Polymorphism of 5’UTR myostatin gene indel (g.1256/TTTTA) and its association with body weight in Boerka crossbred goat

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ABSTRACT

This study aimed to identify the variation of 5’UTR Myostatin (MSTN) gene and its association to body weight in Boer, Kacang, and Boerka goats. DNA samples were obtained from 149 heads of the goats from the Indonesian Goat Research Center, Sungei Putih, North Sumatera. Polymorphism identification was conducted by direct sequencing and PCR-RFLP with Dral as the restriction enzyme for indel g.1256/TTTTA. Analysis of variance for body weight was carried out using the General Linear Model (GLM). The 5’UTR MSTN gene|Dral was monomorphic in Kacang but polymorphic in Boer and Boerka. Genotype frequencies for Boer 0.40(AA), 0.43(AB), 0.17(BB); Kacang 1.00(AA); Boerka 0.53(AA), 0.37(AB), 0.10(BB). Genotype allele frequency for Boer 0.62(A), 0.38(B); Kacang 1.00(A); Boerka 0.72(A), 0.28(B). Genotype allele frequency for AB paling tinggi pada Boer, namun frekuensi genotipe AA paling tinggi pada Kacang dan Boerka. Indel g.1256/TTTTA berpengaruh signifikan (P<0.05) hanya pada bobot lahir (BW) kambing Boer, namun tidak signifikan terhadap parameter bobot badan lainnya baik pada kambing Boer maupun Boerka. Genotipe AA memiliki bobot lahir paling tinggi (P<0.05) dari AB, namun tidak berbeda nyata dengan genotipe BB. Indel g.1256/TTTTA dapat dijadikan sebagai penciri genetik untuk sifat pertumbuhan (bobot lahir) kambing Boer namun tidak pada Boerka.

Kata kunci : 5’UTR, Indel TTTTA, gen MSTN, RFLP, sekuensing

The 5’UTR Myostatine Gene Polymorphisms in Boerka Crossbred Goat (R. Ismail et al.)
INTRODUCTION

Kacang goat is one of Indonesia’s local livestock. These goats were set as Indonesian domestic goat breed based on a decision of the Indonesian Minister of Agriculture No: 2840/Kpts/LB.430/8/2012. The superiorities of Kacang goats are known; have a good environment adaptive ability (Santoso et al., 2016; Septian et al., 2015), quickly produce offspring (Wahyuni et al., 2016), and more resistant to parasitic gastrointestinal infections (Batubara, 2006). As a tropical breed, Kacang goat was prolific (Panjono et al., 2012), smaller body size but had many offspring (Mulyono et al., 2018). Low body weight with an adult male weight of 24.05±3.95 kg makes Kacang goat not as ideal as meat-producing livestock (Batubara et al., 2012; Wahyuni et al., 2016).

One effort to improve the genetic quality of Kacang goats was through crossing with Boer buck goat. Boer goat was a beef type with fast body growth, good meat quality, and parasitic tolerance (Chong et al., 2019; Elieser et al., 2012; Garcia-Muniz et al., 2019). Boer has weaning weights 23.4±9.7 kg at the age of 112 days (Menezes et al., 2016) and adult male 120-150 kg at the age of 2-3 years (Nurgiartiningisih et al., 2006). Boer and Kacang goat crosses were called Boerka (Ginting and Mahmilia, 2008). The Boerka goat was reported to have a birth weight, weaning weights, one-year weight, body weight gain, and carcass percentage higher than the Kacang goat (Doloksaribu et al., 2005; Priyanto et al., 2002; Setiadi et al., 2001; Triyantini et al., 2002). The genetic quality of Kacang goats can be improved through molecular-based growth selection. One gene that can be used was the MSTN gene because that affects the growth of muscle mass (Batubara, 2017; El Shafey et al., 2016).

MSTN was a member of the transforming growth factor-beta superfamily, which is also known as the growth differentiation factor (GDF) 8 (Hayashi et al., 2018). MSTN acts negatively towards the regulation of skeletal muscle mass development (Sun et al., 2020; Yue et al., 2020). The MSTN gene inhibits the work of the Myf5 and MyoD gene factors, which are related to the mechanism of differentiation of precursor cells into myoblasts (McPherron and Lee, 1997). The deactivation of the MSTN gene influences adipose tissue mass in addition to bone muscle mass (Dominique and Gérard, 2006) and associated with muscle hypertrophy (Kvedaras et al., 2019). This gene was specifically expressed during the development of the embryonic phase and adult skeletal muscle. MSTN gene polymorphisms cause growth acceleration and muscle mass of yellow catfish (Zhang et al., 2020), double muscular in cattle and goat (Grisolia et al., 2009; He et al., 2018), affect the carcass quality and lamb meat quality (Grochowska et al., 2019). MSTN gene polymorphisms were exciting things that could be used to increase livestock meat production (Aiello et al., 2018).

Some goats breed shown polymorphisms in the MSTN gene. Several studies reported the existence of 5'UTR polymorphisms in local goats in Iran (Abdolmohammadi et al., 2016), China (Li et al., 2008; Zhang et al., 2012), and India (Singh et al., 2014). 5'UTR was the region of the mRNA upstream from the protein-coding region (Manzella et al., 2020). 5'UTR has an important role such as; affect the level of mRNA transcription, mRNA decay, translation rates (Feng et al., 2019), control the process of translational initiation (Arend et al., 2018), translational repression mediator (Theil et al., 2018), and regulation of gene expression (Araujo et al., 2012; Liao et al., 2013; Zhang et al., 2018). The indel of TTTTA in the 5'UTR goat MSTN gene has a significant effect on body weight and size (Li et al., 2008) on birth weight, 90-day weight, 300 days age weight and birth body length of Boer goat (Zhang et al., 2012), whereas the indel study of the TTTTA in the 5'UTR MSTN
gene in Indonesian goats have never been done.

Therefore, the objective of this study was to
analyze the genetic diversity of the 5'UTR MSTN
gene fragment and associate it with the body
weights of Boer, Kacang, and Boerka goats. The
results of this study are expected to be used as
basic information in the development of goat
breeding programs in Indonesia, especially in the
Indonesian Goat Research Center, Sungei Putih,
Deli Serdang, North Sumatera.

MATERIALS AND METHODS

Animal and Samples

This research was carried out in the animal
molecular genetics laboratory, Animal Science
Faculty, IPB University. One hundred and forty-
ine blood samples were taken from the
Indonesian Goat Research Center, Sungei Putih,
Galang, Deli Serdang, North Sumatera. The
samples consisted of Boer (30 heads), Kacang (29
heads), and Boerka goats (90 heads). The goats
were maintained in one location with the same
management maintenance in a cage and given a
feeding concentrate and forage feed (Indigofera
zoolingeriana, Brachiaria humidicola, Brachiaria
ruziizensis, Pennisetum purpureum cv mott). Data
were collected during the years of 2016-2018 of
body weights at birth (BW), 3 months (M3W), 6
months (M6W), 9 months (M9W), 12 months
(M12W), and Average daily body weight gain
(ADG) between birth to 12 months of age. Blood
samples 3 ml were collected from the jugular vein
using a venoject needle and kept in the vacutainer
tube containing K2EDTA anticoagulant, then
stored in a refrigerator (temperature ± 4°C).

DNA Extraction and 5'UTR MSTN Gene
Amplification

DNA extraction, according to the extraction
procedure of Genomic DNA mini kit (Geneaid
Biotec Ltd). Amplification using the PCR
method with the master cycler gradient machine
(ESCO, Singapore). Amplification was performed
at a total volume of 25 μL consisting of 2.5 μL
(1.1-15.7 ng) DNA templates, 12.5 μL PRIMEGA green master mix, 9.4 μL nuclease-
free water, 0.3 μL (25 pmol) forward primer and
0.3 μL (25 pmol) reverse primer.

The primer design refers to the National
Center for Biotechnology Information (NCBI)
with access number EF591039.1. Primer was
designed using the Primer 3 program, Multiprimer
Analyzer and Primer Stat. The primer design
results were a forward primer 5'-AAGAGCCAATCACAGATCCC-3' and reverse
primer 5'-ACTAGAACACGACTCAGCAG-3'
with a product length of 635 bp. Amplification of
MSTN gene DNA through 5 stages. The first
stage was the denaturation process at 95°C for 5
minutes. The second, third, and fourth stages were
cycles repeated 35 times with steps; denaturation
at 95°C for 10 seconds, annealing at 57°C for 20
seconds, and extension at 72°C for 30 seconds.
The fifth stage ends with elongation primers at
72°C for 5 minutes. DNA amplification products
were extracted on 1.5% agarose gel for 35
minutes and photographed using UV Transilluminator. The use of agarose gel
according to the procedure of Lee et al. (2012).

DNA Sequencing and PCR-RFLP

Ninety-five selected samples amplicon with
a volume of 22 μL for each sample were
sequenced by commercial laboratory service at
First BASE Laboratories (Malaysia). Direct
sequencing using ABI Prism 96-capillary 3730xl
DNA analyzer (Applied Biosystems, USA). Indel
g.1256-1260 (TTTTA/-) was identified using the
PCR-RFLP method on the remaining samples
until all (149) samples were identified. PCR-
RFLP uses the DraI restriction enzyme with a cut
site (TTT[AA]). The RFLP was carried out at a
total volume of 7.2 μL consisting of 5 μL of PCR
(amplicon) products, 0.9 μL nuclease-free water,
0.7 μL buffer enzyme, 0.6 μL DraI enzyme and
incubated at 37°C for 12 hours. RFLP products
were electrophoresed on 2% agarose gel for 43
minutes and photographed using UV Transilluminator.

Data Analysis

Sequencing results were analyzed using the
software Finch TV 1.4, Bioedit 7.2, and MEGA
7.0 Tamura et al. (2013). The allele and genotype
frequency were calculated with Popgene32,
according to Nei and Kumar (2000). Hardy-
Weinberg equilibrium using Popgene32 with chi-
square based on the method of Hartl and Clark
(1997). The ANOVA of the 5'UTR MSTN gene
was analyzed using SAS 9.4 software (SAS
Institute, USA) with a mathematical model of the
General Linear Model (GLM) at level probability
0.05. Further tests were carried out by Least
Square Means to find out the significant
differences between genotypes. A mathematical
model was formulated as follows: \( Y_{ijkl} = \mu + \alpha_i + \beta_j + \epsilon_{ijkl} \)
+ γₖ + zₜ + Єᵢᵣₖₗₘ. Where: μ is the overall mean for each trait; αᵢ is the effect of ith genotype, i is 1,2,3; βⱼ is the effect of jth sex, j is 1,2; γₖ is the effect of kth birth type, k is 1,2,3; zₜ is the effect of lth birth season, l is 1,2; Єᵢᵣₖₗₘ is random error.

RESULTS AND DISCUSSIONS

Polymorphisms in 5'UTR MSTN Gene

The 5’UTR MSTN gene was successfully amplified using primer at an annealing temperature of 57°C for 20 seconds, with a length of PCR product was 635 bp (Figure 1). These results indicate that fragments have excellent specifications and can be further processed through direct sequencing analysis. Visualization of direct sequencing results on finch TV shows that there were three different banding patterns; normal, deletion, and double band until the end of the product sequence (Figure 2). The alignment of sequencing results for the EF591039.1 genebank shows the five base pairs indel (g.1256/TTTTA), but no other SNPs were found (Figure 3). This is in agreement with previous researches which reported the presence of the same indel in eighteen local Chinese goat breeds (Li et al., 2008; Zhang et al., 2012), four Iranian goat breeds (Abdolmohammadi et al., 2016) and seven Indian goat breeds (Singh et al., 2014). But, the Indian goats have shown some new SNP besides the TTTTA deletion. Boer and Boerka goats have shown polymorphisms, but monomorphism in Kacang goat (Figure 3). Amplification RFLP product of the 5’UTR MSTN gene (Figure 4) showed three genotypes; AA (635 bp), BB (430 bp, 205 bp), and AB (635 bp, 430 bp, 205 bp).

Genotype and Allele Frequency of 5’UTR MSTN Genes

PCR-RFLP shows that the 5’UTR MSTN gene in Boer and Boerka goats has three genotypes; AA (deletion), AB (heterozygous), and BB (normal). But the Kacang goat has only one genotype AA (deletion). The highest AA genotype frequency was in Boerka goats (0.53), the AB genotype frequency was highest in Boer goats (0.43) while the BB genotype had the lowest frequency among the others. Li et al., (2008) and Zhang et al., (2012) reported three genotypes (AA, AB, BB) in local Chinese goats with the frequency of AA and AB dominant than BB. Genotype frequencies in seven local Indian goats have only two genotypes (AA, AB) (Singh et al., 2014). One local Iranian goat breed was shown three genotype polymorphisms (AA, AB, BB), but three other breeds have only two genotypes (AA, AB). The results of this study indicate a pattern of genotype frequency in local goats in 4 countries (Indonesia, India, Iran, and China). Chinese local goats have three genotypes, Iranian have 3 and 2 genotypes, local Indian goats have two genotypes, while local Indonesian goats (Kacang) only have one genotype. This is related to the position of the country to the equator. Where the area around the equator is a tropical region bounded by latitude (Reis et al., 2018). Latitude forms 3 main climates (cold zone, temperate climate zone, and hot lowland). In this zone, certain varieties of plants and animals are formed that can adapt to the environment (Asfaw et al., 2019).

This study found two alleles (Table 1) A and B with the highest frequency in A. A allele in Boer goats was 0.62 and 0.72 in Boerka goats. So, the 5’UTR MSTN gene in Boer and Boerka goats were polymorphic because it had at least two alleles with a relative frequency greater than 0.01 (1%) (Nei and Kumar, 2000).

Hardy-Weinberg Equilibrium of 5’UTR MSTN Gene in Population

The genotype balance of 5’UTR MSTN gene in the population was tested by chi-square (χ²).

Figure 1. Amplification PCR Product of 5’UTR MSTN Gene Fragment using 1,5% Agarosa Gel. M = Marker 100 bp DNA; 1,2,3,........13 = Sample Code
The $\chi^2$ test aims to determine whether the population was still in Hardy-Weinberg Equilibrium (HWE) or not. The population was said to be balanced if the calculated $\chi^2$ value was smaller than $\chi^2$ tables ($P<0.05$) (Allendorf et al., 2013). Boer, Kacang, and Boerka goats were still in HWE (Table 1). The frequency of alleles and genotypes does not change from generation to generation indicates that the population was in balance (Allendorf et al., 2013). HWE only applies to populations that are ideal where there are no disturbances that affect genotype and allele frequencies (Waples and Allendorf, 2015). Therefore the overall principle of HWE makes several assumptions such as; random matting, unlimited population sizes, no mutations, no selection, single population, no migration, non-overlapping generations, and diploid inheritance (Meirmans, 2018).

Heterozygosity was used to measure the level of genetic diversity in a population-based on allele frequencies. If the objective heterozygosity ($Ho$) value is greater than the expected heterozygosity ($He$), the population is diverse (Sharma et al., 2016). Based on Table 1, the five base pairs indel (g.1256/TTTTA) MSTN gene in Boer and Boerka goats has low diversity due to the value of Ho less than He. Ho and He values

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that were not significantly different indicated that there had been selection activities and there was no random matting (Allendorf et al., 2013; Kolenda et al., 2019) in the growth in birth weight of the Boer goat, but not significantly in Boerka goats. However, the indel did not show any significant effect on other parameters such as M3W, M6W, M9W, M12W and ADG, both on Boer and Boerka 04±0.82 (Zhang et al., 2008), 3.22±0.13 (Browning et al., 2011) and 3.41±0.80 (Menezes et al., 2016).

The AA genotype in Boer goat birth weight (BW) was significantly different (P<0.05) from the AB genotype but not substantially different from the BB genotype. The AA genotype tends to have a higher body weight than the BB genotype in birth weight (BW) and 3 month age weight (M3W). Although it has no real effect, the AB genotype has a bodyweight that tends to be higher in; 6 months (M6W), 9 months (M9W), 12 months (M12W) of age and daily weight gain (ADG). In this case, the BB genotype has a bodyweight that is almost the same as the AB genotype compared to the AA genotype. BB genotype in Boerka goats tends to have the highest body weight in BW, M3W, M9W, M12W, and ADG. Although the three genotypes in Boerka goats did not show statistically significant differences, the highest tendency for body weight was found in BB and AA genotypes, compared to AB genotypes respectively.

Genotype AA was significant (P<0.05) for the highest birth weight in Boer goat. This shows that 5'UTR MSTN|DraI can be used to select for birth weight traits in Boer goat in Indonesia. Previous studies found a significant association

### Table 1. Result of Statistical Analysis in the Indel 5'UTR MSTN Gene in Boer, Kacang and Boerka Goat

| Marker     | Goat Population | (n) | Genotype Frequency | Allele Frequency | Ho | He | \(\chi^2\) |
|------------|-----------------|-----|--------------------|------------------|----|----|-----------|
| Indel      | Boer            | (30) | AA (12) AB (13) BB (5) | 0.62 0.38 0.43 0.47 0.30 (ns) |
| g.1256 / TTTTA | Kacang         | (29) | 1.00 (29) - - - | 1.00 - - - - |
|            | Boerka          | (90) | 0.53 (48) AB (33) BB (9) | 0.72 0.28 0.37 0.41 0.95 (ns) |

df = 1; \(\chi^2\) table = 3.84; ns = non significant
between the indel g.1256/TTTTA markers on the growth traits in several goat breeds in China. Li et al., (2008) report the results of the same study in which the AA genotype significantly affected birth weight up to 3 months of age in eighteen local Chinese goat breeds. The AB genotype in Boer goats was known to have a significant effect (P<0.05) on birth weight, 90-day weight, and 300-day weight (Zhang et al., 2012). However, Bi et al., (2020) reported different results in which the AA genotype in Shaanbei White Cashmere goat had no significant effect on body weight, but had a significant effect (P<0.05) on body size such as body height and height at the hip cross. All of these studies confirm that the g.1256/TTTTA 5’UTR MSTN gene indel can be used as a marker gene for the growth traits of goats. But differences in livestock environments between countries have led to variations in genotypic expression that control the nature of the growth.

CONCLUSION

The five base pairs indel g.1256/TTTTA of 5’UTR MSTN gene fragment was polymorphic in Boer and Boerka, but monomorphic in Kacang goat. Boer, Kacang, and Boerka goats were still in Hardy-Weinberg equilibrium (HWE). Association of the polymorphism of the MSTN | 5’UTR gene Dral shown a significant difference in birth weight (BW). Genotype AA has only affected the growth of the highest birth weight in Boer goats, but it does not affect the other bodyweight parameters, both in Boer and Boerka goats. Therefore the molecular selection of the MSTN | 5’UTR gene Dral could be used to find the best Boer birth weight with AA genotype, but it is not suitable for Kacang and Boerka goats.

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