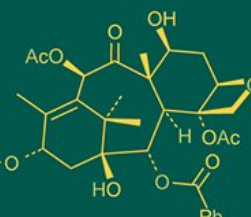
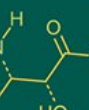
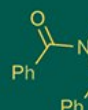


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Studies on cpt1a gene polymorphism during the transition period in Holstein Friesian cows affected by ketosis

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Abstract

The present study aimed to investigate the polymorphism of the CPT1A gene (Carnitine Palmitoyl Transferase 1A), which is expressed in the liver and red blood cells, about ketosis in sixteen lactating Holstein Friesian cows during the post-partum transition period in the Prabhani district of Maharashtra. This investigation focused on the association between CPT1A gene polymorphism and ketosis in these cows.

DNA was isolated from blood samples of the Holstein Friesian cows and the CPT1A gene polymorphism was detected using Polymerase Chain Reaction (PCR) followed by sequencing. The sequencing data were compared against a reference sequence to identify any gene polymorphisms. Additionally, the biochemical markers and hematological indices were compared with the existing literature. The results showed that for one of the primers, the single-stranded DNA bands matched in pattern across all experimental samples, indicating monomorphism. Notably, the known mutant alleles of the CPT1A gene were not observed in this study. In conclusion, this study found no evidence of polymorphism in the CPT1A gene, indicating that there is no association with ketosis in Holstein Friesian cows during the post-partum transition period.

Keywords: Sequencing, Ketosis, Holstein Friesian cows, CPT1A gene

Introduction

Bovine ketosis is a metabolic disorder found in dairy cattle, characterized by elevated levels of ketone bodies. This condition poses a significant challenge for dairy producers around the globe. It is estimated that approximately 22.7% of dairy cows experience subclinical ketosis (SCK), indicating that nearly one-fifth of the bovine population could be affected (Kroezén et al., 2017) [5]. In India, ketosis in dairy cows represents a serious health issue that negatively impacts both milk production and the overall welfare of the animals. Various studies have shown that the prevalence of ketosis varies by region. For example, research in the Bikaner region reported an overall prevalence of postpartum ketosis at 11.42% (Nazeer et al 2019) [13].

The genes CPT1A and CPT1B are involved in the transport of long-chain fatty acids into the mitochondria, where they are utilized as an energy source (Soares et al., 2020) [14]. CPT1 is one of three enzymes that comprise the Carnitine Palmitoyl Transferase (CPT) system and plays a crucial role in regulating the metabolism of long-chain fatty acids (McGarry and Brown, 1997). Previous studies have examined the activity and function of CPT1 in ruminants at various stages of lactation to better understand the metabolic changes that occur during ketosis and hepatic lipidosis (Knapp, 1990; Mizutani et al., 1999) [4, 11]. Aiello et al. (1984) [1] found that increased gluconeogenesis and ketogenesis may be linked to higher activity of CPT1 during early lactation due to greater negative energy balance (NEB). Knapp (1990) studied non-ketotic, non-lactating, and lactating dairy cows and found no differences in CPT1 activity. Similarly, Mizutani et al. (1999) [12] reported no variations in CPT1 activity among cows in early, mid, and late lactation. Dann and Drackley (2005) [2] investigated how prepartum nutrient intake and postpartum health status influence hepatic CPT1 activity, suggesting that changes in this gene might not be involved in the etiology of ketosis in dairy cows.

CPT1A encodes proteins that regulate the rate at which activated fatty acids enter the mitochondria for oxidation. Functional mutations in either gene could affect the fate of fatty acids that accumulate in the liver when a cow is in negative energy balance (Kroezen et al., 2017) [5]. Kroezen et al. (2018) [6] identified four single-nucleotide polymorphisms (SNPs) within the CPT1A gene that are associated with the first lactation estimated breeding value (EBV) for ketosis (FDR <0.05). Absolute measurements of mRNA transcripts for the CPT1A gene revealed that its expression was at least ten times higher than that of CPT1B, establishing CPT1A as the dominant variant (McCarthy et al., 2010). These genes are involved in the oxidation of fatty acids and the synthesis of ketone bodies; previous studies have reported that the expression of the ACSL1 and CPT1A genes is significantly increased in cases of subclinical ketosis in dairy cows (Zhu et al., 2019). In cattle (*Bos taurus*), the CPT1A gene is located on chromosome 29 and spans approximately 60 kilobases (kb), consisting of 19 exons. The gene encodes a protein made up of 773 amino acids (Gobin et al., 2003) [3].

Structurally, the CPT1A protein features a short N-terminal regulatory domain (residues 1–47), a mitochondrial intermembrane domain (residues 74–102), two transmembrane domains (TM1: residues 48–73; TM2: residues 103–122), and a catalytic domain that extends from residues 123 to 773. The N-terminal region undergoes conformational changes that are essential for regulating enzyme activity, particularly in response to malonyl-CoA (Lopes-Marques et al., 2015) [8].

Sr. No.	Groups of Animals	Particulars of animals	No. of Animals
1	Group I	Ketosis affected Holstein Friesian cows	08
2	Group II	Ketosis non-affected Holstein Friesian cows	08
Total number of experimental animals			16

Blood specimens of 6 mL each were collected from 16 experimental subjects via jugular venipuncture using vacuum tubes (vacutainers) containing EDTA (0.5 mM, pH 8.0) as the anticoagulant during the early morning hours. Immediately after collection, the blood specimens were transported to the laboratory on ice packs and stored at -20°C for subsequent analytical procedures. Genomic DNA was isolated using the HI Pura blood genomic DNA miniprep purification kit (HI Media Pvt. Ltd.) in accordance with the standard protocol provided with the kit. The DNA isolation procedure followed the sequential steps outlined in this standard protocol. The quantity and quality of the genomic DNA were assessed using a Nanodrop spectrophotometer, while the integrity was evaluated using agarose gel electrophoresis. The optical density (OD) values at 260/280 ranged from 1.60 to 1.80, indicating a high level of purity. The stock DNA was diluted in a Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA) to achieve a working concentration of approximately 50 ng/μL.

The integrity of the isolated genomic DNA samples was further confirmed by subjecting the samples to 0.8% agarose gel electrophoresis at 75 volts and staining with ethidium bromide dye for visualization of DNA bands under a UV transilluminator. Only the DNA samples that showed

Materials and Methods

The experiment involved 265 lactating Holstein Friesian cows, aged between 3 to 9 years, selected from organized farms in and around Parbhani district, Maharashtra. All animals were kept under standard management practices in either organized dairy farms or field conditions, which included regular vaccinations and deworming, along with a standard diet and access to water ad libitum.

Fresh urine samples of 10 ml each were collected from 256 seemingly healthy lactating Holstein Friesian cows during the morning milking period. Urine collection was performed using clean, sterilized screw-capped glass tubes, and prior to urination, the genitalia of each cow were cleaned with a potassium permanganate solution. After collection, the urine samples were promptly transported to the laboratory on ice packs to ensure preservation and accuracy of the analysis.

The screened animals (n = 256) were categorized into two groups based on Rothera's test for ketonuria. Cows with a positive Rothera's test were classified into Group I, which consisted of ketosis-affected Holstein Friesian cows (n = 8). This was confirmed by an increased blood concentration of ketone bodies/acetone (blood acetone level > 5.0 mg/dL). The other group, Group II, included ketosis non-affected Holstein Friesian cows (n = 8), which showed a normal blood concentration of ketone bodies (blood acetone level < 5.0 mg/dL) and negative Rothera's test results.

Details of experimental animal

minimal smearing were considered acceptable for further applications.

To prepare the working solution of primers, 5 μL of the stock solution was combined with 45 μL of distilled water. The forward primer for the amplification of the CPT1A gene (119 bp) was 5' ACGCCGTGAAGTATAACCCT 3', and the reverse primer used was 5' CCAAAAATCGCTTGTCCTT 3'. The PCR protocol for amplifying the CPT1A gene included an initial denaturation at 95°C for 5 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The PCR was carried out in a final reaction volume of 25 μL, which consisted of 12.5 μL Hi-Chrome PCR Master Mix, 0.5 μL each of the forward and reverse primers, 1 μL of template DNA, and 10.5 μL of nuclease-free water. This cycle was repeated a total of 35 times.

Results and Discussion

Confirmation of CPT1A-PCR products by agarose gel electrophoresis

Under a UV transilluminator, each amplified PCR product was visible as a single band of the expected size 119 bp. (Fig. 1)

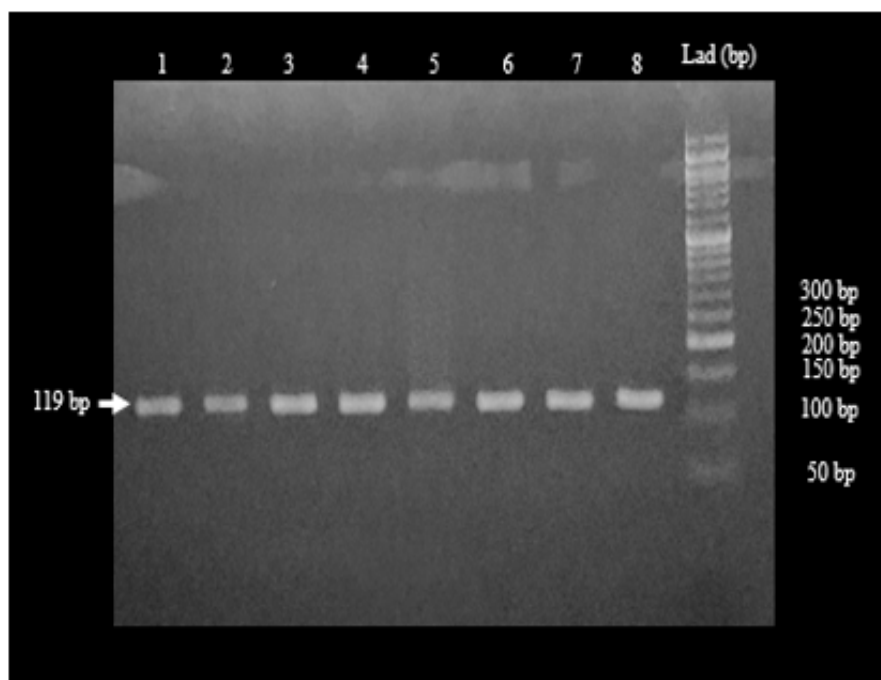


Fig 1: Agarose gel electrophoresis of PCR product of CPT1A gene. “Lad” represents 50bp DNA size marker, Digits 1 to 8 indicate different samples (119 bp).

Sequencing and analysis

Samples from two groups, ketosis-affected (Group I) and ketosis non-affected (Group II), were carefully selected for each PCR product, and the PCR products were custom sequenced by Eurofins Genomics India Pvt. Ltd. in Bengaluru. We aligned the sequence data using MEGA 11 to detect Single Nucleotide Polymorphisms (SNPs). To identify potential polymorphisms, we compared our sequences with the reference sequence of the CPT1A gene.

From our analysis, all 16 samples retrieved from the NCBI database, which consisted of a 119 bp sequence, were found to be identical in both groups, indicating no genetic diversity in exon 1 (119 bp) (Fig. 2). The region of the CPT1A gene, specifically exon 1 (119 bp, XM_027531373), was determined to be monomorphic, meaning there are no variations or polymorphisms present at that specific site within the gene sequence across all samples.



Fig 2: Alignment of sequences of CPT1A gene (119 bp) (exon 1) showing monomorphism with respect to reference sequence

The primary objective of this research was to investigate the polymorphism of the CPT1A gene and its association with ketosis in Holstein Friesian cows during the post-partum transition period. Sequencing analysis revealed that the CPT1A gene was monomorphic, meaning no known mutant alleles were detected in this study. As a result, we could not establish a correlation between ketosis and CPT1A gene polymorphism.

In contrast to our findings, Riani reported that variations in the CPT1A gene (carnitine palmitoyl transferase 1A) were associated with negative energy balance, as well as both

subclinical and clinical ketosis in dairy cows. Additionally, Graber et al. noted that increased expression of ACSL1, PC, and CPT1A genes, which are linked to elevated fatty acid β -oxidation, might occur due to elevated levels of non-esterified fatty acids (NEFA) during the postpartum negative energy balance.

Similarly, Mizutani et al. (1999) ^[11] reported no significant differences in CPT1 activity among cows in early, mid, and late lactation. Additionally, Dann and Drackley (2005) ^[2] found that CPT1A expression did not vary with ketosis, which aligns with our findings. They concluded that the

mitochondrial capacity for long-chain fatty acid oxidation might not be affected, supporting previous data indicating that this enzyme is not strongly linked to the etiology of ketosis (Loor et al., 2007)^[7].

In summary, our study found no polymorphism in the relevant region of the CPT1A gene, making it impossible to confirm any association with ketosis in lactating Holstein Friesian cows.

Conclusion

The present study found that all Holstein Friesian cows are monomorphic for the CPT1A (exon 1) gene in the region (XM_027531373). The changes in the known sequence of the CPT1A gene were not observed in the present study. Hence, the association between ketosis and the CPT1A gene polymorphism could not be established.

This study suggests that a detailed investigation needs to be carried out to find polymorphism/variation at other loci of the CPT1A gene in Holstein Friesian cows and to study a larger sample size (unrelated Holstein Friesian lactating cows) and its association with the gene under study.

Ethical statement

In this study, the experimental measures using Red Kandhari cattle have been conducted after approval from the Institutional Animal Ethical Committee (IAEC) of the College of Veterinary and Animal Sciences, Parbhani (Maharashtra Animal and Fishery Sciences University, Nagpur) vide no. IAEC/157/2025. All the research investigations with Red Kandhari cattle were carried out according to the IAEC guidelines.

Conflict of Interest

No external agency has been involved or funded for conducting this research project. The author does not have any conflict about publishing this article.

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