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Genetic Analysis of Calpain-1 Gene in three different cattle breeds of District Ghotki, Sindh, Pakistan

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Abstract
To solve knowledge bottlenecks, many technologies have been developed or are being developed. In this study, four mutations in the Calpain-1 gene were discovered in three cattle breeds. PCR-gel electrophoresis and DNA sequencing techniques were used to identify the Dhanni cattle breed's mutation 03, the Sahiwal cattle breed's mutation 01, and the non-mutation in the Cholistani cattle breed. AAT, GTT, and TGT replace the hitherto encoded lysine (essential amino acid), phenylalanine (essential amino acid), and tyrosine Acid in all missense mutations found in 2003. (non-essential amino acid). The changed codons are responsible for encoding asparagine, valine, and tyrosine, three important amino acids (non-essential amino acid). Histidine, an essential amino acid with a positively charged imidazole functional group, has been identified as a result of a deletion point mutation in Sahiwali cattle, where the CAC codon was altered to C-C. It is frequently involved in enzyme-catalyzed processes due to imidazole, and the missing codons do not encode any amino acids. Cattle from Cholistani. The Dhanni cattle breed is a good breed for animal husbandry meat properties and is highly useful for breed mixing, according to our findings.
Keywords: CAPN1, Cattle, DNA sequencing, PCR, SNPs

Introduction

Calpain is thought to have a protein modification function by blocking protein breakdown and thereby targeting proteins (Murphy et al., 2006). M-calpain can autolyze from 80 kDa to 78 kDa by boosting its sensitivity to Ca + 2 by more than 10 times. A pre-activated m-calpain is found beneath the surface membrane, and it has been demonstrated to attach to the cell membrane fast (at least partially) and retain a lengthy connection duration in the presence of micromolar Ca + 2. It increases the susceptibility of Ca + 2 m-calpain to autolysis in the presence of phospholipids. Rapid membrane healing is aided by pre-activated calpain, which is known for its reliance on local cytoskeleton reconstruction (Gailly et al., 2007).

-calpain and m-calpain are Ca+2-dependent proteases, and one peptide are included in the original three-molecule complex. After that, it will be possible to make calpain synthetically. A macromolecular protein called calpain, which may induce severe pain and produce cramping, can be the cause of cramping. CAPP1, the active protein of the protease, encodes M-calpain, a proteolytic activity that decreases muscle protein during storage. Modulations of enzyme activity are linked to changes in meat sensitivity (Geesink and Koohmaraie 1999). The CAPN1 gene encodes the enzyme's structural backbone on a vast scale. The acronym for chromosome 29 is CAPN1 (Smith et

al., 2000). This specific protease appears to be the most abundant enzyme in the sample after staining (Koohmaraie 1996).

The concentration of Ca + 2 in free cells rises in muscular dystrophy and other muscle disorders. Increase intracellular Ca + 2 to stimulate calpain action. The structural abnormalities found in atrophic muscle are mimicked when normal muscle myofibrils are treated with m-calpain or an m-calpain inhibitor (Kretzschmar et al., 1989). According to sodium dodecyl sulphate polyacrylamide gel electrophoresis, calpain induces degrading changes in myofibrils comparable to Duchenne muscular dystrophy (SDS-PAGE). In 1979, Miyaura et al. used heterodimers made from skeletal muscle proteins with molecular weights of 78 kDa and 28 kDa. In a side-by-by-side comparison, researchers found that the refined calpain found in the brains of pigs and cattle was composed of 75 kDa (29 kDa) heterodimers with almost similar molecular weights (Nagata et al., 1986). Calpain molecules of various kinds have the same molecular weight due to this phenomenon. Regulation and catalysis are carried out by separate subunits of calpain, each of which weighs around 80 kilodaltons. 80 KDA's catalytic subunit is divided into four sections (Sorimachi et al., 1997). Spontaneous regions are located in Area I; cysteine catalytic regions are in Area II; and the cysteine domain is located in Area III (Storr et al., 2011). Calmodulin and phospholipid binding sites are shown in Figures IV and V. The EF hand is made up of five domain IV and IV subdomains that connect calmodulin and calcium. A lot of study has been done on the CAPN1 gene mutation, which is particularly essential for genetically defining meat quality traits. The genotypic effects of the CAPN1 gene have been examined in several species, primarily sheep and pigs, in Argentina (Crova et al., 2014), New Zealand (Morris et al., 2006), China (Li et al., 2010), and other countries. However, little is currently known about this trait in Pakistani cow breeds. The CAPN1 gene polymorphism was investigated in this study, and the genotype effect was calculated in three distinct cattle.

Materials and Methodology

Collection of samples

Thirty jugular vein blood samples were taken from three different cattle breeds: Sahiwal cattle, Cholistani cattle, and Dhanni cattle. They are all between the ages of 1.5 and two years old and are cared for in compliance with the Canadian Animal Care Commission. Collect a blood sample (5 ml) with a 5 to 10 cc syringe and store it in an EDTA tube (0.5 M). Blood samples are kept at 0°C for future DNA extraction.

Extraction of DNA and quantification

Thermo Scientific Gen JET Genomic DNA Mini Purification Kit # K0781 is used to extract DNA from blood leukocytes. When you use this kit, you will be able to obtain high-quality genomic DNA by scrubbing the spin column with silica gel to remove harmful phenolic chloroform and finish the alcohol precipitation process. To obtain ultra-pure DNA, the complete technique takes 20 to 30 minutes once cell viability is achieved. The nanodropper of Karachi University's HEJ Genome Research Center was used to quantify the DNA concentration of each extracted sample in order to ensure that it was sufficient for amplification. The purity of the DNA was also determined using a 260/280 nm ratio.

Primer Designing PCR Amplification

The CAPN1 gene sequence was obtained from the Ensemble.org genome research browser and used to create primers using online primer 3 software. After that, the primer sequences (Table 1) were checked for specificity on the NCBI website.

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Table. 1 Sequences of forward and reverse primers for amplifying CAPN-1 gene.

Primers	Forward 5` --- 3`	Reverse 5`--- 3`	Bp	Tm
CAPN-1 gene	CGAGCAAGTGCTCTCAGAAG	CTCCGAGTAGCGGGTGATAA	210	56
	GCAGCATGAGTGCCTATGAA	CTCCAAGGAAGGCACCTG	210	56

Prepare the reaction mixture in an Eppendorf tube to amplify the calpain-1 gene. All of the components in the reaction mixture are at optimal concentrations. Transfer 20 l of the reaction mixture to each of the 7 l 2X Red PCR Master Mix, 2 l forward and reverse primers (10 pmol / l), 5 l (100 ng) genomic DNA, and 4 l ddH2O tubes. A thermal cycler was used to do PCR amplification (Bio Rad S1000, USA). The thermal cycling settings were as follows: 1) 94°C pre-denaturation for 5 minutes, 2) 94°C 35 seconds of denaturation for 34 cycles, annealing at 58°C 45 s, and 72° C annealing. Extend for 1 minute; then, at 72°C, extend for 5 minutes. The PCR reaction was carried out according to Zhang et alstandard .'s procedure from 2007.

Data analysis, Sequencing, and Purification

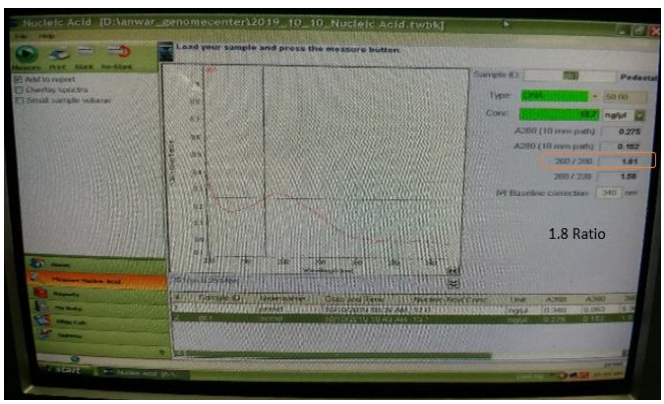
It is necessary to sequence the amplified products obtained from the PCR process. Figure 3 displays the whole CAPN-1 gene sequence that was acquired. Use ensemble.org's sequence alignment tool to do an in-depth analysis of the sequence. Following are the genetic code-based mutations shown in the alignment chart.

Results

Quantification of Extracted DNA and PCR- Gel Electrophoresis

The amount of DNA in the extracted DNA samples was measured with a Nanodrop and found to be in the range of 30-103 ng/l, which was sufficient for PCR amplification. Nano drop was also used to determine the purity of DNA. The investigation was also carried out. The absorbance from 230 nm to 320 nm was measured to detect other possible impurities and assess the purity of the DNA. The ratio of the absorbance at 260 nm divided by the value at 280 nm is the most typical purity calculation. For high-quality DNA, the A260/A280 ratio should be between 1.7 and 2.0. The CAP-1 gene DNA quantification and PCR amplification gel images are shown in Figures 1 and 2.

Fig. 1. DNA quantification using nanodrop method.



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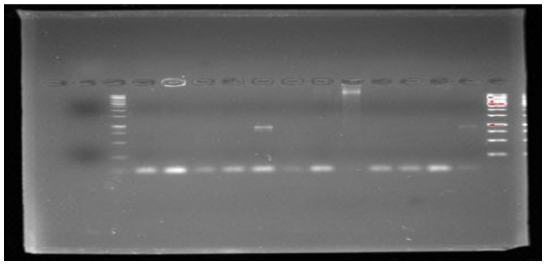

Fig. 2 Shows the PCR ampilied Product of Cap -1 gene on 1.5% Agarose gel

Note: Vertical bands: 1Kb DNA Marker and horizontal bands line: samples

Identification of Single Nucleotide Polymorphism and

The CAPN1 gene of the three cattle has four single nucleotide changes, according to the findings of this study. This genetic variant of the CAPN1 gene was discovered in three cow breeds in Pakistan's Ghotki region. In the Dhanni cattle breed, PCR basic analysis found three SNPs, 01 mutations in the Sahiwal cattle breed, and no mutations in the Cholistani cattle breed. These changes are classed as point mutations in the genetic code. This mutation was found in Dhanni cattle, where lysine was changed to asparagine at 16 base pairs. The mutation was incorrect because the original codon AAA encoding the necessary amino acid lysine was altered to AAT encoding the essential amino acid asparagine. At nucleotide 25, there is still another mutation. The original codon is TTT, which encodes phenylalanine and is altered in GTT when phenylalanine is transformed to valine. The valine amino acid is encoded by GTT. Acid. Another single mutation in the Dhanni cow breed occurs at 20 base pairs, where adenine is changed to guanine. The original codon TAT, which codes for the amino acid tyrosine, is changed to TGT, which codes for cystine. This modification is incorrect.

Only one mutation was found at 68 base pairs in cattle of the Sahiwal breed. A deletion point mutation, the CAC base pair mutation that encodes the histidine amino acid is a C-C deletion mutation. No alterations were discovered in Cholistani cattle using PCR-gel electrophoresis technique and DNA sequencing. The mutation's details and frequency are listed in Table 2, and the nucleotide phloem results are shown in Figure 3, which depicts the mutation sites of the CAPN 1 gene in cow breeds.

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Table-2 Results showing the detailed position and types of point mutation in 03 cattle breeds based on genetic code.

Name of breed	Position of mutation	Original codon	Changed codon	Original amino acid	Changed amino acid	Type of point mutation
Dhanni	16	AAA	AAT	Lysine (Essential Amino Acid)	Asparagine (Essential Amino Acid)	Missense Point Mutation
	25	TTT	GTT	Phenylalanine (Essential Amino Acid)	Valine (Essential Amino Acid)	Missense Point Mutation
	20	TAT	TGT	Tyrosine (Non- Essential Amino Acid)	Cystidine (Non Essential Amino Acid)	Missense Point Mutation
Sahiwali	68	CAC	C-C	Histidine (Essential Amino Acid)	Delete Point Mutation	
Cholistani	No mutation was identified in any sample of this breed					

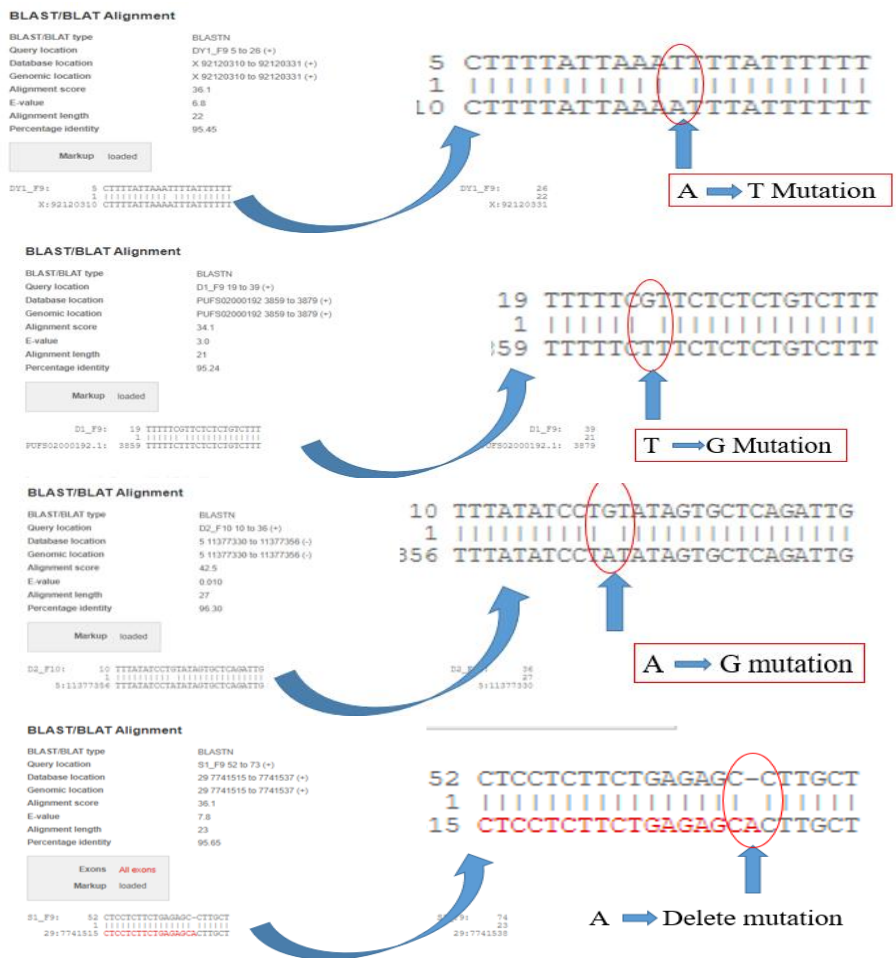

Fig. 3. Nucleotide Blast results through Sequence alignment of the cattle CAPN-1 gene

Discussion

Animal husbandry planning may benefit greatly from the use of DNA markers. The use of DNA markers has revolutionised genetic mapping and the study of animal and plant genetics in general. SNPs (single nucleotide polymorphisms) are the most common kind of genetic variation observed in mammals (sometimes known as "snips"). At least one single nucleotide change is associated with each single SNP. Differences between genes are frequently seen in their DNA. To help researchers find genes associated with animal characteristics or disease expression, they can be used as biomarkers. There may be a greater impact on gene function when SNPs are found in regulatory regions inside or near genes, impacting health, illness and other characteristics such as meat and milk production (Dodgson, Cheng, & Okimoto, 1997).

To solve knowledge bottlenecks, many technologies have been developed or are being developed. The CAPN1 gene of the three cattle has four single nucleotide changes, according to the findings of this study. This genetic variant of the CAPN1 gene was discovered in three cow breeds in Pakistan's Ghotki region. In the Dhanni cattle breed, PCR basic analysis found three SNPs, 01 mutations in the Sahiwal cattle breed, and no mutations in the Cholistani cattle breed. These changes are classed as point mutations in the genetic code. This mutation was found in Dhanni cattle, where lysine was changed to asparagine at 16 base pairs. The mutation was incorrect because the original codon AAA encoding the necessary amino acid lysine was altered to AAT encoding the essential amino acid

asparagine. At the 25th nucleotide, another mutation occurs. The original codon for phenylalanine is TTT, which codes for phenylalanine, and it mutates into GTT, which codes for valine, when phenylalanine becomes valine. The amino acids are the same. Another single mutation in the Dhanni cow breed occurs at 20 base pairs, where adenine is changed to guanine. The original codon TAT, which codes for the amino acid tyrosine, is changed to TGT, which codes for cystine. This modification is incorrect.

Only one mutation was found at 68 base pairs in cattle of the Sahiwal breed. A deletion point mutation, the CAC base pair mutation that encodes the histidine amino acid is a C-C deletion mutation. Despite the use of DNA sequencing technologies and PCR gel electrophoresis, no mutations in the Cholistani breed of cattle were discovered. The new study's findings are in line with Ujjan et alwork .'s from 2011.

Conclusion

In this study, 04 Calpain 1 gene mutations were found in 03 cattle breeds. In addition, 3 mutations were found in Dhanni cattle breeds, 01 mutations were found in Sahiwal cattle breeds, and no mutations were found in Cholistani. Breed cattle with the help of DNA sequencing technology and PCR gel electrophoresis. According to the genetic code, these mutations are classified under the Missense (03) mutation in the Dhanni breed of cattle, which is genetically considered to be a good mutation for meat characteristics. DNA sequencing has also been performed on cattle of the Cholistani breed, but so far no mutations have been found in this breed. In addition, the results show that the Dhanni cattle breed is very useful for mixed breeds and very suitable for breeds with meat characteristics.

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