"GENETIC POLYMORPHISMS OF CSN3 GENE AND ITS EFFECT ON SOME PRODUCTION TRAITS"

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ABSTRACT

Kappa-Casein gene (CSN3) polymorphisms were investigated in one breed of sheep (Awassi) reared in Babylon City. Genomic DNA was extracted from blood of 51 samples of Awassi ewe by using one primer. The primer of PCR were sequenced and prepared by Integrated DNA Technologies Company. PCR productions were loaded on gel electrophoresis. The polymorphism of kappa - casein gene was genotyped by *Hindlll* restriction enzyme (TAKARA), PCR products, Ethidium bromide and 2.5% Agarose gel in horizontal Medi Bio Rad electrophoresis with 0.5 x TBE (100 V. for 60 min.) were loaded to identify of results using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. The present paper revealed three genetic patterns (AA, AB and BB) with two alleles (A and B). The gene frequency for A allele was record high percentage than the other allele (B). The statistical analysis of CSN3 patterns was indicated no significance different on parameters of milk components, while the results appeared positive influence of AB pattern on milk components inside the breed, that is confirm high in heterozygous genotype production (AB) in the sheep are reported in the present study. Furthermore, the first observation for Kappa casein patterns of Awassi sheep breed in Babylon city, Iraq was reported by the present paper. In addition, the population of kappa casein gene with AB genotype was the best choice for good fat and protein percentage of milk composition in the breed is previous reported.

Key words: Iraqi Awassi Sheep, genotype, CSN3 gene, QTL, RFLP-PCR.

"التباين الوراثي لجين ال CSN3 و تأثيره على بعض الصفات الانتاجية"

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المستخلص

ان التباين الوراثي للجين CSN3 تم الكشف عنه في سلالة الاغنام العواسية و التي تمت تربيتها في محافظة بابل. تم عزل الحمض النووي الجينومي من الدم ل 51 نعجة عواسية. تم تجهيز دليل تفاعل البلمرة المتسلسل (البادىء) من قبل شركة Integrated DNA Technologies. رحلت نواتج ال PCR على الرحلان الكهربائي للهلام باستخدام بادىء واحد. ان التباين الوراثي لجين الكابا – كازين تم التعرف عليها بواسطة إنزيم القطع HindIII المجهز من شركة (TAKARA)، منتجات PCR ، بروميد الإيثيديوم و 2.5% من جل الاكاروز في جهاز horizontal Medi Bio Rad electrophoresis مع 0.5 X TBE لمدة 60 دقيقة و بفولتية 100 فولت و ذلك للتنبؤ بالنتائج باستخدام تقنية تغاير قطع التقييد لتفاعل البلمرة المتسلسل (PCR–RFLP). كشفت النتائج عن وجود ثلاثة أنماط وراثية (AA ، AB و BB) مع أليلين (A و B). ان قيمة التكرار الجيني للاليل A أعلى من قيمتها مع الاليل الآخر (B). أشارت نتائج التحليل الإحصائي لجين CSN3 الى عدم وجود فروق معنوية لمقاييس مكونات الحليب، في حين يوجد تاثير موجب للشكل الجيني AB مع مكونات الحليب داخل السلالة، و هذا يؤكد ارتفاع انتاجية الشكل الجيني AB في الاغنام. علاوة على ذلك، ان دراستنا الحالية سجلت المشاهدات الاولى للاشكال الجينية لجين الكابا كازين في الاغنام العواسية لمحافظة بابل. بالاضافة لذلك، ان الافراد الحاملة للشكل الجيني AB من جين الكابا كازين هي افضل اختيار من حيث ارتفاع النسبة المئوية لكمية الدهن و البروتين في الحليب في السلالة السابقة الذكر.

الكلمات المفتاحية: الاغنام العواسية العراقية، CSN3جين، التركيب الوراثي، الصفات الكمية، RFLP–PCR

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INTRODUCTION

The List of World Watch for Animal Diversity of Domestic Animals, approximately 30% of all resources of genetic are facing extinction currently and about one domestic animal breed is being lost per week. This leads to domestication of some species from animals having desirable characteris-tics for mankind. There are over 800 breeds of sheep in the world, in a variety of sizes, shapes, types and colors (4). Sheep in India and Arabian country have originated from their wild ancestor ovis orientale visnei (24). The Iraqi genetic resources in sheep are poor and reflected by the presence of three major breeds of sheep such as; Awassi, Arrabi and Karradi (1). Sheep production traits affected by genotypic and environmental variation, therefore the improvement of this variation will be lead to increase animal production (19) otherwise; the changes in genetic structure have caused the genetic variability in animal populations. The improvement of genetic to livestock has been depending on the breeding selection with superior of phenotypes mainly. In addition, with genetic science advancement during 20^{th} century, the knowledge of population genetics will be great using for the genetic improvement of animal populations (26). Thus, the applica-tions of bio techniques of population genetics were used for genetic improvement of livestock.The milk of sheep considered to be high of nutritional value with high concentrations of fats, proteins, vitamins and minerals, in comparison with milk components of other animal domestic species (3). There are many genes are responsible for milk components such as: CSN2, CSN3, DGAT, SDS, β-LG...etc. Therefore, many studies about CSN3 gene studied from researcher for different aspects and breeds (5, 8, 14, 17, 23, 25), in sheep specifically (6, 7, 16, 27), for important of this gene in genetic improvement and milk production. In addition, the proteins of ruminant milk consist of four genes for casein: αS1-, β-, αS2- and κ-casein (CSN1S1, CSN2, CSN1S2 and CNS3 respectively). These proteins are represent for nearly 80% of proteins milk of bovine (13), which are classified as fraction soluble (11). These genes are correlated with parameters of performance that explain the genetic variance and can improve the prediction of breeding value. The κ-casein molecule is called a single-chain polypeptide that plays an important and vital role in the chemistry of milk (8). In comparison to other casein types, CSN3 gene are related with higher fat, protein and has influence of significant on milk properties (12). Because RFLP-PCR are simple and cheap, therefore it used with *Hind*III (Promega) restriction enzyme from many researcher on CSN3 gene in animals (18, 22, 25), while this study on CSN3 gene with PCR-RFLP technique are the first study for Awassi sheep in Iraq. The study was aimed to determine possible κ-casein gene polymorphism and their correlated with milk components among Awassi sheep animals from Babylon City in Iraq by using PCR-RFLP method, otherwise, to identification of linkage between DNA marker with loci controlling quantitative characters (QTL) using PCR-RFLP.

MATERIALS AND METHODS

To prove the feature of RFLP-PCR, we attempt to find out the diversity within Awassi sheep breed by using the variability of alleles. Females from this breed were selected randomly from different places in Babylon City, Iraq. Jugular vein is the best site to bull of blood from ruminant animals. Milk and blood samples were collected from same ewes. The blood was collected from these animals by EDTA-tubes that using for collect the blood to isolate the DNA from it. After collection, blood was kept in ice container under 4ºC, and then the samples were transferred to the laboratory. According to the method (1), genomic DNA extract was done. To amplify a fragment of DNA, we used the primer are reported in table1. This primer was prepared by Integrated DNA Technologies Company (Table 1). The total volume of PCR was carried out in 20 µl encompass [30 mM KCl, 10 mM Tris–HCl (pH 9.0), 250 µM of each dNTP "dATP, dCTP,dGTP and dTTP", 1.5 mM $MgCl_2$, 1 U Top DNA polymerase] these components were prepared by (BioNeer Company, Korea) under name PCR Premix and mixed with 3µl from Genomic DNA and primer. All these ingredients have been subjected to the PCR into the Biotechnology lab, Department of animal resources,

Agricultural College, Al-Qasim green University. The principle of PCR that used at the present study were applied at 95°C for 5 min followed by 30 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 4 min. according to the steps of PCR. The samples were screened for *CSN3* gene polymorphism by using PCR-RFLP test with *HindIII* restriction enzyme (TAKARA) at 37°C for overnight to carry out the genotyping. The fragments of restriction that obtained were checked on 2.5% Agar-ose gel with Ethidium bromide in horizontal medi Bio Rad electrophoresis in 0.5 x TBE (100 V. for 60 min.). Gel electrophoresis was showed under UV Trans-illuminator with Nikon camera. Sheep milk consists of many components that are assist of growth and health of mammals. Milk checking in Lab of Milk analysis, Faculty of Food Science, Al-Qasim green Univer-sity was done. After collection the milk, kept the samples in ice cool under 4ºC then sent to milk analysis lab. It is keep it in water bath under 40ºC for heat the milk then filtration and washing milk analysis device (Lacto flash) by Ringer solution. To analyze milk sample, should be take 10 ml from each sample and show the results.

Table 1. Primer sequences of CSN3 locus (Schlieben et al., 1991).

Locus	Primer sequence
CSN3	F 5'-GCT GAG CAG GTA TCC TAG TTA T-3'
	R 5'-CTT CTT TGA TGT CTC CTT AGA G-3'

F = forward, R = reverse

To calculate of allele frequency and genotype for CSN3 gene, should be use counting method (9). In the studies of large population, chi square ($\chi 2$) test were used to evaluate Hardy- Weinberg equilibrium (HWE). To analyze the effect of kappa casein genotype on milk component traits such as: fat, protein and solids-not-fat were used Statistical Analysis System (SAS) software (20) by completely randomized design (ANOVA) accord-ing to the following statistical model:

$Yij = \mu + Gi + eij$

Where

Yij: The analyzed trait of each sheep

M: The overall mean

Gi: The fixed effect of the i^{th} genotype

eij: The random error

RESULTS AND DISCUSSION

Some of the quantitative traits Loci (QTL) in sheep are represented by milk production and milk components. By using data, our observation of RFLP-PCR is a suitable tool for estimated of genetic variability in Awassi sheep. Specific primer were using to amplify of 420 bp kappa-casein gene fragment in sheep breed (Fig. 1), that is agreement with(27), while many size CSN3 gene fragments with different primer sets were amplified by (15). By *Hindlll* restriction enzyme with PCR products, three genetic patterns (AA, AB and BB) were appeared using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) analysis (Fig. 2).

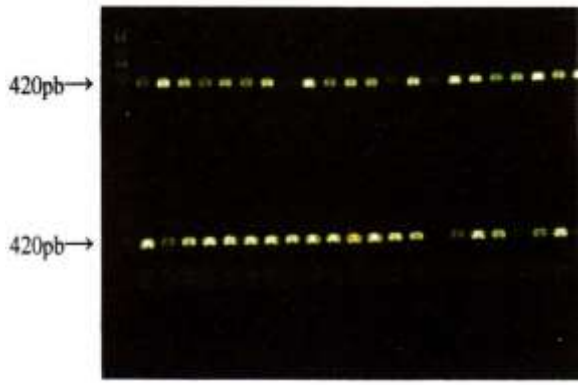

Figure 1. PCR product was showing by Ethidium bromide with Agarose gel for CSN3 gene (M: 100 pb ladder, two lanes: 420 bp PCR product of CSN3 gene).

Many researchers have revealed three genotypes in Italian Sarda and Serbian Pramenka sheep breeds by (10), in New York East Friesian sheep by (23), in Iranian Indigenous Zel sheep by (27).

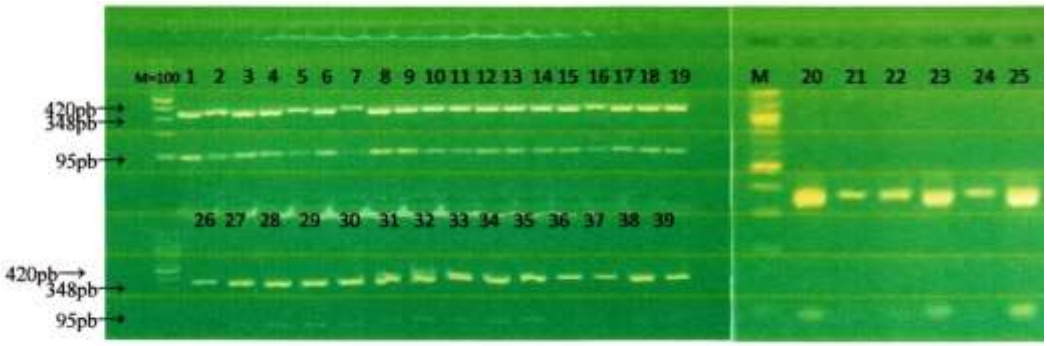

Figure 2. PCR-RFLP analysis for CSN3 gene with 420 bp fragment on 2.5% Agarose gel. 1- PCR product (420 bp), 2-DNA ladder (100bp), 3-AA pattern (95bp and 348bp), 4- AB pattern (95bp, 348bp and 420 bp), 5- BB pattern (348bp). 6- The samples (1-6, 8-19, 27- 35, 38 and 39) AA, (7, 20, 23, 25) AB, (26, 36, 37, 21, 24) BB.

Table 2. Genetic Polymorphism frequencies for CSN3 gene in Awassi sheep milk (P<0.05).

Sheep (n=51)	Genotype			Allele		X^2
	AA	AB	BB	A	B	32.21**
Number	36	8	7	80	22	
Frequency	0.706	0.157	0.137	0.7843	0.2157	

Other researchers have observed just two genetic patterns in three Italian sheep breeds by Ceriotti et al. (2004), in Croatian Pag sheep by Feligini et al. (2005), while Othman et al. (2013) when they studied on three Egyptian sheep breeds, they reported monomorphic genotype that possess one genetic pattern. However, Corral et al. (2010) were recorded five alleles in Merino sheep breed for CSN3 gene by use microsatellite method. The frequency of A allele (0.7843) was higher than the second allele (B) 0.2157 (Table 2). The results confirmed the total mean of fat, total mean of protein and total mean of SNF percentage were 8.56068, 4.3059 and 12.78844 respectively, furthermore the total variance of fat, total variance of protein and total variance of SNF percentage were 5.9319, 0.38049 and 63.5524 respectively. The statistical analysis of data were appeared significant between AB genotype of CSN3 gene (9.15857±0.75 and 4.34285±0.31) with increase of percentage to fat and protein milk compared to AA (8.43235±0.45 and 4.33617±0.10) and BB (8.61999±0.68 and 3.9100±0.14) genotypes (P<0.05) (Table 3). Furthermore, the higher Solids-non-fat was showed with AA patterns than the other two genotypes. (23) and (27) they observed no significant differences between CSN3 gene conformational genotypes with protein, fat and SNF. These results are agreed with the present study in percentage of fat, protein and SNF; otherwise, there are no effects of significant to CSN3 polymorphism on milk composition. The studies of CSN3 gene with PCR-RFLP technique are very few for component of milk sheep breeds around the world therefore, the present observation and other results of researchers was not completed so, the present paper carried out to provide many and new information for Kappa Casein gene in Awassi sheep breed tried to successful of breeding selection with assisted of marker and improvement programs subsequently.

Table 3. Mean and Standard deviations of milk components in Awassi sheep of different CSN3 patterns

Parameter gene	Genotype	Fat%	Protein%	S.N.F.%
Total:		M.= 8.56068	M.= 4.3059	M.= 12.78844
		SD=2.43555	SD= 0.61684	SD= 7.97198
		Var.= 5.9319	Var.= 0.38049	Var.= 63.5524
CSN3	AA	8.43235±0.45	4.33617±0.10	13.19714±0.53
	AB	9.15857±0.75	4.34285±0.31	11.70857±0.84
	BB	8.61999±0.68	3.9100±0.14	10.5410±0.38

Var.=Variance, SD= Standard deviations, M.= Mean and S.N.F= Solids-non-fat.

PCR-RFLP technique was used to detect the genetic polymorphism of CSN3 gene for 51 populations of Awassi sheep. The gene frequency for A allele was record high percentage than the second allele (0.7843 vs. 0.2157). The statistical analysis of CSN3 patterns was confirm no significance different on parameters of milk components, while the results appeared positive influence of AB pattern on milk components within breed, that is confirm high in heterozygous genotype production (AB) in the sheep are reported in the present study. Furthermore, the first observation for Kappa casein pattern of Awassi sheep breed in Babylon city, Iraq was reported by the present paper. In addition, the population of kappa casein gene with AB genotype was the best choice for good fat and protein percentage of milk composition in the breed is previous reported.

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