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Detection of F94L marker in myostatin (*MSTN*/*Taq*I) gene of Indonesian Sumba Ongole cattle (*Bos indicus*)

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ABSTRACT

Sumba Ongole (SO) cattle are Ongole cattle from India which were imported to Indonesia during the Dutch colonial era and were well adapted on Sumba Island, East Nusa Tenggara, Indonesia. Sumba Ongole (SO) cattle are known for high meat production. This research was conducted to identify one point mutation of g.415C/A (F94L marker) in the myostatin (*MSTN*) gene (exon 1) using PCR-RFLP method with *Taq*I restriction enzyme (T*CGA). DNA samples of SO bulls and cows (Total 153) from Sumba Island were used in this study. Results revealed that all samples in this study had CC genotype with C allele as the common allele. The mutation point of g.415C/A was not found in this study. However, one mutation point of g.400G/A was detected in this study without amino acid changes (synonymous). It was concluded that *MSTN*/*Taq*I gene in the present study is monomorphic and cannot be used as the genetic marker for productive traits of SO cattle.

Keywords: F94L marker, *MSTN*/*Taq*I gene, Sumba Ongole (SO) cattle

Sumba Ongole (SO) cattle is one of the Indonesian local beef cattle developed by the process of breeding imported cattle, namely 'Ongole Cattle' from the Nellore-Ongole region in Prakasam District (Andhra Pradesh State), Southeastern coast of India. The SO cattle is capable of adapting well for many years to tropical conditions in Indonesia, especially on East Sumba Island, East Nusa Tenggara (Hartati *et al*. 2015). The SO cattle have a high potential for meat production in the intensive management system. The selection of SO cattle to increase meat production can be performed with the molecular selection method. One of the potential advantages of molecular selection is marker information which can be used to predict an animal's genotype before its actual performance recording for a particular trait (Kumar *et al*. 2020, Mohammadabadi 2021). One of the candidate gene that can be used for molecular selection is the myostatin (*MSTN*) gene. The bovine *MSTN* gene located at chromosome 2 (Charlier *et al*. 1995, Smith *et al*. 1997) and consists of two introns and three exons (Tantia *et al*. 2006). *MSTN* gene is a major negative regulator of skeletal muscle mass, muscle growth and development, and causes a variety of metabolic changes (Sheng *et al*. 2021, Wu *et al*. 2022).

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Previous studies reported that one point mutation (g.415C/A) occurred in exon 1 of the *MSTN* gene (GeneBank: AY794986.1) in Jersey (Esmailizadeh *et al*. 2008), Wagyu (Alexander *et al*. 2009), Rubia Galega × Nelore and Canchim cattle (Curi *et al*. 2012). One point mutation (g.415C/A) changed the amino acid from Phenylalanine (TTC) to Leucine (TTA) and was mentioned as the F94L marker (Smith *et al*. 2000, Abe *et al*. 2009). On the other hand, determination of gene polymorphism is important in farm animals breeding (Mohammadabadi *et al*. 2011, Gooki *et al*. 2019) in order to define genotypes of animals and their associations with productive, reproductive and economic traits (Gholamhoseini *et al*. 2018). Hence, this research was carried out to detect the F94L marker in the *MSTN*/*TaqI* gene of SO bulls using PCR-RFLP method. The result of this study can be used as early information for obtaining the genetic markers in SO cattle in the future.

MATERIALS AND METHODS

Experimental animals, blood sample collection and DNA extraction: The experiment was conducted under the guidelines of the Indonesian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the Indonesian Ministry of Agriculture Animal Ethics Committee (Balitbangtan/Lolitsapi/Rm/08/2018). Sumba Ongole (SO; 153 heads) cattle from Sumba Island of Indonesia were enrolled in this study for the DNA analysis. Fifty *longissimus dorsi* meat tissue samples were collected from 50 bulls from slaughterhouse. While, 103 blood samples were collected from SO cattle from smallholders belonging to 28 bulls (250-300 kg / 3 years of

Forward >>

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254                gcatgtt tgtggaggga aaacactaca tcctcaagac tagaagccat
301 aaaaatccaa atcctcagta aacttcgcct ggaaacagct cctaacatca gcaaagatgc
361 tatcagacaa cttttgccca aggctcctcc actcctggag ctgattgatc agtt*cgatgt
421 ccagagagat gccagcagtg acggctcctt ggaagacgat gactaccacg ccaggacgga
481 aacggtcatt accatgccca cggagtgtga gtagtcctgc tggtgca
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<< Reverse

Fig. 1. Target sequence in the exon 1 of *MSTN* gene of *Bos indicus* (GenBank: AY794986.1) along 274 bp and primer position (underlines).**Taq*I restriction site (underline). One SNP of g.415C/A (in the *Taq*I restriction site) was present in many cattle breeds and changes in the amino acid from Phenylalanine (TTC) to Leucine (TTA) or F94L were present.

age), 50 cows (220-300 kg / 3-4 years of age) and 25 calves (80-120 kg / about 12 months). The meat tissue samples were taken into a bottle containing 99% Ethanol. The blood samples were collected with venoject and vacutainer tube containing EDTA. Both samples were stored at -20°C until used for DNA extraction analysis. Total 20 mg of meat tissue and 200 µL of blood sample were taken into 1.5 mL microtube for DNA extraction analysis using DNA Extraction Kit (Zymo Research, USA) following the manufacturer's protocol. The obtained DNA product was stored at -20°C until used for PCR analysis.

PCR amplification: Around 274 bp of *MSTN* gene's amplicon was amplified from the extracted DNA with the primer pairs designed from GenBank: AY794986.1. i.e. Forward: 5' -GCA TGT TTG TGG AGG GAA AA -3' and Reverse: 5' -TGC ACC AGC AGG ACT ACT CA -3' (Abe *et al*. 2009) as illustrated in Fig. 1. The PCR reaction was performed in of 20 µL volume consisting of 3 µL DNA, 10 µL of PCR mix (Bioline Mytaq, USA), 0.4 µL forward and reverse primers and 6.2 µL NFW (Nuclease Free Water). The PCR program was performed in the PCR machine (Veriti Applied Biosystem, USA) with pre-denaturation temperature (95°C for 1 min), 35 cycles of denaturation (95°C for 15 sec), annealing (57.1°C for 15 sec), extension (72°C for 10 sec) and final extension (72°C for 5 min) .The PCR product was visualized using 1.5% agarose gel (Thermo Scientific, USA) in the gel documentation (Infinity VX2, France). The amplicons were digested using restriction enzymes. The size of the DNA fragment was compared with a 50 bp DNA marker (Thermo Scientific, USA).

PCR-RFLP: The genotyping of the *MSTN* gene in animals under study was performed with PCR-RFLP method in 10 µL volume consisting of 3 µL PCR product, 0.2 µL of *Taq*I restriction enzyme (T*CGA), 1.0 µL of 10× Buffer tango and 5.8 µL of aquabidest. The RFLP product was separated with 2.5% agarose gel, stained with gel red, using electrophoresis method at 120 V for 50 min. The sequencing analysis was performed in this study using two samples of PCR product (20 µL) to confirm the findings through a commercial laboratory service (PT. Genetika Science, Indonesia). Therefore, the statistical analysis in this study was based on Nei and Kumar (2000), i.e. allele frequency, genotype frequency, expected heterozygosity (He), observed heterozygosity (Ho), number of effective allele (Ne), polymorphic informative content (PIC) and Chi square ($\chi 2$).

RESULTS AND DISCUSSION

The amplification of *MSTN* gene fragments in this study was successful at annealing temperature of 57.1°C showing the *MSTN* gene at exon 1 position with a PCR product of 274 bp. The PCR product was vizualized using 1.5% agarose gel (Fig. 2). Restriction results from *MSTN* gene fragments showed that all SO cattle in this study had CC genotype with C allele as the common allele (Fig. 3). The statistical analysis showed that genotypic and allelic frequencies of *MSTN*-F94L in SO cattle were monomorphic with CC genotype and C allele frequencies of 1, respectively (Table 1). The F94L polymorphism did not occur in the *MSTN* gene of SO cattle and was marked by the CC genotype. However, a mutation site of g.400G/A was present in animals studied, without amino acid changes (synonymous) as presented in Fig. 4.

Absence of F94L marker occurred in many *Bos indicus* breeds of India (Tantia *et al*. 2006) and some *Bos taurus* breeds such as Wagyu, Salers, Angus, Polled Hereford,

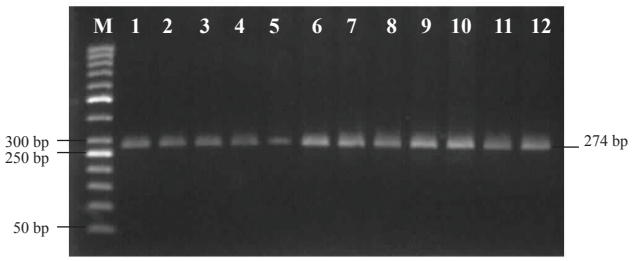

Fig. 2. Amplification of *MSTN* gene (274 bp) in SO separated in 1.5% agarose gel. Line 1-12: DNA samples; M: DNA ladder 50 bp.

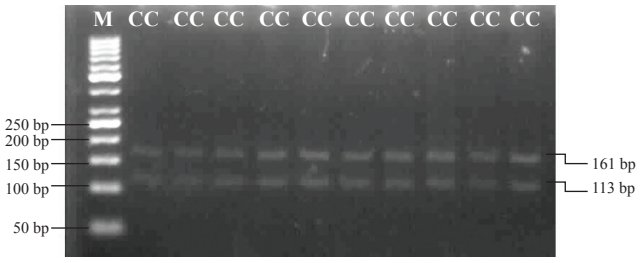

Fig. 3. RFLP analysis in *MSTN*/*Taq*I of SO cattle in 2.5% agarose gel showed monomorphic CC genotype (161 bp and 113 bp) as the common genotype. M: DNA ladder 50 bp.

Table 1. Polymorphism of *MSTN*/*Taq*I gene in SO cattle

Genotype frequency (N)			Allele frequency		H_o	H_e	n_e	PIC	χ^2
CC	AC	AA	C	A					
1.00 (153)	0.00 (0)	0.00 (0)	1.00	0.00	0.00	0.00	1.00	0.00	-

N, number of observation; H_o, observed heterozygosity; H_e, expected heterozygosity; n_e, number of effective allele; PIC, polymorphic informative content; χ^2, Chi square.

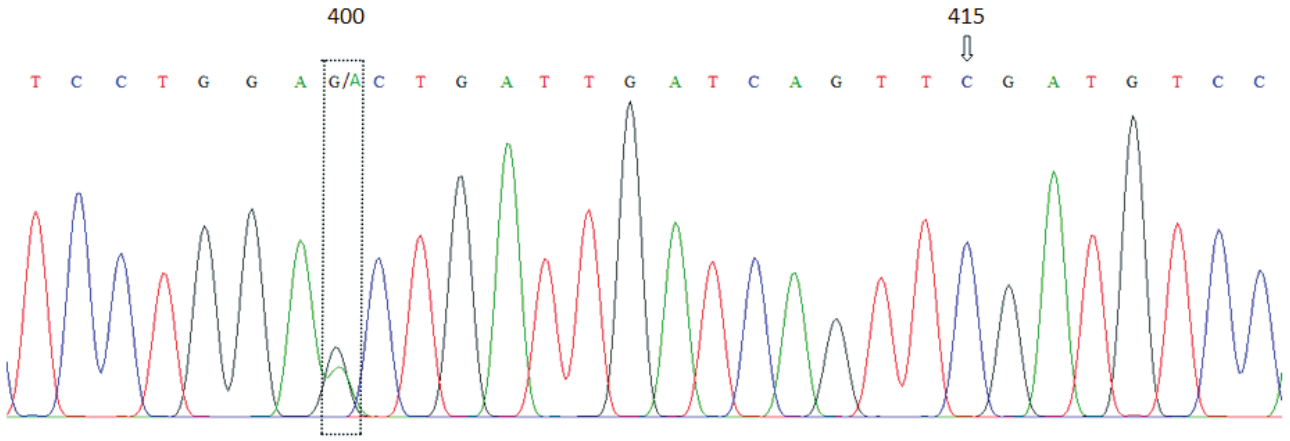

Fig. 4. Part of chromatogram in the exon 1 of *MSTN* gene in SO cattle. A synonymous mutation (g.400G/A) occurred in 400th nucleotide (box) and F94L marker at 415th nucleotide (arrow) was not detected. M: DNA ladder 50 bp.

Hereford, Gelbvieh, Charolais, Jersey, Brahman, Holstein, Shorthorn and Maine Anjou (Alexander *et al*. 2009, Vankan *et al*. 2010). Moreover, Vankan *et al*. (2010) reported that F94L mutation was present in Limousin (94.2%), Droughtmaster (4%), Piedmontese (2%) and Simmental (0.8%). Mutation of g.400G/A was present in Japanese Black × Limousin and Wagyu cattle (Alexander *et al*. 2009, Abe *et al*. 2009). Commonly the genetic characterization of g.400G/A in *Bos taurus* and *Bos indicus* breeds had G and A alleles respectively (Tantia *et al*. 2006). In reproductive traits of beef heifers, genotype AA at F94L had highest birth weight rather than CC and AC genotypes (P<0.05). In addition, the CC genotype had higher reproductive tract weight, number of follicles, follicular fluid volume, follicular fluid estradiol and granulosa cell weight rather than the AA genotype, but not significantly different (Cushman *et al*. 2015). In beef meat quality, the A allele at F94L had higher LMA than the C allele in Limousin cattle. Meanwhile, the C allele had higher monounsaturated fatty acid (MUFA) rather than the A allele in Wagyu cattle (Alexander *et al*. 2009). Also, the C allele at F94L had higher values in fat-related traits such as BMS, crude fat (%) and RFA to *M. longissimus, M. semispinalis* capitis and *M. trapezius* (%) rather than the A allele in Japanese Black × Limousin cattle (Abe *et al*. 2009).

Similar to this study, Anwar *et al*. (2020) reported that the F94L marker was not detected in Bali (*Bos javanicus*), Sumbawa (*Bos indicus*) and Friesian Holstein (*Bos taurus*) cattle. Contrastly, the F94L marker was detected in Pasundan cattle (*Bos indicus* × *Bos javanicus*) with low frequency (Anwar *et al.* 2020). However, detecting another mutation site in a different region is important to obtain the candidate marker in this cattle. Absence of F94L mutation in SO cattle can be caused by natural selection and close breeding that reduces genetic diversity. Previous study reported that bovine *MSTN* was polymorphic with many mutations that occurred from promotor to 3'UTR regions (Tantia *et al*. 2006, Abe *et al*. 2009). So, further study to detect single nucleotide polymorphism (SNP) in the *MSTN* gene of SO cattle is important for developing a marker assisted selection (MAS) program. Moreover, the cross-breeding program between selected Limousine bulls (with AA genotype) and SO cows is important to be performed in the future with an intensive management system.

In conclusion, the F94L marker in the *MSTN* gene of SO cattle was not detected. The CC genotype (Phenylalanine) was the common genotype with the C allele as the common allele in this cattle. Therefore, one synonymous mutation (g.400G/A) was present in the *MSTN* gene of SO cattle.

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REFERENCES

Abe T, Hasebe H, Okumura T, Kuchida K, Kawamura T and Kobayashi E. 2009. Detection of DNA polymorphisms in the *MSTN* gene and the effect of F94L on meat quantity and

quality traits in Japanese Black × Limousin F2 population. *Journal of Animal and Veterinary Advances* **8**(8): 1609–15.

Alexander L J, Kuehn L A, Smith T P L, Matukumalli L K, Mote B, Koltes J E, Reecy J, Geary T W, Rule D C and MacNeil M D. 2009. A Limousin specific myostatin allele affects longissimus muscle area and fatty acid profiles in a Wagyu-Limousin F_2 population. *Journal of Animal Science* **87**(5): 1576–81.

Anwar S, Volkandari S D, Wulandari A S, Putra W P B, Sophian E and Said S. 2020. Detection of F94L mutation of the *MSTN* gene in four Indonesian local cattle breeds. *Journal of the Indonesian Tropical Animal Agriculture* **45**(1): 7–14.

Charlier C, Coppieters W, Farnir F, Grobet L, Leroy P L, Michaux C, Mni M, Schwers A, Vanmanshoven P, Hanset R and Georges M. 1995. The mh gene causing double-muscling in cattle maps to bovine Chromosome 2. *Mammalian Genome* **6**(11): 788–92.

Curi R A, Krauskopf M M, Hadlich J C, Fortes M R S, Vankan D M, Silva J A V, de Oliveira H N and da Mota M D S. 2012. Candidate SNP's for carcass and meat traits in Nelore animals and in their crosses with *Bos taurus*. *Pesquisa Agropecuaria Brasileira* **47**(2): 294–301.

Cushman R A, Jr Trait R G, McNeel A K, Forbes E D, Amundson O L, Lents C A, Lindholm-Perry A K, Wood J R, Cupp A S, Smith T P L, Freetly H C and Bennett G L. 2015. A polymorphism in myostatin influences puberty but not fertility in beef heifers, whereas μ-calpain affects first calfbirth weight. *Journal of Animal Science* **93**(1): 117–26.

Esmailizadeh A K, Bottema C D K, Sellick G S, Verbyla A P, Morris C A, Cullen N G and Pitchford W S. 2008. Effects of the myostatin F94L substitution on beef traits. *Journal of Animal Science* **86**(5): 1038–46.

Gholamhoseini G F, Mohammadabadi M R and Asadi F M 2018. Polymorphism of the growth hormone gene and its effect on production and reproduction traits in goat. *Iranian Journal of Applied Animal Science* **8**(4): 653–59.

Gooki F G, Mohammadabadi M, Fozi M A and Soflaei M. 2019. Association of biometric traits with growth hormone gene diversity in raini cashmere goats. *Walailak Journal of Science and Technology* (WJST) **16**(7): 499–508.

Hartati Y T, Utsunomiya, Tad S, Sonstegard, José F G, Jakaria and Muladno. 2015. Evidence of *Bos javanicus* × *Bos indicus* hybridization and major QTLs for birth weight in Indonesian Peranakan Ongole cattle. *BMC Genetics.*

Kumar M, Ratwan P and Dahiya S P. 2020. Potential candidate gene markers for milk fat in bovines: A review. *Indian Journal of Animal Sciences* **90**(5): 667–71.

Mohammadabadi M R, Soflaei M, Mostafavi H and Honarmand M. 2011. Using PCR for early diagnosis of bovine leukemia virus infection in some native cattle. *Genetics and Molecular Research* **10**(4): 2658–63.

Mohammadabadi M R. 2021. Tissue-specific mRNA expression profile of ESR2 gene in goat. *Agricultural Biotechnology Journal* **12**(4): 169–84.

Nei M and Kumar S. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

Sheng H, Guo Y, Zhang L, Zhang J, Miao M, Tan H, Hu D, Li X, Ding X, Li G and Guo H. 2021. Proteomic studies on the mechanism of myostatin regulating cattle skeletal muscle development. *Frontiers in Genetics* **12**: 1–18.

Smith J A, Lewis A M, Wiener P and Williams J L. 2000. Genetic variation in the bovine myostatin gene in UK beef cattle: Allele frequencies and haplotype analysis in the South Devon. *Animal Genetics* **31**(5): 306–09.

Smith T P, Lopez-Corrales N L, Kappes S M and Sonstegard T S. 1997. Myostatin maps to the intervalcontaining the bovine mh locus. *Mammalian Genome* **8**(10): 742–44.

Tantia M S, Vijh R K, Kumar S T B, Mishra B and Reecy J M. 2006. Comparative analysis of GDF 8 (myostatin) in *Bos indicus* and *Bos taurus*. *DNA Sequence* **17**(4): 311–13.

Vankan D M, Waine D and Fortes M. 2010. Real-time PCR genotyping and frequency of the myostatin F94L mutation in beef cattle breeds. *Animal* **4**(4): 530–34.

Wu D, Gu M, Wei Z, Bai C, Su G, Liu X, Zhao Y, Yang L and Li G. 2022. Myostatin knockout regulates bile acid metabolism by promoting bile acid synthesis in cattle. *Animals* **12**(2): 1–13.