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Gene Polymorphism of CSN1S1 and CSN3 Gene Associated with Casein Production Milk Trait in Iraqi Buffaloes (Bubalusbubalis)

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Abstract

Twenty-four blood samples of lactating Iraqi buffaloes were analyzed for the presence of CSN1S1 and CSN3 genes using two pair primers to amplify 314bpand 453bp DNA fragment, respectively. Most investigated samples showed the presence of the CSN1S1 gene. In contrast, all samples showed the presence of CSN3 gene. Sequence analysis of both CSN1S1 and CSN3 genes were analyze using Blast algorithmic tool. The results showed that most of the investigated animals resemble those of the Middle East and Asian buffaloes at a percentage of 100% .Moreover, some of investigated genes showed little differences than animals of the other countries by having polymorphism genes posing DNA mutations. Most mutations were either insertion or transition mutation and located between 10936-10938 region. In conclusion, the mutations occur in these genes, which play a role in milk productivity may face unflavored climate condition in the summer in the south of Iraq.

Keywords: *Polymorphism, CSN1S1, CSN3, casein, milk, buffaloes.*

Introduction

Domestic water buffalo (Bubalusbubalis) is an important milk source in many worldwide countries. About 185 million animals were found around the world(1). Buffalo is of great importance and superiority to domesticate cattle due to its high capacity for climate changes, good nutritional benefits and disease resistance. Buffalo milk contains 7% fat, 16%solid not fat (lower phospholipids and cholesterol levels, and a higher saturated fatty acid ratio (2). For best animal production, genotype selection of the animals must be used according to their productivity(3).Animals have a high ability for genetic improvement in the production of milk and meat (4). As in other bovines, buffalo milk is having four types of casein, αS1, αS2, β and π casein. They are under control of CSN1S1, CSN2, CSN1S2, and CSN3 genes. The most dominant one is β (53.45%), followed by αS1, αS2 and π at 20.61%, 14.28% and 11.66%, respectively (5). Casein proteins are high in an essential amino acid such as lysine which is not found in many plant sources (6). They also have a high concentration of phosphate that compound which allows high amounts of calciumto bind tocasein(7). The purpose of this work was to characterize the genetic polymorphism of the CSN1S1and CSN3 gene in the Iraqi buffaloes.

Materials and Method

Animals and sample collection: A total of 24 blood samples were collected from healthy lactating Iraqi buffaloes (Bubalusbubalis) from different farms of Basrah governorate.

DNA Extraction and Primers: Whole blood of buffalo was used for DNA extraction by ReliaPrep™ Blood gDNAMiniprep System. The primers used for CSN1S1 amplification were previously used (Chessaet al.,2007), with a fragment length of 314bp.

F: TGGATGCCTATCCATCTGG

R: CACTGCTCCACATGTTCCTG.

The primers used for CSN3 amplification with 453bp fragment were described by (8) and have been

used by [9]. The 453bp fragment of CSN3 covers most of the exon IV coding region [10]. They have the following nucleotide sequences:

F: 5'-3'TGTGCTGAGTAGGTATCCTAGTTATGG;

R: 5'-3'GCGTTGTCTTCTTTGATGTCTCCTT

Polymerase Chain Reaction: The PCR reaction contained (25µl) including 5 µl of DNA. The following conditions were used for CSN1S1 gene: initial denaturation for 5 min. at 94 ◦C; 35 cycles of denaturation for1 min at 94 ◦C; annealing for 45 s at 60 ◦C; elongation for 80 s at 68–72 ◦C and a final extension for 7 min. at 72 ◦C. While the following cycling conditions were used for CSN3 gene: initial denaturation for 3 min. at 94 ◦C; 35 cycles of denaturationfor 1 min at 94 ◦C; annealing for 45 s at 60 ◦C; elongation for 80 s at 72 ◦C and a final extension for 10 min at 72 ◦C.

The PCR reaction products were electrophoresed on 1.5% agarose gel stained with ethidium bromide to test the amplification success.Agarose gel was run at 75 V for 4 hrs. Gels were examined and photographedunder UV illumination (E - graph – ATTO -Japan). Fragment size of approximately 314bp and 453bpwas verified as positive for CSN1S1and CSN3 genes, respectively. A 100bp DNA ladder (Bioneer, Korea) was used as a molecular size standard.

DNA Sequencing:Purification of PCR products were done by using GeneJET™PCR Purification Kit (Fermentas #K0701). The product thensequenced by Bioneer ABI 3730XL DNA analyzer. Blast analysis wasdone using Blast directory of the NCBI at (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Twenty-four lactating Iraqi buffaloes were analyzed for the presence of CSN1S1 and CSN3 genes using two pairs of primers for the amplification of 314bpand 453bp DNA fragment, respectively.

PCR results of CSN1S1 gene: The fragment size of approximately ~314bp was verified as positive for CSN1S1 gene. Most investigated samples showed the presence of this gene (Fig. 1).

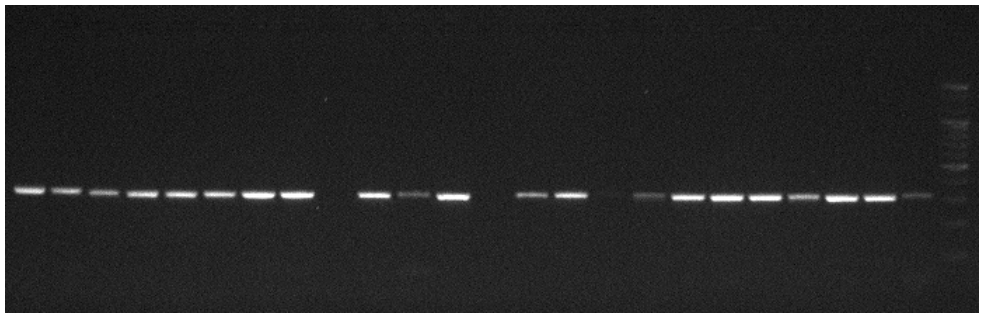

Figure 1: Agarose gel electrophoresis showing the presence of 314bp CSN1S1 gene in blood samples of Bubalusbubalis.

PCR results of CSN3 gene: Fragment size of approximately 453bpwas verified as positive for CSN3 genes. All investigated samples showed the presence of this gene (Fig 2).

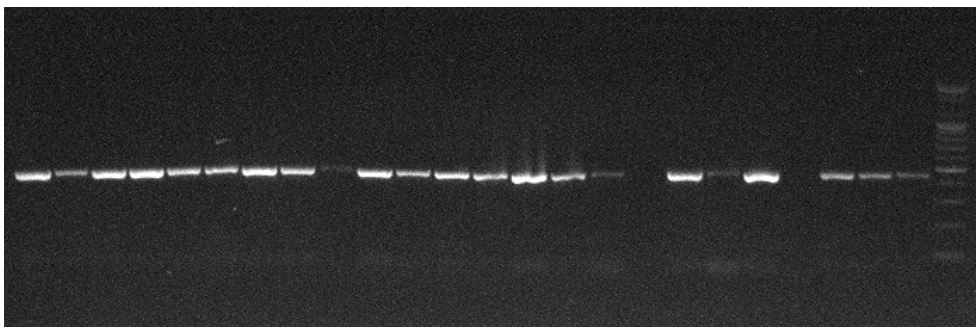

Figure 2: Agarose gel electrophoresis showing the presence of 453bp CSN3 gene in blood samples of Bubalusbubalis.

Sequencing Results: Sequence analysis of both CSN1S1 and CSN3 genes were analyze using Blast algorithmic tool. The results showed that most of the investigated animals resembled those of the Middle East and Asian buffaloes at a percentage of 100%. Moreover, some of investigated genes showed little differences than animals from other countries by having polymorphism genes posing DNA mutations.

Sequencing results of CSN1S1 gene: Results of sequencing of this gene showed 100% identity with Egyptian animal with accession numbers KC577235.1 and JQ670674.1. Only two strains showed insertion mutation at location 67 (Table 1).

Table 1: Results of sequencing of CSN1S1 gene

Source	Identities	GenBank	Nucleotide	Location	Mutation	Sample No.
B.bubalis Egypt	100%	KC577235.1	-	--	-	S2, S3, S6,S13, S15,S17-S23
B.bubalis Egypt	99%	JQ670674.1	- >G	67	Insertion	S4
B.bubalis Egypt	100%	JQ670674.1	-	-	-	S5
B. bubalis Egypt	99%	KC577235.1	- >C	67	Insertion	S7

Sequencing results of CSN3 gene: The results of sequencing of this gene were listed in table 2. The comparison was done with the most resembled Indonesian animals of Bubalusbubalis with accession number MF679163.1. Most investigated samples showed 100% identity whereas five samples showed variable sequence having one or more mutations. Most mutations were either insertionor transition mutation and located between 10936-10938 regions.

Table 2: Results of sequencing of CSN3 gene

Source	Identities	GenBank	Nucleotide	Location	Type of mutation	No. of sample
B.bubalis Indonesia	100%	MF679163.1	-	--	-	N3,N5, N7,N10, N11,N14
B.bubalis Indonesia	99%	MF679163.1	T>C	11131	Transition	N12
B.bubalis Indonesia	99%	MF679163.1	- >C G>A	10936 10937	Insertion Transition	N13
B.bubalis Indonesia	99%	MF679163.1	- >A	10894	Insertion	N15
B. bubalis Indonesia	99%	MF679163.1	- >A - >A	10894 10938	Insertion Insertion	N17
B.bubalis Indonesia	100%	MF679163.1	-	--	-	N18,N20, N21,N22
B.bubalis Indonesia	99%	MF679163.1	- >C G>A	10937 10938	Insertion Transition	N19

Phylogenic tree of the sequenced results showed that both genes (CSN3 andCSN1S1)of local animal are closely related to those previously registered in GenBank from Egypt, Brazil, India, China and Indonesia (Figure 3, A & B).

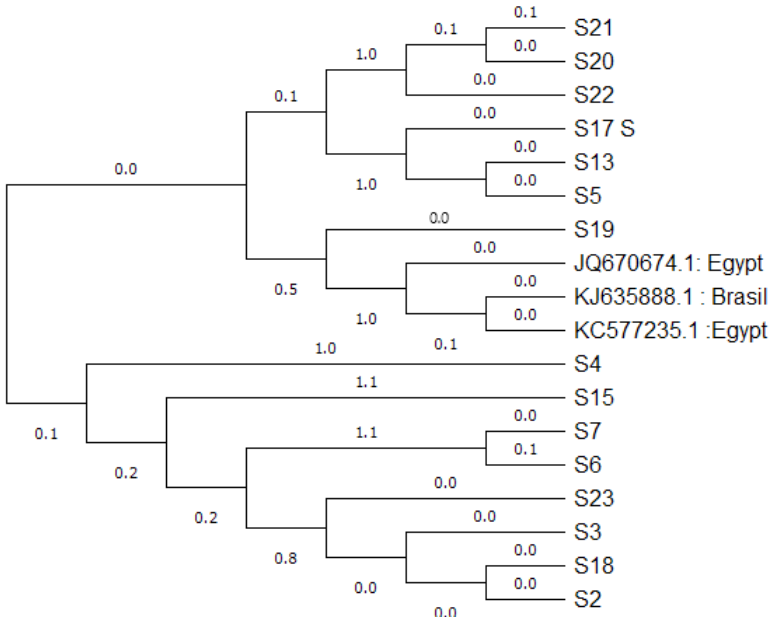

Figure 3: The neighboring phylogenic tree of A= CSN3; B= CSN1S1 genes of local animal compared with that previously registered at GenBankfrom different countries.

Discussion

In order to select more efficient breeding of farm animals there is aninterest in applying genetics technology of specific gene markers associated with productivity characteristics (Spelman et al., 1998). In order to improve animal characteristics and productivity, animals should be selected periodically based on the genotype that reflects their productivity and national economy[11]. CSN1S1 Polymorphism and CSN3 exon IV have not been previously investigated in Iraqi buffaloes. In this study, for the first time we report here, sequence analysis in 24 unrelated Iraqi buffaloes lactating has characterized CSN1S1 Polymorphism and CSN3 exon IV.

Several genes, including casein genes, control the milk trait. CSN1S1, CSN1S2, CSN2, and CSN3 are closely linked to four casein genes. They code a(S1) and a(S2), b, and K casein, respectively. Approximately 80% of the total milk protein is kappa casein [12] CSN1S1 gene sequencing results showed 100% identity with Egyptian animals. [13] confirmed the monomorphism at 178Ser by analyzing Indian buffalo's full CSN1S1 mRNA (accession number: DQ111783). However, a different variant was detected at 192Glu (GAA) versus 192Gly (GGA). Variation of Glu/Gly was detected in bovine animals as investigated by [14]. The Mediterranean type belongs to the Egyptian and Italian buffalo. Mediterranean buffalo appeared as descendant of Indian Murrah buffalo. The long isolation lead to development of some unique characteristics [15].In this study, location 67 strains showed only two additional mutations. It was noted that there were two variants of CSN1S1 in buffalo from Egypt. The variation occurred in the CSN1S1 (178Ser (TCA)/178Leu (TTA) 178 mature protein codon and have been reported in Italian buffalo and referred to HE573920 and HE573919 by [16]. CSN3 gene sequencing results have been analyzed and most of the samples showed 100% identity with Bubalusbubalis Indonesian animals. Between the locations 10936-10938, there was a change in insertion or transition. Exon IV was involved in most of the CSN3 polymorphism. Exon IV mutations are responsible for gene expression differences[17]. The samples of animal blood were collected from buffaloes known for their high production of milk and meat. High temperatures, drought and high water salinity, unflavored climate conditions duringthe summer in the south of Iraq may result in increasing the prevalence of mutations in genes coding for high productivity and higher resistance of animals. Using nucleotide sequence analysis, CSN3 polymorphism has been investigated in buffalo over the past decade. Two variants of nucleotides in codons 135 Thr (ACC)/Ile(ATC) and 136Thr (ACC/ACT) (silent mutation) were reported in Italian [18], Bulgarian[19], and genomic library of water buffalo [20]. Buffalo

polymorphism was investigated using the method of PCR–RFLP. BB monomorphic were mentioned in Egyptian buffalo [21], in Pakistani buffalo[22],and in [23].

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Ethical Clearance: Not required

Conflict of Interest: None

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