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Variations of Exon 2-3 of the Branched Chain Keto Acid Dehydrogenase E1 Subunit Alpha (BCKDHA) Gene and Its Flanking Intronic Region in Madura Cattle

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Abstract

Indonesia is a country with high biodiversity and genetic resources. One of them is Madura cattle, which derived from crossing between exotic cattle, namely zebu cattle, with local Indonesian cattle, namely Bali cattle. Branched-chain alpha-keto acid dehydrogenase is an enzyme complex that metabolizes branched-chain amino acids, namely valine, leucine and isoleucine. This study aimed to analyze exons 2-3 variations of the BCKDHA gene and its flanking region in Madura cattle. The sequences of it were obtained using DNA sequencing techniques. A total of seven variations were found, one in the flanking region adjacent to the 5' end of exon 2 of the BCKDHA gene. The A392G variation in exon three did not cause any amino acid changes. No variation was found in exon 2, indicating that the exon regions of the BCKDHA gene were more conserved than introns in the Madura cattle.

Keywords: BCKDHA gene, Exon, Flanking region, Madura cattle

Introduction

Madura cattle are dispersing fairly throughout Indonesia, like in Sumatra, Java, Kalimantan, Sulawesi, West Nusa Tenggara and East Nusa Tenggara. Madura cattle are local Indonesian cattle that have good adaptability to tropical environments, poor feed quality, resistance to tick infestations, and high carcass quality.

Madura cattle are one of Indonesia's local cattle breeds derived from crosses of zebu cattle (*Bos indicus*) and Bali cattle or banteng (*Bos javanicus*). Based on the local wisdom of Madura, Madura cattle can be divided into three groups: karapan cattle, sonok cattle, and beef cattle. Karapan cattle is a traditional bull racing tournament from Madura Island, while sonok cattle or female cow beauty contest is a Madura tradition, where the assessment is based on the beauty of its decoration, cleanliness, and the harmony of its walks in front of the jury. Beef cattle are a type of cattle raised for meat.

Since the beginning of the 20th century, other cattle breeds have been prohibited from entering Madura Island to maintain their purity (Martojo 2012). The utilization of cattle in the tradition of the Madurese

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community aims the selection cattle based on their designation, which has performance that is in accordance with the tastes of the community. Selection in this way will likely influence the variation of genes involved in energy metabolism. The variation found is likely to be differentiated following a pattern based on the designation of the cattles.

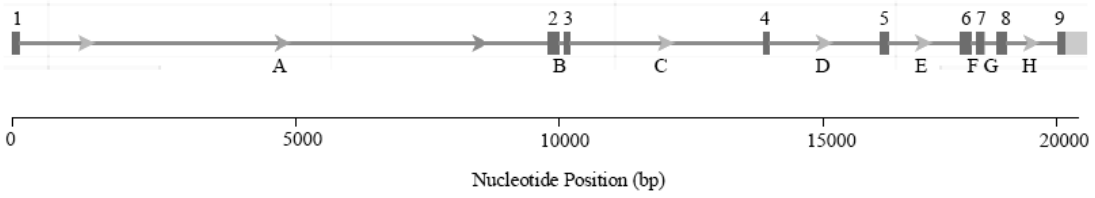
Branched-chain alpha ketoacid dehydrogenase (BCKDH) is one of the significant enzyme complexes in the mitochondrial inner membrane that metabolizes branched-chain amino acids (BCAAs) as valine, leucine, and isoleucine. The BCKDH complex consists of α and β-ketoacid dehydrogenase subunits (E1α and E1β) and two other subunits (Patel and Harris 1995). Figure 1 shows that the E1α subunit in cattle is encoded by the branched-chain α-ketoacid dehydrogenase E1α (BCKDHA) gene located on chromosome 18, with a gene length of about 20575 bp and consists of 9 exons and eight introns (Elsik *et al*. 2009).

The BCKDH gene has been used as a marker to detect a disease, namely Maple Syrup Urine Disease (MSUD). This disease is an autosomal recessive metabolic disease that affects the metabolism of BCAAs. Under normal circumstances, the excess protein will trigger BCAAs to produce energy. BCAAs will be degraded by the branched-chain α-ketoacid dehydrogenase (BCKD) enzyme complex. In MSUD, if one or several genes encoding the components of this enzyme complex are mutated, BCAAs cannot enter the mitochondrial metabolic chain, resulting in accumulation in the blood and urine. This accumulation causes the patient's urine to have a maple syrup-like odour (Fisher *et al*. 1991).

MSUD has been identified in humans and cattle (Fisher *et al*. 1991; Chuang *et al*. 1993; Dennis and Healy 1999). Based on previous research, it was shown that a mutation in the form of substitution of 248C/T (CAG to TAG) at codon 6 in exon 2 of the BCKDHA gene could cause a stop codon that results in premature termination during translation in Polled Shorthorn cattle (Zhang *et al*. 1990). Exon 2-3 in the bovine BCKDHA gene has a short nucleotide sequence compared to other exons (Figure 1), making it possible to study the region entirely and its flanking region.

Variations found in the BCKDHA gene may affect the encoded amino acids and the efficiency of the BCKDHA enzyme in BCAA metabolism. Characterization of genes involved in energy metabolism needs to be done to improve the quality of Madura cattle in livestock breeding schemes. This study aimed to analyze the variation of exon 2-3 of the BCKDHA gene and its flanking region found in exon 2-3 in Madura cattle. It is hoped that the nucleotide sequence obtained can be used as a reference for

Figure 1
BCKDHA gene structure based on B. taurus genome sequence. Numbers 1 to 9 represent the exon. Letters A to H denote intron (Elsik et al. 2009; Zimin et al. 2009).



further research on other Indonesian local cattle.

Research Methods

DNA extraction and amplification

The DNA samples used were Madura cattle blood samples collected based on the designation of the cattle, Karapan cattle from Sampang Regency, and Sonok and beef cattle from Pamekasan Regency. Genomic DNA was extracted from blood using the Genomic DNA Mini Kit for Fresh Blood from Geneaid.

Primers used to amplify the sequence target were designed online using Primer3 (Koressaar *et al*., 2018). The primer sequence to amplify exon 2 and 3 and the polymerase chain reaction conditions are AF503F 5'-GGCTGCATCACCAGCAAY-3' and AF504R 5'- GATCCGGTTCAACCTTCTGC-3'. DNA amplification was carried out in a final volume of 25 µL containing 50-100 ng of genomic DNA, one µL of 10 pM solutions of each primer, and 1X final concentration of GoTaq® Green Master Mix. The DNA amplification was performed using ESCO *Swift Maxi Thermal Cycler.* Reaction conditions were an initial denaturation **at** 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 60°C for 1 minute and extension at 72°C for 1 minute, ending with a final extension at 72°C for 5 minutes.

DNA Sequence analysis

Sequencing reactions were performed using a BigDye Terminator by commercial institutions of sequencing services and carried out from both directions, forward and reverse. The nucleotide sequences obtained were edited using the BioEdit program version 7.2.5 (Hall, 1999) and aligned with the BCKDHA gene sequences in *B. taurus and B. indicus* available in GenBank. The alignments obtained were then corrected manually by using the eye. Variation analyses were conducted using MEGA11 (Tamura *et al*., 2021).

Research Results and Discussion

The primer pair AF503 and AF504 successfully amplified the target region with a length of 666 bp. The alignment results of the nucleotide sequences were compared with the nucleotide sequences of the BCKDHA gene in *B. taurus* available from GenBank. The final length of the target sequence was 643 bp after editing.

A total of seven variations were found in the form of nucleotide base substitution and heterozygous alleles or double peaks in the sequence chromatogram. Double-peaks chromatograms were found both in the forward and reverse directions.

Variations of intron one and intron 3 (partial), intron two and exon 3 of Madura cattle against *B. taurus* can be seen in Table 1. Intron 3 region adjacent to the 5' end revealed the highest variation compared to another intron. In addition, the highest variation was found in beef cattle 5, as many as six variations out of a total of seven variations. Meanwhile, no variation in the beef cattle 1 sample was found when compared to the order of *B. taurus*. The 392A>G variation found in exon 3 is a heterozygous allele, so it does not cause a change in amino acids, even though it is located in the second base of the triplet codon. Variations in the form of nucleotide base substitution are all found in the intron region so that it will not affect the amino acids formed.

Variations can be found in coding (exon) and non-coding (intron) regions. These variations can affect the function or expression of genes, leading to undesirable conditions, such as genetic

disorders. There is no variation found in exon 2. This is probably because the coding region tends to be more conserved compared to the non-coding region, especially in the protein functional region (Ibeagha-Awemu *et al*., 2008).

According to variations in the BCKDHA gene discovered in this study, Madura cattle are the offspring of a cross between indigenous Indonesian cattle and exotic cattle like zebu and taurine cattle. Madura cattle have a genetic makeup that includes zebu and taurine cattle based on molecular genetic markers on the Y chromosome, whereas Madura cattle have zebu cattle and Bali cattle, or *banteng*, based on mitochondrial DNA and microsatellites. To boost the productivity of Madura cattle, taurine cattle have been imported to the island as bulls (Mohamad *et al.*).

Table 1
Variations of exon 2-3 and its flanking intronic region of BCKDHA gene on Madura cattle

No	Breed	Nucleotide base position						
		Intron 1	Intron 2	Exon 3	Intron 3			
		8	270	392	473	538	564	598
1	*B. taurus* NW001493616	G	G	A	A	G	G	C
2	Karapan cattle 1	.	.	.	G/A	.	G/T	.
3	Karapan cattle 2	G/A	.	G/A	G/A	G/A	.	.
4	Karapan cattle 3	G/A	G/A	.	G/A	G/A	.	C/T
5	Sonok cattle	.	G/A	.	G/A	G/A	.	.
6	Beef cattle 1	.	.	.	.	.	.	.
7	Beef cattle 2	A	A	.	G	A	.	C/T
8	Beef cattle 3	G/A	A	.	G	A	.	C/T
9	Beef cattle 4	G/A	.	G/A	G/A	G/A	.	.
10	Beef cattle 5	G/A	G/A	.	G	G/A	G/T	C/T

Conclusion

A total of 7 variations were determined successfully. The type of variation is the substitution of nucleotide bases and heterozygous allele or double chromatogram peaks. No variation was found in exon 2, and the intron three region adjacent to the 5' end revealed the highest variation. It indicates that the exon regions of the BCKDHA gene in Madura cattle are more conserved than introns.

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