 0.61, 0.39 respectively. χ^2 calculated lower than χ^2 standard, in average daily milk yield (ADMY) and total milk yield (TMY) there is a significant with TT vs TA, 0.872, 0.809 g/day, 119.84, 111.16 kg/day respectively, whereas milk composition there are a significant difference between genotypes, TA vs TT in lactose and solid nonfat 4.84, 4.44 and 8.72, 8.20 respectively. A difference between genotyping in ADM, TMY, LAC and SNF was observed so can be used a genetic marker in improvement programs that uses in goats breeding and farm animals.

علاقة تعدد المظاهر الوراثية لجين بيتا لاكتوكلوبيولين β -LG مع بعض صفات انتاج الحليب ومكوناته في الماعز المحلي

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الخلاصة

تهدف هذه الدراسة الى كشف تعدد المظاهر الوراثية لجين β-*LG* وتأثيرها على انتاج الحليب وتركيبه الكيميائي في الماعز المحلي. في هذا البحث اخذت 75 معزة بعمر 1.5-5.5 سنة، فترة التجربة كانت من 31-1-2023 الى 28-5-2024، تحديد التراكيب الوراثية لهذه العينة بواسطة تقنية PCR-SSCP ومن بعد ذلك اجراء تقنية تتابع القواعد النيتروجينية لتشخيص التغايرات الحاصلة بين مظهر وراثي واخر. تم حساب التكرار الاليلي وتكرار التراكيب الوراثية بواسطة برنامج PopGene للمعلوماتية الحيوية، اما عينات الحليب فقد اخذت في الصباح وتم تحليلها مع كل وقت. أظهرت النتائج وجود اليلين هما T وA، كان تكرارهما 0.81 و0.19 على التوالي بينما تكرار التراكيب الوراثية TT وTA كان 0.61 و0.39 على التوالي. معدل انتاج الحليب اليومي والكلي قد سجل تفوقاً معنوياً لصالح التركيب الوراثي TT وبلغت قيمتها872 غم/يوم و 119.84 كغم/موسم مقارنة بالتركيب الوراثي TA وكانت قيمها 809 غم/يوم و 111.16 كغم/موسم على التوالي، وبخصوص التركيب الكيمياوي للحليب وجد تفوقاً معنوياً للتركيب TA على التركيب الوراثي TT في كل من اللاكتوز والمواد الصلبة اللادهنية اذ بلغت 4.84 و4.44 ، 8.72 و 8.20 %على التوالي. الاختلافات الحاصلة بين التراكيب الوراثية يمكن ان تستخدم كمؤشر وراثي في برامج التحسين التي تستخدم في أنظمة تربية وتحسين الماعز وحيوانات المزرعة.

الكلمات المفتاحية: الماعز، جين البيتالاكتوكلوبيولين، التركيب الوراثي، صفات الحليب.

INTRODUCTION

Goat is one of the oldest animal species that was domesticated in Mesopotamia and then later spread to all parts of the world Approximately 10,500 years ago, its play a major role in the livelihood of a wide proportion of small and marginal holders (Saleh *et al.,*, 2023). in this time, updates and changing in molecular genetics have led to the identification of multiple genes, genetic markers, important genes associated with economic traits of interest in livestock. MAS, is a breeding strategy that uses molecular markers to identify and select specific traits in animal and plants, such as resistance to diseases or improved tolerance to environmental stresses (Chukwu *et al.,* 2019). There are a lot of genes that is approved in breeding of ruminant, *β-LG* one of genes uses in MAS and breeding program, it plays a crucial role in the milk quality (Kahilo *et al.,* 2014). There are a lot of papers and searches refers to genetic effective of this gene with milk yields in goats and sheeps (Kumar *et al.,* 2006 ; Garzon and Martinez, 1992) . It has been proven that polymorphisms of the β-LG gene affect milk characteristics (Hedayat *et al.,*, 2020) Single nucleotide polymorphisms have been reported for the β-LG gene in several such as saanen goat breeds. β-LG encode to a polypeptide has molecular weight 18 KDa with 162 amino acids (Gharedaghi *et al.,*2016). expressed of β-LG has a high-level during lactation,

especially in the mammary gland (Gündüz and Biçer,2023). This study aims to detection of β-LG polymorphisms in local goats and its effect on some economic traits.

MATERIALS AND METHOD

This study included several steps, firstly collection of samples, DNA extraction, amplification of target sequence, PCR-SSCP, Sequencing, then analysis of Data by statistical and software.

Ethical Approval

All applicable national and international guidelines for the care and use of animals were followed.

Collection of blood and milk

In this study, 75 blood samples were collected from animals by vacutainer needle and placed in EDTA tubes 6 ml that are in ruminant Research Station / Agricultural Research Department, Abu Ghraib site - Iraqi Ministry of Agriculture. All animals are raised under the same system management and feeding. Data of milk yield and composition were recorded every week in morning for five months, Milk components were analyzed by milk analyzer (Julie 7z) in same the station.

DNA isolation and PCR amplification.

Isolation of gDNA by procedure of (Geneaid Biotech Ltd®., Taiwan) using Genomic DNA Mini Kit following the recommended procedure, isolation DNA was kept and stored at –20 C° Until uses. checked The DNA quality by electrophoresis fig (1), and the purity was measured the by a nanodrop. DNA extraction were done in genetic engineering and biotechnology laboratory in Biotechnology department- University of Samarra.

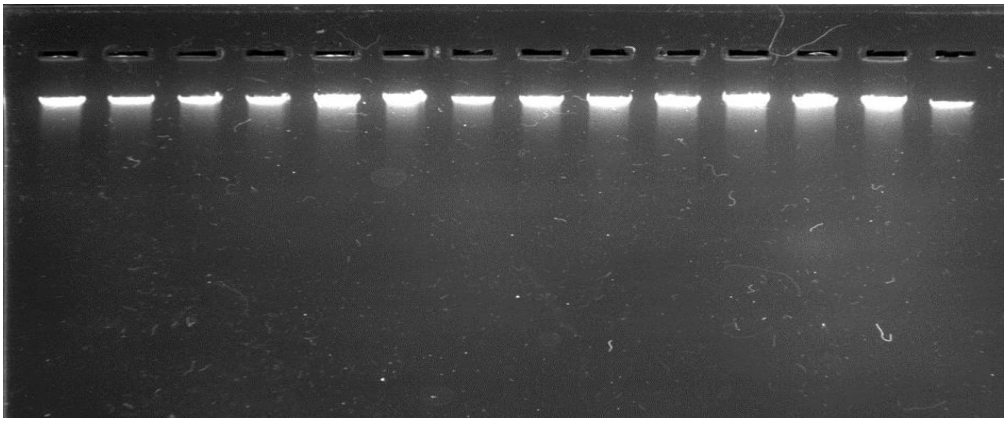

Fig (1): Electrophoresis of gDNA (agarose 0.8%).

Sequence of primer β-LG in this study were taken by (Gündüz and Biçer, 2023) and (Z33881.1). one pairs of primer in this study it was used to amplify β-LG accession number (334bp) as shown in table (1). For promoter region and 5' UTR .

Table 1: Primers description of β-LG gene

Gene	**Sequence** 5′→ 3′	**Tm (C°)**	**Region**	**PCR product (bp)**
β-LG_F	CGGGGATGAGCCAAGTAGGA	61.05	promoter region and 5' UTR	334
β-LG_R	AACCCGACGTCACAGCCTCT	63.61		

Tm = primer melting temperature

PCR reaction was a volume 25 µl , protocol is, initial denaturation 94 was C° 5 min, followed by 35 cycle, second denaturation 94 C° for 30 Sec, Tm for annealing was 63 C° for 30 sec, initial extension 72 C° for 30 sec, then final extension 10 min. and hold for some time, As shown in Table 2. Amplification was done by Thermal cycler (applied biosystems, Taiwan)

Table 2: Amplification of PCR For β-LG gene

Steps	**Time**	**Temp** C°	**Cycles**
First denaturation	5 min	94	1
Denaturation	30 sec	94	
Annealing	30 sec	63	
Extension	30 sec	72	35
Final extension	10 min	72	1
Hold	∞	4	

Result of PCR product fig (2) was analyzing by SSCP technique after denatured by heating and cooling then electrophoresis in PAGE, then uses sequencing to diagnostic the variation in sequence of nucleotides.

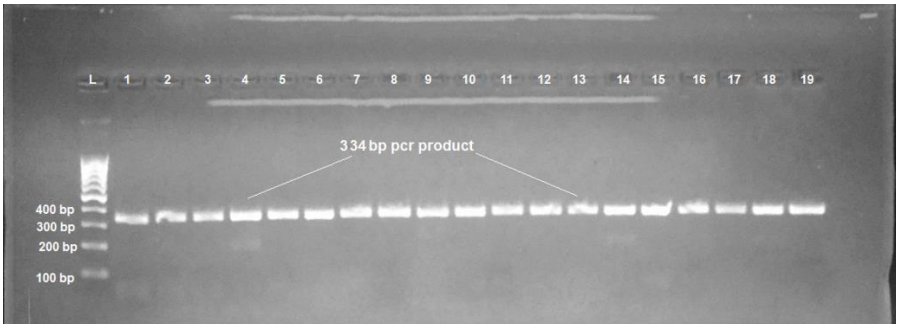

Fig (2): electrophoresis of PCR product (2% agarose).

Statistical analysis

Data for this study was statistically analyzed by (SAS 2012), F-test were tested the relation between polymorphism of gene with productive traits at significance level ($P \leq 0.05$).

genotypic and allelic frequencies was calculated by POPgene32. And Chi square value by $\chi^2 = \Sigma \frac{(o-e)2}{e}$. Statistical model was**:** $\mathbf{Y_{ij} = \mu + G_i + e_{ij}}$.

μ: mean, G_i: effect of genotype: Random error that is normally distributed with a mean equal to zero and a variance of $\sigma^2 e$.

RESULTS AND DISCUSSION

Take 3µl from PCR product and mix with 3µl SSCP loading dye, after that denature of sample in 90 C° for 10 min, and taken out after this period directly to a containing icebox for two minutes in the ice and loaded in gel. This technique shows two patterns of samples fig (3).

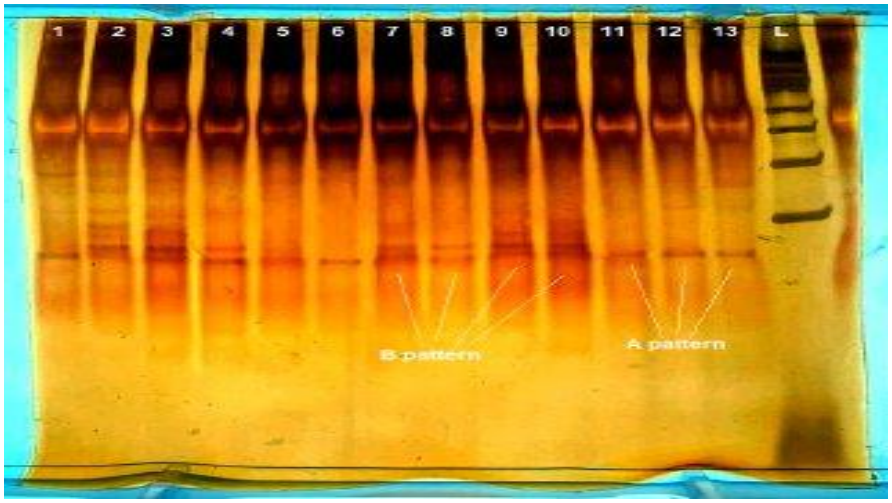

Fig (3): Patterns of genotypes β-LG gene

Allele and genotype frequency estimated and calculated Hardy-Weinberg equilibrium by POPgene32(Yeh *et al.,,* 2000) software packages of the population Table (3) show

percentage of Allelic and genotypic frequencies in population and number of animals per genotype. In current study, there is two alleles of this gene: T and A with three genotypes: Allele T was significantly higher than A allele 0.81 and A 0.19 respectively, concerning genotypic frequency homozygous TT of β-LG gene shows a significant superior it was 0.61, on other hand heterozygous genotype TA was lowest values was 0.39. There is no difference observed among individuals within group.

Table (3): Percentage of Allelic and genotypic frequencies

Genotype	No	Genotype frequencies
TT	46	0.61
TA	29	0.39
Total	75	100 %
Chi χ^2	---	3.92 *
	Allele frequency	
T		0.81
A		0.19
	* ($p \leq 0.05$)	

Tow samples were taken per genotype to sequenced for promoter and 5` UTR region from β-LG gene, after performing SSCP Technique. The alignment results of the β-LG 334 bp amplicons referred to the presence of two patterns of genetic representations in the 186th position between the two assigned genotypes (Fig. 4)**.**

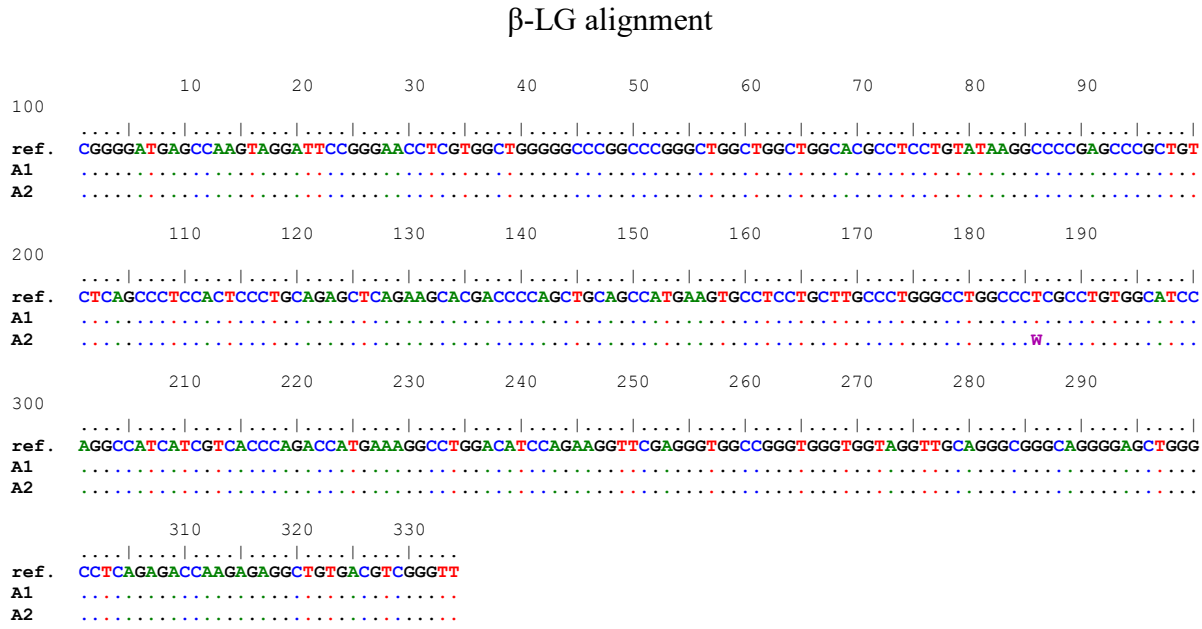

Fig (4): Alignment of samples with reference.

In the position 186th there are a difference between a wild and mutant genotype, reference or wild has T in this position, and on other hand a mutant genotype has W that refers to either A or T in this position.

Sequence of β-LG

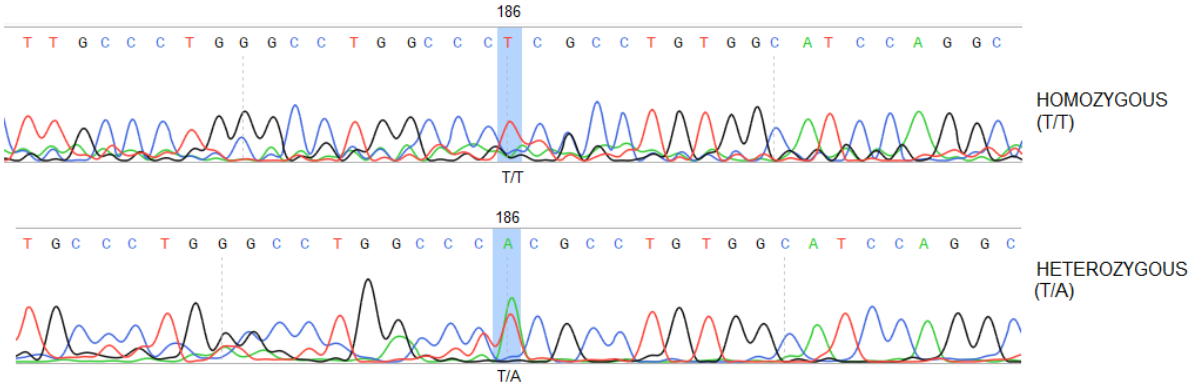

Fig (5): variation between homozygous and heterozygous in β-LG.

Result in table 4 shows the TT of β-LG have significant effect (p≤ 0.05) in ADMY and TMY vs AT it was 0.872, 0.809 g/d, 119.84, 111.16 kg respectively. On other hand, no significant differences were observed between genotypes in each of LP and peak. This study reported the presence of two genotypes in local goats, this result may be return for inbreeding or acclimation (fitness). Results shows similarity to same studies such as (Gündüz and Biçer, 2023) which shows wild genotype better than mutant in ADMY, TMY in kills dairy goat, in addition (Işik, 2017) that mentioned the wild genotype were significant (p≤ 0.05) in milk yield for sannen breed. In other hand, mentioned (EL Hanafy, 2015) in his study at three breeds of Saudian goats (Ardi, Habsi, Harri), the wild genotype (AA) better than AB and BB in milk yield during 16 weeks. While (Ambarwati *et al.,* 2017) didn't shows any differences between genotypes.

Table (4): Effect of genotypes of β-LG in milk production

Genotype	N	ADMY (Gram/day	LP (Day)	TMY (Kilogram)	Peak (Day)
TT	46	0.872±0.01[a]	137±1.09	119.84±2.24[a]	39.13±0.90
TA	29	0.809±0.01[b]	137±1.45	111.16±1.91[b]	39.93±1.57
Total	75	*	NS	*	NS

Different letters within column indicating of significant differences between genotype (p≤ 0.05)
N: number of animals, ADMY: average daily milk yield; LP: length period, TMY: total milk Yield, NS: non-significant

In this study result table (5) of chemical analysis shows a significant difference ($p \leq 0.05$) between genotypes, TA genotype it was better than TT in lactose and solid nonfat 4.84, 4.44 and 8.72, 8.20 % respectively. Whereas other components FAT and Protein didn't any differences between the genotypes. These results it agree with Khaldi et, all. (2023) at Tunisian goats in lactose only, wild genotype CC had superior CT. whereas other chemical characteristics, our study it's not match with some studies such as Wardani et, all (2022) at Indonesian Senduro goats and Darwish et, all. (2022) at Egyptian goats in addition Ambarwati et,all. (2019) at Turkish Saanen goats.

Table (5): Effect of genotypes of β-LG in milk components

Genotype	N	Lactose	FAT	Protein	Solid non fat
TT	46	4.44±0.02 [b]	2.02±0.03	2.83±0.02	8.20±0.04 [b]
TA	29	4.84±0.01[a]	2.05±0.03	2.81±0.03	8.72±0.02 [a]
Total	75	❋	NS	NS	❋

Different letters within column indicating of significant differences between genotype ($p \leq 0.05$)

CONCLUSION

Results of current study refers to a significant correlation between β-LG polymorphism and ADMY, TMY, lactose, solid non fat. So, this gene is important for improvement the productive and some economical traits in goat and farm animals generally. Some studies that have conflict results it concern the relation of polymorphism of this gene with milk yield and characteristics, this variation of these results may be return to number of samples that studied it and breed. So, must be study including a large number of animals and more specific about study region of gene. Suggested this study dependence β-LG as a marker gene for milk yield and chemical component especially 5`UTR and Promoter in local goats. It is possible to carry out a wider study and of different breeds.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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