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GENETIC STRUCTURE ANALYSIS OF KAPPA-CASEIN GENE/ HINDIII AND ITS RELATIONSHIP WITH SOME PRODUCTIVE TRAITS IN IRAQI COWS POPULATION (COMPARATIVE STUDY)

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ABSTRACT

Caseins are a family of milk proteins that exist in several molecular forms (alpha s1, alpha s2, beta and kappa) with variant alleles of each. Kappa-casein (k-Cn) gene exists commonly as A or B variants and are of particular importance to the quality of the milk. This study aimed to evaluate the genetic polymorphism within k-Cn using PCR-RFLP technique as well as the association of these genotypes with milk production and body weight within Iraqi and hybrid bovine as a comparison study. Genomic DNA extracted from 130 healthy bovine (80 Iraqi, 50 hybrids) and amplified using primers that were designed from the cattle κ-Cn, and the structural gene polymorphism was applied using certain restriction enzyme (HindIII) throughout PCR-RFLP technique. There were three genotypes observed, the distribution of the three genotypes and allele frequency were calculated according to Hardy-Weinberg equation, AA=45 (55.32%), 21 (44.89%), LV=29 (38.12%), 25 (44.22%) and VV=6 (6.57%), 4 (10.89%) for Iraqi and hybrid bovine respectively. The results indicated that κ-Cn genotype (AA) increased significantly ($P<0.10$) in Iraqi bovine as compared with hybrid, moreover, κ-Cn genotype (AA) significantly decreased with milk production ($P<0.05$, 0.01) in Iraqi bovine as compared with hybrid cows. In contrast, there was no relationship between κ-Cn genotypes and body weight in both Iraqi as well as hybrid bovine. It can be concluded that the κ-Cn genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the quality of production of this herd.

KEYWORDS: bovine, Kappa-casein, *Hind*III, PCR-RFLP, University of Baghdad, Iraqi Bovine Population.

INTRODUCTION

In marker-assisted selection of dairy livestock, some genes are proposed as potential candidates associated with dairy performance traits. Among different candidates, Casein proteins and their genetic variants have been extensively studied, and reported as important factors associated with lactation performance, milk composition and cheese yield efficiency.[1]

The casein genes are tightly linked and inherited as a cluster so they have a potential value and can play an important role in marker-assisted selection for milk traits.[2] The kappa-casein (κ-Cn) gene has been broadly studied due to its influence on the manufacturing properties of milk, vital role in the processing properties of milk by providing colloidal stability to the casein micelle.[3,4]

Its molecule is a single-chain polypeptide of 169 amino acid (a.a.) with a molecular weight of 19.2 KDa.[5] The bovine κ-Cn gene located on chromosome 6q31 with an overall length of approximately 13kb possesses 5 exons and four introns with most of the coding sequence of the mature κ-Cn protein located in the fourth exon.[6]

Moreover, nine variants have been described in bovine, the most frequent being the A and B alleles. In fact, the point mutation in exon four of k-Cn gene results in two allelic variations A and B.[7] The A and B variants occur in amino acids located relatively close to several glycosylation sites such as amino acids in position 136 and 148 primary structure. In this variation, the threonine is replaced by isoleucine in position 136 a.a, whereas, aspartic acid is replaced by alanine in position 148 a.a for A and B, respectively.[8,9,10]

However, literature reported contradicting results between milk protein polymorphism and production traits relationship[11,12,13,14,and15], while some studies elucidated no significant associations.[16] A possible wide-scale screening of bovine for κ-Cn genotype requires a fast, efficient and low-cost method, independent of age and gender.

This study aimed to evaluate the genetic polymorphism within k-Cn using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique as well as the association of these genotypes with milk production and body weight within Iraqi and hybrid bovine as a comparison study.

MATERIALS AND METHODS

Genomic DNA extraction

The total numbers of blood samples were taken from *vena jugulars* of 131 pubered healthy bovine (80 local Iraqi, 51 hybrids) with age ranged between 3-10 years, and were accomplished by reserving them in EDTA tubes at -20˚C.[17] Genomic DNA was extracted from whole blood samples with isolation kit, ReliaPrep™ Blood gDNA Miniprep System, (Promega, USA). Moreover, the DNA concentration was estimated and the samples were diluted to 30ng/µl in TE at least 24 hours prior to the reaction.

Polymerase chain reaction (PCR)

Genotype analyses were performed using (PCR-RFLP) technique. A 379 bp, fragments of exon 4 in bovine κ-Cn gene were amplified by PCR using forward and reverse primers according to Pipalia *et al*., (2001) (Table 1, Table 2).[18]

Amplification of fragments of the k-Cn gene was done by using PCR technique. The polymerase chain reaction for the κ-Cn gene was performed in a 25µl reaction mixtures (Promega, USA), containing (2x PCR reaction buffer, 3mM $MgCl_2$, 400 µM dNTPs, 10 U Tag DNA polymerase), 5µl template genomic DNA, while κ-Cn gene primer 0.5µl, so far, the sterile water was 19µl respectively. The PCR products were electrophoresed on 1.5% agarose gel stained with Ethidium bromid at constant voltage (5v/cm^2) for 1hour to test the amplification success.[19]

Table 1: The sequence and information of primers used in this study.

Primer	Sequence		Product size	Restriction Enzyme	Reference
κ-Cn	F	5′- CAC GTC ACC CAC ACC CAC ATT TAT C-3′	379 bp	*Hind*III	Pipalia *et al.*(2001)
	R	5′-TAA TTA GCC CAT TTC GCC TTC TCT GT-3′			

Table 2: PCR amplification program of kappa-casein gene.

Step	Kappa-Casein		
	Temp./C°	Time/ Min.	Cycles
Initial Denaturation	95	5	1
Denaturation	95	1	35
Annealing	56	1	
Extension	72	1	
Final Extension	72	10	1

Restriction fragment length polymorphism (RFLP) technique

The PCR products for the tested fragments were digested with the restriction enzyme *Hind*III. The restriction mixture for each sample was prepared by adding 2µl of 10 × restriction buffers to 7 units of the appropriate restriction enzyme and 0.2µl BSA; the volume was completed to 20µl by sterile water. This restriction mixture was mixed with PCR product (~10µl) and incubated at 37˚C for 3 hours in water bath. The digested PCR products were electrophoresed on 3% agarose gel at 5v/cm^2 for 1.5hour, staining with ethidium promide to detect the different genotypes of the two tested sequences by UV-transilluminator and finally documented in gel doc system.[8]

RESULT AND DISCUSSION

There is a considerable interest in the application of molecular genetics technologies in the form of specific DNA markers that are associated with various productivity traits to promote farm animals with an advantage for inheritable traits of meat more efficient and relatively easy selection and breeding of and milk productivity. Many candidate genes have been identified and selected for analysis based on a known relationship with productivity traits.[4]

The amplified PCR products (κ-Cn gene) were visualized as a single band of expected size under the UV with the marker, which was 379bp (Figure 1).

Figure 1: PCR product of bovine κ-Cn gene with size of 379bp. The product was electrophoresis on 2% agarose gel at 5 volt/cm^2 for 1hour. PCR product was visualized under U.V.

We can easily differentiate between 3 different genotypes, AA genotype which lacks restriction site for *Hind*III in 379bp fragment, hence it remains undigested and yield only one fragment of 379bp. BB genotype has one restriction site, yielding two fragments of 225 and 154bp, and AB genotype reveals 379, 225 and 154bp fragments (Figure2). In addition, these variations in genotypes observed in both local and hybrid bovine. In fact, variant alleles (A and B) differ at amino acids 136 and 148. At position 136, Thr (ACC) is changed to Ile (ATC) and at position 148, Asp (GAT) is changed to Ala (GCT) for A and B, respectively [8, 9, 10]. These results supported by.[20,21,22]

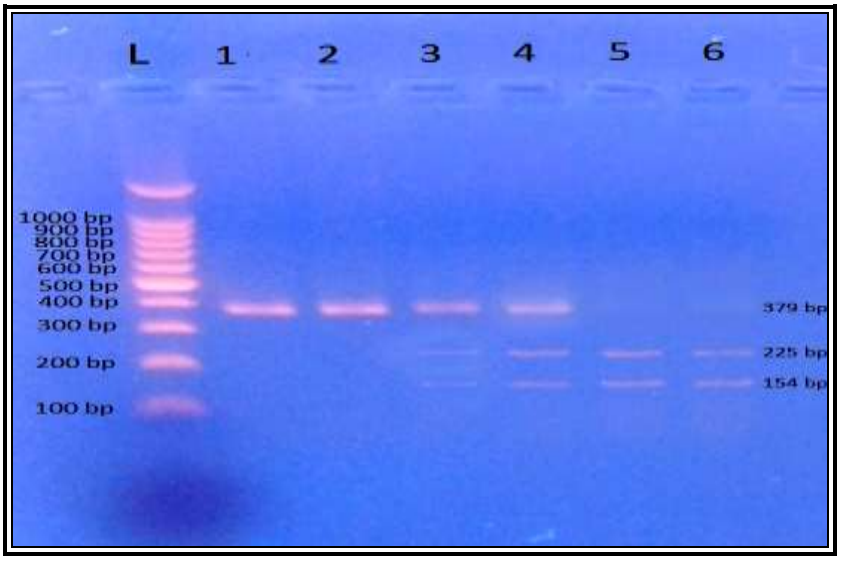

Figure2: **The digestion of PCR products (379bp) of κ-Cn gene with *Hind*III enzyme. The product was electrophoresis on 3% agarose gel at 5volt/cm^2 for 1.5 hour, Visualized under U.V light. Lane L: DNA ladder (100-1000), Lane (1, 2): the undigested PCR products of the κ-Cn gene with size of 379bp which represented dominant homozygous AA genotype, Lanes 3, 4: the digested form which represented the heterozygous AB genotype. Lanes 5, 6: digested form which represented the recessive homozygous BB genotype.**

At the κ-Cn gene, variant A, was predominant in both local and hybrid breeds, throughout homozygous AA in local and heterozygous AB in hybrid breeds, as compared with B allele throughout homozygous BB. The distribution of the three genotypes and allele frequency was calculated according to Hardy-Weinberg equation, AA=45 (55.32%), 21 (44.89%), LV=29 (38.12%), 25 (44.22%) and VV=6 (6.57%), 4 (10.89%) for Iraqi and hybrid bovine respectively. The results indicated that κ-Cn genotype (AA) increased significantly with P-value 2.71 (P<0.10) in Iraqi bovine as compared with hybrid breeds (Table 3). These results

supported by[10,14,22], while disagree with[4,19], this is may be due to their studied samples which were buffaloes κ-Cn genotypes.

Table 3: **The genotype of bovine κ-Cn genotypes and allele frequency calculated according to Hardy-Weinberg equation distribution among samples.**

	Genotypes		
	AA	**AB**	**BB**
Local	45	29	6
Expected H-W Freq.	44.25 55.32%	30.49 38.12%	5.25 6.57%
Allele Frequencies	A= 119 (74%), B= 41 (26%)		
Hybrid	21	25	4
Expected H-W Freq.	22.45 44.89%	22.11 44.22%	5.45 10.89%
Allele Frequencies	A= 67 (67%), B= 33 (33%)		
Chi- Square Value	*P-Value = **2.71**		

***** (P<0.10)

However in this study, κ-Cn genotype AA was associated with the low average breeding value for milk yield in Iraqi bovine as compared with AB hybrid cows, moreover, genotype (AA) significantly increased with low milk production with P- value 7.0 (P<0.05, 0.01) in Iraqi bovine as compared with hybrid bovine. In contrast, there was no relationship between κ-Cn genotypes and body weight in both Iraqi as well as hybrid bovine (Table 3).

Table 4: The comparison of cow κ-Cn genotypes with milk production and body weight between Iraqi and hybrid bovine.

	Milk Production (Litter/day)		
Genotypes	**AA**	**AB**	**BB**
Breed	Mean±S.E.	Mean±S.E.	Mean±S.E.
Local (Iraqi)	3.47±o.17*	3.52±0.18	3.17±0.48
Hybrid	4.81±0.27	4.81±o.27	5.38±0.38
	Body Weight (Kg)		
Genotypes	AA	AB	BB
Breed	Mean±S.E.	Mean±S.E.	Mean±S.E.
Local (Iraqi)	152±3.26	157.67±4.09	141.67±7.92
Hybrid	184.29±4.12	178.42±5.72	187±11.09

***** *(P<0.05, 0.01)*

Rachagani and Gupta[23, 24] analyzed the allelic variants of the κ-Cn gene in Sahiwal and Tharparkar bovine breeds; it has been shown genotype BB of the κ-Cn gene had more influence on the milk yield. While Marziali and Ng-Kwai-Hang[25], suggested that AA

genotype more influence with immune histocompatibility and immune response than BB genotype.

In conclusion, this study indicated that the κ-Cn/AA genotype was more influence in Iraqi bovine, reflecting their immunity for extreme environment features as well as infectious diseases. In contrary, the quantity and quality of milk decreased with this genotype.

According to above, this study indicated that the κ-Cn genetic variants may be used as a genetic aid through increasing the frequency of desired genotypes to improve the quality of production of this breed. Therefore, it has been proposed to increase the frequency of κ-Cn/ BB genotype within breeding programs, preferring sires with the favorable kappa-casein genotypes.

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