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Genetic Analysis of Milk Gene (B-Casein) in BOS INDICUS Cattle

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Abstract

Because of their physiologically active constituents, milk and other dairy products are considered healthful foods. The goal of this study is to look at the -casein genetic polymorphism in Pakistani cattle. Casein research in dairy products and animal husbandry has piqued attention in recent years, because casein is a source of biologically active peptides that may be helpful to health as well as a source of high-quality protein. Furthermore, the polymorphism of -casein and its link to milk production characteristics, composition, and quality has led several efforts to assess the allelic distribution of the -casein locus as a potential trait marker. Dairy goods are dairy products. There are, however, few data on -casein variation in Pakistani dairy cows. We obtained a blood sample, amplified it with PCR, measured it with a Nanodrop spectrophotometer, and genotyped the PCR product using gel electrophoresis and DNA sequencing to estimate the B-casein trait (CSN2) Genetic polymorphism. Cholistani, Sahiwali, and Thari are three separate cows. In the Cholistani and Sahiwali dairy cow breeds, bioinformatics analysis of DNA sequencing of the casein gene found seven nucleotide alterations. In the Taliban race, there is no mutation. These changes were classed as a 02 point mutation in the Cholistani cow breed and a 05 point replacement in the Sahiwali cattle breed, but not a modification in the Thari cattle breed, according to the genetic code. Our findings can be utilized as a guide and reference for selecting high-quality milk for industrial usage, as well as for hybridization and genetic enhancement initiatives.

Keywords: DNA quantification, Cattle, PCR, Gel Electrophoresis

INTRODUCTION

Milk is the primary source of critical nutrients and important raw materials acquired from dairy cows in human diets (Reinhardt et al., 2011). S1 (CSN1S1, 39-46 percent whole casein), S2 (CSN1S2, 8-11 percent), (CSN2, 25-35 percent), and kappa (CSN3, 8-15 percent) are composed of the two main proteins -lactalbumin and -lactoglobulin (16 percent), a small part consists of low-weight peptone/peptide molecules (3 percent), and Milk fat globules constitute membrane protein (MFGM) (1 percent) (

There are around 800 breeds of domestic cattle, which are arbitrarily split into two species: Bos taurus (taurine) and Bos indicus (indicin or zebu). Bradley and Magee (2006) identified several morphological distinctions between indica and pit bulls, the most noteworthy of which being the indica's large hump on the shoulder and neck. Increased heat tolerance, lesser sensitivity to

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gastrointestinal wall ticks and parasites, and lower metabolic rate and nutritional requirements are only a few of the physiological advantages of indicine cattle over bullfighting bulls.

It's unclear where Indicin and Taurine came from. The mitochondrial DNA sequence revealed a 250,000-year difference between the two types, indicating that there are at least two different centres (Bradley et al., 1996). The Y chromosome data also supports multiple centres and highlights the importance of genetic infiltration in the evolution of modern livestock (Pérez-Pardal et al., 2010).

The global cattle herd distribution demonstrates that taurine is primarily used by cattle in European countries, while taurine is primarily used by cattle in India, Brazil, the southern United States, northern Australia, and southern China. Human nutrition is primarily provided by cattle breeds designed for the production of beef and dairy products. They specialise as ruminants in turning low-quality food into energy-dense fat, milk, and muscle. Because of this, humans have exerted substantial selection pressure on these species by generating tiny groups of ancestors with interesting characteristics. Furthermore, less productive varieties are frequently disregarded, resulting in their extinction, as has previously occurred with 209 livestock breeds (Food and Agriculture Organization of the United Nations, 2007). These changes have had a significant influence on species, especially since wild cattle are no longer present and all genetic variation is now found in farmed animals. As a result, a greater understanding of animal genetics may be able to help us mitigate some of these negative consequences.

The quality of dairy products, curdling ability, and cheese manufacturing are all affected by milk protein concentration, which has a substantial economic impact on the dairy business. 2006, Wedholm et al. The four caseins (CN), S1-, S2-, -, and κ-CN, as well as two whey proteins, -lactalbumin (-LA) and -milk Globulins (-LG), account for roughly 90% of the total composition of Dutch milk proteins. 2010; B et al.

Milk protein components can thus be incorporated in the selection criteria of dairy breeding programmes seeking to improve cheese-making qualities, given the adoption of quick and effective technologies such as spectroscopy. These characteristics are determined using Fourier transform infrared (FTIR). Ferragina and colleagues, 2015. Increasing the nutritional value of milk by changing the protein level is another option. Schopen and colleagues, 2009.

Four caseins compose more than 75% of total milk protein (S1-, S2-, B-CN-, and K-CN). Genes encoding these proteins may be found in a 250-kb segment of BTA 6, which has four of them (Hayes and Petit, 1993). In addition to CSN1S1, CSN2 and CSN3 each code for a different amino acid and are found in the following gene sequences: (Threadgill and Womack, 1990) In addition to supplying infants with calcium, phosphate and amino acids (amongst other things), casein and casein have an impact on milk production and cheese manufacturing capabilities (Nilsen et al., 2009). The protein and DNA levels of casein have thus been intensively studied, particularly at the protein level. Cattle are known to carry it. All of the bovine casein genes' polymorphism has been found (Farrell et al., 2004).

As one of the four casein milk proteins, b-casein is critical to milk's structure. There are four casein genomes on chromosome 6, each with 209 amino acids in their protein chain. There have been two studies on this topic: one by Farrel and colleagues (2003); the other by Truswell and colleagues (2005). Casein, the most abundant protein in milk, accounts about 45 percent of the genome's copy number. The A1 and A2 genetic variants of CSN2 are most common (Farrell et al., 2004; Kami et al., 2007). For the first time since its discovery by Halle'n et al. in 2003, the B-casein gene has been characterised (2007). Casein has been shown to include a variety of active peptides known to aid in

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the management of stress and pain (Nguyena et al., 2015). Dairy products' nutritional and technical properties are greatly influenced by casein, a protein found in milk.
Milk protein is highly polymorphic and encoded by genes with a wide range of variation. Milk proteins that are genetically different from one another can only be created by small alterations or deletions in the polypeptide chain (Nilsen, Olsen et al., 2009). Many studies have shown that milk protein from different breeds of cow has high levels of genetic variation. colleagues of Caroli's (2008) (Caroli and colleagues, 2009). A study by Caroli and colleagues was published in 2009.

Milk protein polymorphism and economically relevant production factors, as well as the potential link between milk composition, quality, and quantity depending on genetic code, have all been extensively studied. This (Oner and Elmaci, 2006). This study's objective is to investigate the genetics of Pakistani cow breeds' casein CSN1 genes.

Material and Method:

Sample collection and DNA Extraction

The study was conducted on three cattle breeds namely Cholistani, Sahiwali and Thari cattle breed and Blood specimens were obtained from the jugular vein of cattles. The Animals were maintained at cattle farms in Ghousala, Shaikh Chock Gambat, District Khairpur Mir's, Pakistan. Mubeen Ahmed Phulpoto cattle farm Wisryo Wahn and Naheed Qazi cattle farm, District Sukkur. Fakir Dairy and Cattle Shadi Shaheed Road Near Dargah Long Fakir Khairpur Sindh, Pakistan.
DNA was extracted from blood leucocytes using Thermo Scientific's Gen JET genomic DNA purification micro kit# K0781. After cell lysis releases the highly pure DNA, the whole process takes around twenty to thirty minutes. Nanodrop machines at the Genome Research Centre (HEJ, University of Karachi) evaluated the extracted sample DNA concentration to ensure enough amplification. DNA purity was also assessed using a 260/280 nm ratio**.**

Primer Designing and PCR amplification

CSN2 gene was enhanced in PCR with primers PF11 5'-CAGGAAGATCCGTTGGAGAG3 CF13 5'-ACAGCCTCCCACAAAACATC3 and CF12 5'-CCTAACAGCCTCCCACAAAA3.The PCR reaction mixture contained 12.5µl Master Mix (Green Master Mix, Promega, Madison, WI, USA),10pmol of every primer, 50 mg of DNA template and residual amount, nuclease free H2O was added Zhang et al., 2007 to full the amount of 25µl PCR Mixture. PCR enhanced in thermal cycler (Applied Biosystem®2720, USA).

Gel Electrophoresis

TAE buffer is solution comprising of a blend of Tris base acetic acid and EDTA. The PCR product was run on the 1.5% Agar gel (AG) and 2µl ethidium bromide (EtBr) was used to make it visible in TAE as the running buffer at 70V current for 60 minutes and the bands were watched under UV Trans illuminator (Gel doc system) to study the fragment measured by 1kb ladder.

Bioinformatic analysis

The PCR product was commercially purified and sequenced from Macrogen company of Korea (https://dna.macrogen.com/) and obtained sequence data was subjected to Nucelotide blasting and online sequence alignment was performed and mutations were evaluated, and percentage of mutation was directly counted through MS Excel.

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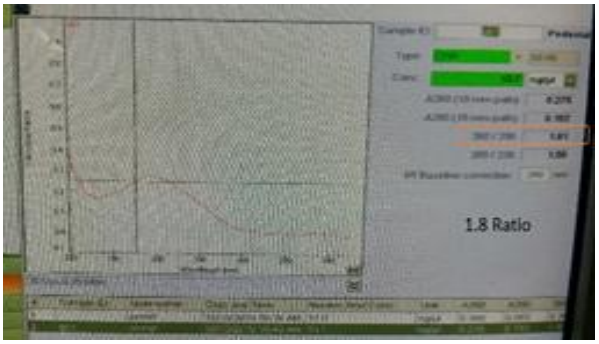
Results

Quantified DNA samples

The amount of DNA in extracted DNA samples was measured using a nanodrop spectrophotometer, and the Gel Documentation system was found to be in the range of 30-103 ng/l, which is adequate for PCR amplification. The results of DNA quantification are given
under Graph 1:



Graph 1: Quantification of DNA using the Nanodrop Technique

SNPs identification

The mutations were analyzed by Blasting on Emseble.org and position of nucleotide change was noted from the PCR product of CSN2 gene. The Size of PCR product is given as under Figure 1 and detailed information of the mutations are provided under table 1 and percentage of mutation is given under table 2,3.

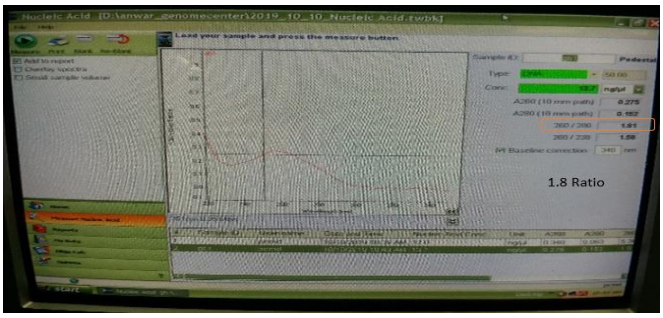


Table 1 Describes the types of mutations and their number

samples	Original codon	Modified codon	Amino Acid	Mutation
Cholistani (CH3)	TTT	TTG	Phenylalanine Leucine	01
Cholistani (CH4)	GGA	CGA	Glycine Arginine	01
Sahiwali (S2)	AGG	AGC	Arginine Serine	
Sahiwali (S2)	ACT	ACC	Threonine Threonine	02
Sahiwali (S3)	CAG	CAT	Glutamine Histidine	
Sahiwali (S3)	AGA	ATA	Arginine Isoleucine	03
Sahiwali (S3)	CAG	CAT	Glutamine Histidine	

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Original Amino acid	Modified Amino Acid	Original Amino acid	Modified Amino Acid	Point Mutation
Phenylalanine	Leucine	Essential	Nonessential	Missense
Glycine	Arginine	Essential	Nonessential	Missense
Arginine	Serine	Essential	Nonessential	Missense
Threonine	Threonine	Essential	Essential	Silent
Glutamine	Histidine	Essential	Nonessential	Missense
Arginine	Isoleucine	Essential	Nonessential	Missense
Glutamine	Histidine	Nonessential	Essential	Missense

Table 2 Describes the detailed information of chnaged amino acids.

Table 3 Show the percentage of Mutation

Gene	Breed	Mutation	% Formula	% of Mutation
CSN2	Cholistani	02	02/211*100	0.94%
	Sahiwali	05	05/211*100	2.36%

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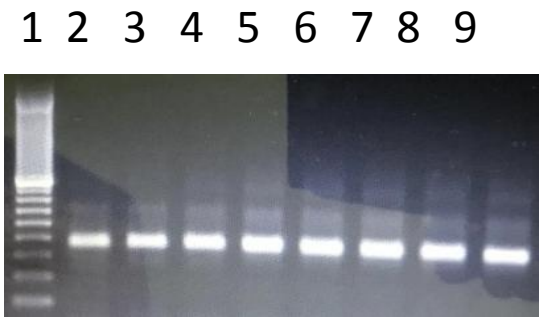

Note:
M= Marker 1kb
PCR product size 211bp

Figure 1 Show the Size of PCR amplified product of CSN 1 gene

Results of current study give complete genomic data and explain the comparison of mutations and percentage in across all three cattle breeds. Two mutations were discovered in the Cholistani breed, five mutations in the Sahiwali breed and no mutation was found in Thari breed. Based on the genetic code, these alterations are classified as point mutations. In Cholistani breed Phenylalanine change into Leucine in which essential amino acid TTT change into non-essential amino acid TTG. Glycine change into Arginine in which essential amino acid GGA change into non-essential amino acid CGA. In Sahiwali cattle breed Argnine change into Serine in which essential amino acid AGG change into non-essential amino acid AGC. Threonine change into Threonine in which ACT essential amino acid change into essential amino acid ACC. Glutamine change into Histidine in which essential amino acid CAG change into non essential amini acid CAT. Arginine change into Isoleucine in which essential amino acid AGA change into non essential amini acid ATA. Glutamine change into Histidine in which essential amino acid CAG change into non essential amini acid CAT.

Discussion

Estimating genetic and phenotypic relationships between the quantity, ratio, and daily yield of milk protein fractions is critical in order to better understand how casein and whey protein respond to dairy cow decisions. The comparable direction of phenotype and genetic association, according to Cheverud Sodini et al., could be attributed to the same direction of environmental influence as genetic influence. The synthesis of casein and whey protein is controlled by the same biological process. Pegolo and colleagues, 2018. In reality, there is a lot of linkage disequilibrium in the genomic areas that affect the expression of whey protein and casein. 2012, Huang et al.

The results of this analysis revealed that the three cattle had a total of 07 mutations in the CSN2 gene. Three indigenous goat breeds from Khairpur District, Sindh Province, Pakistan, have genetic diversity in the CSN2 gene. In the Cholistani breed, PCR basic analysis found two beneficial mutations, five mutations in the Sahiwali breed, and no mutation in the Thari breed. These changes are classed as point mutations in the genetic code. The Cholistani variety showed this alteration, with phenylalanine being converted to leucine at 41 base pairs. The non-essential amino acid alanine's initial codon TTT has been altered to TTG. At 140 bp, the essential amino acid GGA is changed to the non-essential amino acid CGA in glycine modified with arginine.

In Sahiwali cattle breed we found five mutation one is silent mutation which is Threonine change into Threonine in which ACT essential amino acid change into essential amino acid ACC at the bp. Other four are Missense in which Glutamine modified into Histidine in which original CAG alter into CAT. Arginine modified into Isoleucine in which original codon AGA alter into ATA. Glutamine modified into Histidine in which original codon CAG alter into CAT.

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