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Polymorphism of Growth Hormone Msp1 Enzyme-Restriction Associated with Production Performance of Ongole-Crossbred Cattle Mated by Artificial Insemination Technique

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ABSTRACT

The most important polymorphism is mutation (transition of Thymine (T) to Cytosine (C)) at intron 3, detected by *MspI* enzyme-restriction in cattle. Uncontrolled breeding by artificial insemination (AI) technique could cause genetic in equilibrium of genotype frequency in animal population as a part of non random mating system. The objective of this research was to identify growth hormone (GH) gene polymorphism using *MspI* enzyme-restriction and its association with production performance including body weight (BW), chest girth (CG), body length (BL) and average daily gain (ADG) in Ongole-crossbred cattle. Total of 74 blood samples were collected from cows and their progenies at the AI service center of North Sulawesi province, Indonesia. Blood samples were analyzed for presence of GH gene using PCR-RFLP method involving *Msp1* enzyme-restriction and visualized on 1.2 % agarose gel. Data were adjusted for 5 years old within parental cows and for 10 months old within their progenies for elimination of different age effects. Data were analyzed using statistical program in Excel XP. The results showed that cows and their progenies with $Msp1^{+/-}$ heterozygous genotype performed the outstanding average of CG, BL and BW compared with those averages in both $Msp1^{+/+}$ and $Msp1^{-/-}$ homozygous genotypes. Although, the $Msp1^{+/+}$, $Msp1^{+/-}$ and $Msp1^{-/-}$ genotypes did not affect ADG of the progenies, these genotypes can be used as the candidate genes in Ongole-crossbred cattle to improve their production performance. The AI technique should be maintained for breeding in increasing the favorable $Msp1^{+/-}$ heterozygous genotype.

KEYWORDS: growth hormone, $Msp1^{-/-}$ genotypes, ongole-crossbred cattle, polymorphysm

INTRODUCTION

The Indonesian cattle breeds are supposed to be of unknown compositions of mixed breed origin. The Ongole crossbred cattle is composed of crossing among zebu (*Bos indicus*), banteng (*Bos javanicus*), and other Indonesian local indigenous breed, which has not been documented and is so far only supported by preliminary molecular analysis [1]. They have adapted to harsh environment under hot and humid climate as well as low-quality feed to produce meat and power to plough a farm land prior to planting. This Ongole crossbred cattle is dominant breed and plays important role for increasing income of smallholder animal agriculture in Indonesia including North Sulawesi, Indonesia.

Growth hormone (GH) in beef cattle plays a vital role in post-natal growth and general metabolism [2]. Therefore, GH has been the most intensive object of studies and research in ruminant animals to relate the mutation of GH with the productive traits [3]. With the development of molecular biology and biotechnology, scientists are able to achieve more accurate and efficient selection goal by marker-assisted selection (MAS). Growth hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner, the pattern of which plays an important role in postnatal longitudinal development, tissue growth, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism [4]. Effects of GH on growth are observed in several tissues, including bone, muscle and adipose tissue, so that GH gene, with its functional and positional potential, has been widely used for marker in several livestock species, including the cattle such as *Bos taurus* and *Bos indicus* [5]. It has been reported that the restriction fragment length polymorphisms (RFLP) of GH were associated with body weight in Grati dairy cows [6].

The studies of GH gene *MspI* locus have been reported in Ongole crossbred cattle [7][8], Brahman cattle [5], Indian Zebu cattle [9], West coastal Sumatera cattle [10], and Grati dairy cows [6]. Their studies indicated that *Msp1*+/+ and *Msp1*+/- genotypes can be used as the candidate genes in cattle selection for breeding program. These genotypes had a stronger correlation to the higher body weight than *Msp1*-/- genotype in Grati dairy cows [6]. In contrast, these genotypes did not strongly correlate with body weight, chest girth and body length in the Indonesian local West coastal Sumatera cattle breed [9]. The objective of this research was to identify growth hormone (GH) gene polymorphism using *MspI* enzyme-restriction and its association with

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production performance including body weight (BW), chest girth (CG), body length (BL) and average daily gain (ADG) of Ongole-crossbred cattle mated by artificial insemination (AI) technique in North Sulawesi province.

MATERIALS AND METHODS

Animal and Blood Samples Collection

This study was carried out in AI service centre of North Sulawesi Province Indonesia where Ongole-crossbred cattle are bred by the artificial insemination (AI) technique for their improvement and distribution to farmers. Total of 76 cattle were used in this study, comprising of 37 cows (age ranging from 4 to 5 years old) and their 37 female progenies (age ranging from 8 to 10 months old) as well as two Ongole bulls of approximately 10 years old. All cows were reared under private areas belong to farmers with unknown ancestors. Progenies were born from those cows mated by AI technique using germ plasma (semen) of the two Ongole bulls called "Kirsta" and "Tunggul" from "the AI bull germ plasma centre" located at Singosari, East Java province, Indonesia.

Prior to blood collection, body weight of animal was determined by using a digital weighing scale. The parameters of chest girth and body length of the cattle were measured using measuring tape when they were standing as described in Ozkaya and Bozkurt [11]. Blood samples of the cows, their progenies and the two Ongole bulls as source of germ plasmas (mated by AI) were collected during July 2011 from Jugular vein of the cattle in 10 ml EDTA (10%) tubes during the *Msp1* selection experiment and stored in the refrigerator (4°C) until ready for DNA isolation.

DNA Extraction

The genotyping process was conducted at the Biotechnology Laboratory, Department of Biological Science, Faculty of Mathematics and Natural Science, Sam Ratulangi University, Manado. Genomic DNA from whole blood of Ongole crossbred cows, bulls and their calf were purified by standard protocol using proteinase K digestion as described by DNA extraction kit (AxyPrep Blood Genomic DNA Miniprep kit, AXYGEN Biosciences, Union city, CA, 94587, USA).

Genotyping for GH and Allele Identification

Following the genomic DNA isolation, the animals were genotyped for GH locus using PCR-RFLP (Polymerase chain reaction-restriction fragment length polymorphism) and 1.2% agarose gel electrophoresis [12]. Amplification of the fragment of 327 bp at intron 3 [13] was done with PCR using forward primer 5'-CCCACGGGCAAGAATGAGGC-3'; reverse primer 5'-TGAGGAACTGCAGGGGCCCA-3' [14]. The reaction mixture of PCR was performed by using 1x Taq pol 25 µl of master mix (Axygen Biosciences, CA, USA).

To digest this fragment, a protocol RFLP with restriction enzyme *Msp1* was used to recognize the particular site of C↓CGG. The PCR product of GH gene was digested at 37° C for 3 hours by *Msp1* enzyme. Reaction consisted of 2 µl Buffer V2 10X, 7.5 µl H_2O, 0.5 µl Enzyme *Msp1* (20 U/µl), and 10 µl PCR product. PCR protocols to amplify the fragment were by the initial denaturation temperature step at 94 °C for 5 min for 1 cycle followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, elongation at 72 °C for 30 s and a final extension at 72 °C for 1 min. [15]. Following the end of PCR and RFLP process, the products were then subsequently electrophoretic using 1.2% agarose gel to identify polymorphism of allele based on the length of the band as presented at Figure 1.

Data Analysis

PCR-RFLP data were analysed by allele frequency [16]. The allele frequency was calculated by the methods as follows:

$$x_i = \frac{(2n_{ii} + \sum n_{ij})}{2N}$$

Note; x_i is the *Msp1*+ allele frequency

n_{ii} is the number of cattle with the genotype of *Msp1*+/+,

n_{ij} is the number of cattle with the genotype of *Msp1*+/-,

N is the total number of cattle tested.

The equilibrium test of the observed *Msp1*+ genotype frequency compared with the expected *Msp1*+ genotype frequency was calculated using Chi-square test ($\mathcal{X}^2$) [17] as follows:

$$\mathcal{X}^2 = \sum \frac{(f_o - f_e)^2}{f_e} = \sum \frac{f_o^2}{f_e} - N$$

Note: $\mathcal{X}^2$ is the Chi-square distribution,

f_o is the observed frequency of the ijkth cell, and
f_e is the expected frequency of the ijkth cell.

$$f_{e-ijk} = \frac{\sum f_{e-i} \; x \; \sum f_{e-j}}{\sum f_{e-ijk}}$$

$\sum f_{e-i}$ is the total of observed frequency of the ith row;
$\sum f_{e-j}$ is the total of observed frequency of the jth column; and
$\sum f_{e-ijk}$ is the total of observed frequency of the ijkth cell.

In this study, the total of 37 parental cows were divided into 20 superior body weight animals (cow weights heavier than at least one fifth standard deviation above the mean) and 17 inferior body weight animals (cow weights lighter than one and half standard deviation below the mean) among cow population (n = 363 heads, with body weight mean of 440.20 ± 58.03 kg) as described in Paputungan *et al.* [18].

The data of the parental cows within 5 years of age were used in this study. The female progenies used in this study comprised of 15 heads within 5 days old, 10 heads within 20 days old, 3 heads within 30 days old and 9 heads within 50 days old. Data of the female progenies were corrected by adjusting for the 50 days old age for the first weighing (first standard) and for the 345 days old age for the second weighing (second standard) for elimination of different age effects of animals using the formula [10] as follows:

$$x_{i-corrected} = \frac{\bar{x}_{standard}}{\bar{x}_{observed}} \; x \; x_{i-observed}$$

Comparison of the means of body measurement variables within animal genotype was tested using *t* test [19] as follows:

$$t = \frac{\bar{x}1 - \bar{x}2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x}_1)^2 + \sum_{i=1}^{n}(x_i - \bar{x}_2)^2}{n_1 + n_2 - 2}}$$

Note: $\bar{x}_1$ and $\bar{x}_2$ are mean of genotype 1 and 2, respectively;
n_1 and n_2 are sum of the animals with genotype 1 and with genotype 2, respectively.
Data were analysed using software of the statistical program function in Excel XP (2007).

RESULTS AND DISCUSSION

Polymorphism Detection

The *Msp1* digestion of the PCR products produced the fragments of 104 bp and 223 bp for allele *Msp1+* and of 327 bp for allele *Msp1-* (Figure 1). These alleles were the same with research reported by Zhou *et al.* [20] using *Msp1* enzyme restriction in Beijing Holstein and Maylinda [6] using *Msp1* enzyme restriction in Grati dairy cows. The difference of these two fragments of *Msp1+* and *Msp1-* alleles was caused by mutation of Cytosine (C) to Thymine (T) [21]. Genotype *Msp1+/+* consisted of two bands (104 bp, 223 bp), genotype *Msp1+/-* consisted of three bands (104 bp, 223 bp, 327 bp), genotype *Msp1-/-* consisted of one band (327 bp).

The *Msp1* enzyme recognized only the restriction site of four nucleotides for C↓CGG (Figure 2). Gene variation of GH locus for *Msp1* in cattle was detected in the position of intron 3 [21] at the sequence position of 1547 based on nucleotide sequence from GenBank, number: M57764.1 (http://www.ncbi.nlm.nih.gov) sourced in Gordon *et al.* [13] accessed on March 26, 2012 (Figure 2). Mutation occurred on DNA level due to nucleotide changes, either transition or insertion [22]. Based on the difference of nucleotide restriction sites of each allele, the mutation of Cytosine (C) into Thymine (T) occurred due to nucleotide transition. The transition of C into T changed the restriction site of *Msp1* enzyme [21].

Growth Hormone *Msp1* Polymorphism in Superior and Inferior Cow Groups

The number of parental cow matching pair with its female progeny for this study was only 37 heads. Because of large variation in smallholder cattle farms at the Tumaratas village under study, the animal body weights involved in this study was divided into superior cow group (SCG) of 20 heads and inferior cow group (ICG) of 17 heads (Table 1).

The number parental cows showing polymorphism at GH locus bearing *Msp1*$^{+/+}$, *Msp*1$^{+/-}$ and *Msp1*$^{-/-}$ genotypes were 5, 14 and 18 heads, respectively. It was found that the frequency of *Msp*1^{+} and *Msp1*$^{-}$ alleles was 0.45 and 0.55 under the SCG, respectively; and 0.18 and 0.82 under the ICG, respectively (Table 1). The heterozygosis level at this locus was 0.32 which was higher than the accepted minimum polymorphism value of 0.01 reported by Dorak [23], indicating that Ongole crossbred parental cow was polymorphic. This result indicated that in Ongole crossbred cow there was high variability in the GH locus and offers opportunity to apply GH genotype for selection criteria.

Based on the Chi square test, it was indicated that allele frequency in both cow groups were not under genetic equilibrium (Table 1). However, the all parental cows mated by AI technique using sperms of bull Krista ($Kr^{+/+}$) and Tunggul ($Tu^{-/-}$) reproduced the progenies with different allele frequency. It was found that the frequency of $Msp1^{+}$ and $Msp1^{-}$ alleles in the progenies born by the SCG was 0.50 and 0.50, respectively. In addition, the frequency of those in the progenies born by the ICG was 0.21 and 0.79, respectively (Table 1). Based on the Chi square test, it was revealed that allele frequency in the progenies born by both cow groups were under genetic equilibrium. Factor affecting genetic equilibrium was animal selection such as using selected male and female animal parents for the next generation [24][25]. In this case, the heterozygous genotype frequency of GH gene of *Msp1*+/- was not found in the ICG (Table 1) causing the instability of genotypic and allelic frequencies of GH gene in cow population. This heterozygous genotype frequency was found only in group of the SCG indicating a trend of heterocyst effect inherited by alleles of both *Msp1*+ and *Msp1*-. Heterosis was defined as a productive trait advantage of outstanding progeny inherited from crossing of both parents producing lower productive trait average compared with that of their progeny [26]. This finding suggests that in equilibrium of allelic frequency in parental cow generation could be stabilized by breeding program using desirable selected genotype of bulls to produce generation of the genotype progenies being under genetic equilibrium (Table 1).

Genotypic Association of GH *Msp1* with Production Performance of Cow Groups

In this study, the $Msp1^{+/+}$ and $Msp1^{-/-}$ genotypes gave less influence on body weight (BW) and body length (BL) than those of the $Msp1^{+/-}$ genotype (463.00 and 462.25 vs 465.57 kg for BW; 140.50 and 137.25 vs 145.71 cm for BL) under superior cow group (SCG). In contrast, the $Msp1^{-/-}$ genotype gave more influence on chest girth than those of the $Msp1^{+/+}$, $Msp1^{+/-}$ genotypes (182.25 vs 179.50 and 179.50cm) under SCG (Table2). Heterozygous genotype of the $Msp1^{+/-}$ not found under inferior cow group (ICG). Moreover, body weights in both $Msp1^{+/+}$ and $Msp1^{-/-}$ genotypes did not differ significantly ($p>0.05$) under (ICG). The $Msp1^{+/+}$ genotype gave less influence on chest girth (CG) than that of the $Msp1^{-/-}$ genotypes (154.00 vs 164.43 cm) under ICG. In contrast, the $Msp1^{+/+}$ genotype gave more influence on body length than that of the $Msp1^{-/-}$ genotypes (142.33 vs 130.07 cm) under ICG (Table 2). The combination of both larger CG and longer BL of animal may attribute to the heavier body weight. Moreover, heavier body weight of cow may also be attributed to higher body fat reserves causing lower milk production of dam [27]. Some researchers reported that heavier body weight cows did not affect milk yield because leaner cows increased their feed intake and fatter cows tended to deplete labile fat reserves, suggesting that milk yield was maintained at the expense of the body weight [15][3].

The growth of animals is under the hormonal control of GH, growth hormone receptor (GHR) and insulin-like growth factor 1(IGF-1) [28]. Polymorphism occurring in the regulatory region (promoter region) and coding region (exons) of the gene responsible for those three hormones would influence the expression of the genes and the function of protein during the translation process [2].

This study revealed that superior animals differ genetically from inferior animals mainly in their regulation of nutrient utilization and the GH release [29]. Therefore, genotypic association of GH locus with the performance of parental cow in this study indicated that the homozygous $Msp1^{-/-}$ genotype was more responsible for development of animal chest girth (CG), while the homozygous $Msp1^{+/+}$ genotype was more responsible for development of animal body length (BL). The heterozygous $Msp1^{+/-}$ genotype would indicate a trend of heterosis effect. This genotype was more responsible for the animal BL and animal body weight (BW). This is in agreement with some reports [26][30][31] who stated that heterocyst effect was a productive trait advantage of outstanding progeny inherited from crossing of both parents producing lower productive trait average compared with that of their progeny.

Genotypic Association of GH *Msp1* with Production Performance of the Progenies

In this study, the means for the CG, BL, BW and ADG of the female progenies by the genetic classes born in each parental cow group are presented in Table 3. Superior cow weight had significantly positive effects ($p<0.05$) on CG, BL and BW of the progenies at the age of 50 days old.

The results indicated that the heavier cows had allocated more nutrients to the development of the calf during the fetal and suckling periods, resulting in the larger CG and heavier BW of the calves. The results are in agreement with those reported by Paputungan and Makarechian [27] who found that cows with heavier weights gave birth to heavier calves. However, parental cow weights gave less influence on the CG, BL and ADG of their progenies (Table 4).

The genotype of GH locus was a significant source ($p<0.05$) of variation in BW of progenies at the age from 50 days to 345 days old. The homozygous $Msp1^{-/-}$ genotype of the progeny may contribute to development of the chest girth (CG) compared with the homozygous $Msp1^{+/+}$ genotype (140.50 vs 137.25 cm) born by superior parental cow weight. In addition, the homozygous $Msp1^{-/-}$ genotype of the progeny may also contribute to development of the chest girth (CG) compared with the homozygous $Msp1^{+/+}$ genotype (140.42 vs 137.00 cm) born by inferior cow weight (Table 4). The heterozygous $Msp1^{+/-}$ genotype would indicate a trend of heterocyst

effect for all progenies born by both superior and inferior parental cow weights. This genotype may contribute to the animal CG, BL and animal body weight (BW). In this study, the animal progenies with the heterozygous $Msp1^{+/-}$ genotype performed the outstanding average of CG, BL, BW and ADG compared with the average of those in both homozygous $Msp1^{+/+}$ and $Msp1^{-/-}$ genotypes of progenies (Table 4). This also is in agreement with the report of Fahmy [26] who stated that heterocyst effect was a productive trait advantage of outstanding progeny inherited from crossing of both parents producing lower productive trait average compared with that of their progeny. In recent study, the animal genotypes of $Msp1^{+/+}$, $Msp1^{+/-}$ and $Msp1^{-/-}$ did not affect the ADG of the progenies at the age of 295 days.

CONCLUSION

The homozygous $Msp1^{-/-}$ genotype may contribute to the development of animal chest girth (CG), while the homozygous $Msp1^{+/+}$ genotype may contribute to development of animal body length (BL). The heterozygous genotype of $Msp1^{+/-}$ contributed to higher animal CG, BL, BW and ADG in term of the heterocyst effect. Therefore, the $Msp1^{+/+}$, $Msp1^{+/-}$ and $Msp1^{-/-}$ genotypes can be used as the candidate genes in Ongole crossbred cattle to improve body weight and morphometric measurements. The AI technique should be maintained for breeding in increasing the favourable *Msp1*+/- heterozygous genotype.

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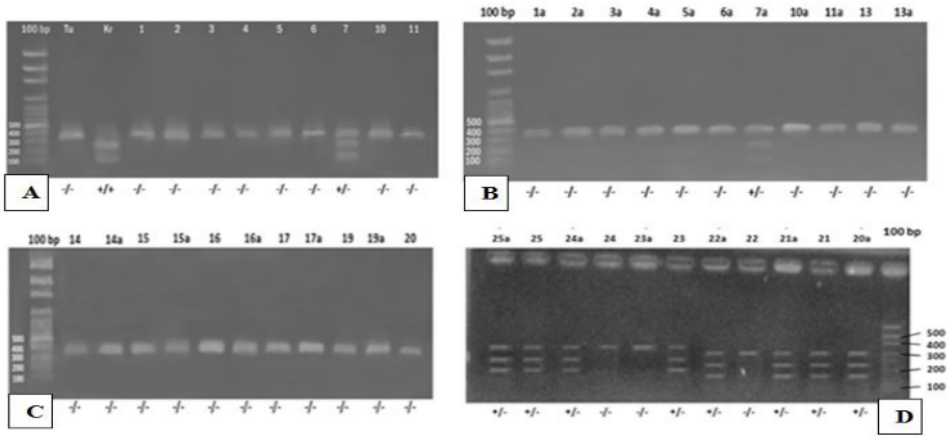

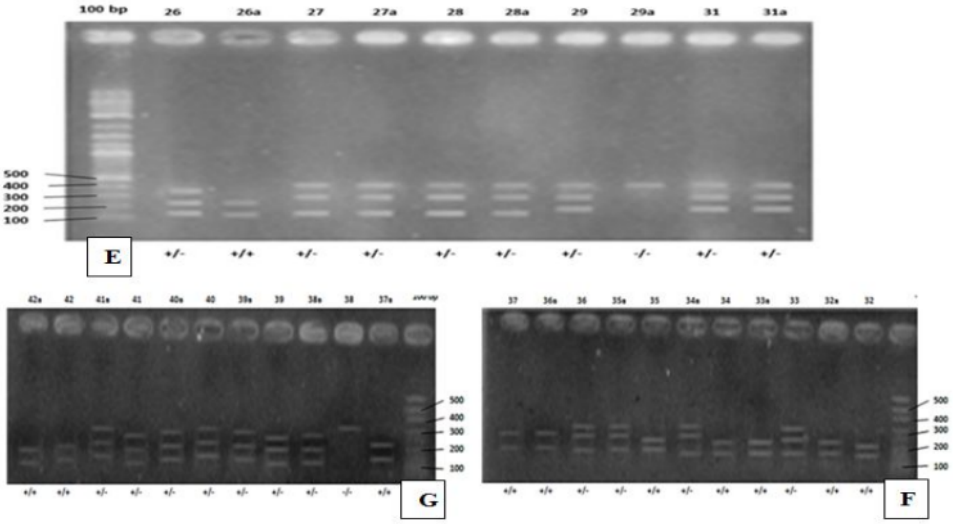

Figure 1. Genotyping Results of *Msp1* Enzyme Restriction in GH Locus Detected through Agarose Gel Electrophoresis. (**A**) Tu= Tunggul (Ongole bull, source of sperms for AI), Kr= Krista (Ongole bull, source of sperms for AI), Cows (1, 2, 3, 4, 5, 6, 7, 10, 11) mated by AI method using sperms of Tu; (**B**) Cow (13) and Progenies (1a, 2a, 3a, 4a, 5a, 6a, 7a, 10a, 11a, 13a) of cows mated by AI method using sperms of Tu; (**C**) Cows (14, 15, 16, 17, 19) and their progenies (14a, 15a, 16a, 17a, 19a) mated by AI using sperms of Tu and Cow (20) mated by AI using sperms of Kr; (**D**) Progeny (20a) of Cow (20) mated by AI using sperms of Kr, Cows (21, 23, 25) and their progenies (21a, 23a, 25a) mated by AI using sperms of Tu, Cows (22, 24) and their progenies (22a, 24a) mated by AI using sperms of Kr; (**E**) Cow (29) and its progeny (29a) mated by AI using sperms of Tu, Cows (26, 27, 28, 31) and their progenies (26a, 27a, 28a, 31a) mated by AI using sperms of Kr; (**F**) Cows (32, 33, 36, 37) and their progenies (32a, 33a, 36a) mated by AI using sperms of Kr, Cows (34, 35) and their progenies (34a, 35a) mated by AI using sperms of Tu; (**G**) Progeny (37a) of Cow (37) mated by AI using sperms of Kr, Cows (38, 39, 40, 41, 42) and their progenies (38a, 39a, 40a, 41a, 42a) mated by AI using sperms of Kr.

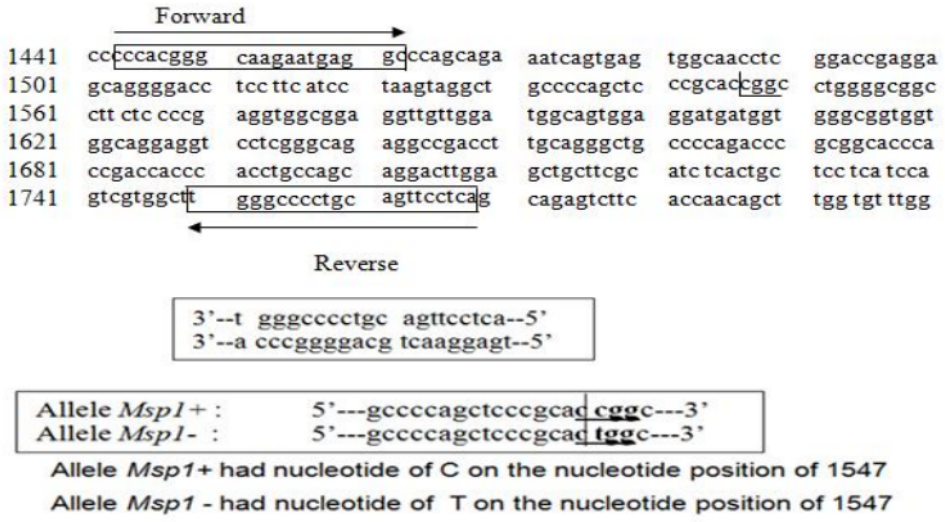

Figure 2. Fragment difference of GH gene and restriction site of *Msp1* enzyme based on GH gene sequence in cattle accessed from *GenBank*, number: M57764.1 (http://www.ncbi.nlm.nih.gov), sourced in Gordon et al. (1983) accessed on March 26, 2012.

Table 1. Genotype and allele frequencies of *Msp1* $^{+}$ and Msp1^{-} at GH locus in Ongole crossbred cows and their progenies reproduced by AI technique

Allele Frequency of Parental Cows	Genotype Frequency of Parental Cows (♀) Mated by AI Technique (♂$Kr^{+/+}$) and (♂$Tu^{-/-}$)						Allele Frequency of Progenies
	♀(*Msp1*$^{+/+}$) x		♀(*Msp1*$^{+/-}$) x		♀(*Msp1*$^{-/-}$) x		
	♂$Kr^{+/+}$	♂$Tu^{-/-}$	♂$Kr^{+/+}$	♂$Tu^{-/-}$	♂$Kr^{+/+}$	♂$Tu^{-/-}$	
[1)]**Superior cow group (SCG):**	SCG:	SCG:	SCG:	SCG:	SCG:	SCG:	Born by the SCG:
***Msp1*$^{+}$ = 0.45**[*)]	1	1	9	5	2	2	*Msp1*$^{+}$ = 0.50 [eq)]
***Msp1*$^{-}$ = 0.55**[*)]							*Msp1*$^{-}$ = 0.50 [eq)]
n = 20							n = 20
[2)]**Inferior cow group (ICG):**	ICG:	ICG:	ICG:	ICG:	ICG:	ICG:	Born by the ICG:
***Msp1*$^{+}$ = 0.18**[*)]	2	1	NA	NA	2	12	*Msp1*$^{+}$ = 0.21 [eq)]
***Msp1*$^{-}$ = 0.82**[*)]							*Msp1*$^{-}$ = 0.79 [eq)]
n = 17							n = 17

$Kr^{+/+}$ =Bull Krista with genotype of *Msp1*$^{+/+}$; **$Tu^{-/-}$** =Bull Tunggul with genotype of *Msp1*$^{-/-}$.
[1)] Superior cow group was cow weights heavier than 450 kg per head.
[2)] Inferior cow group was cow weights lighter than 350 kg per head.
NA = Not available (there is no animal observed with heterozygous genotype of *Msp1*$^{+/-}$).
[*)] The values denote that allele frequency of these groups was not in genetic equilibrium based on the Chi square test, $X^2 = 8.93 > X^2_{.05}\{1\} = 3.84$;
[eq)] The values denote that allele frequency of these groups was in genetic equilibrium based on the Chi square test, $X^2 = 3.73 < X^2_{.05}\{1\} = 3.84$.

Table 2. PCR-RFLP product and mean of chest girth (CG), body length (BL) and body weight (BW) in each genotype of the Ongole crossbred parental cows

Genotype Frequency	CG (cm)	BL (cm)	BW (kg)
Superior cow group (SCG):	SCG:	SCG:	SCG:
***Msp1*$^{+/+}$ = 2**	179.50 ± 0.71^{a}	140.50 ± 0.71^{a}	463.00 ± 4.24^{ab}
***Msp1*$^{+/-}$ = 14**	179.50 ± 1.70^{a}	145.71 ± 0.73^{b}	465.57 ± 2.47^{a}
***Msp1*$^{-/-}$ = 4**	182.25 ± 1.71^{b}	137.25 ± 1.26^{c}	462.25 ± 0.50^{b}
Subtotal = 20	180.05 ± 1.93^{a}	143.50 ± 3.66^{w}	464.65 ± 2.70^{y}
Inferior cow group (ICG):	ICG:	ICG:	ICG:
***Msp1*$^{+/+}$ = 3**	154.00 ± 3.46^{c}	142.33 ± 2.31^{d}	347.67 ± 1.53^{c}
***Msp1*$^{+/-}$ = NA**	NA	NA	NA
***Msp1*$^{-/-}$ = 14**	164.43 ± 1.50^{d}	130.07 ± 0.83^{e}	347.21 ± 1.63^{c}
Subtotal = 17	162.59 ± 4.49^{y}	132.23 ± 4.94^{x}	347.29 ± 1.57^{z}

The values bearing different superscript in the same column differ significantly ($p<0.05$).
NA = Not available (there is no cow observed with heterozygous genotype of *Msp1*$^{+/-}$).

Table 3. PCR-RFLP product and mean of chest girth (CG), body length (BL) and body weight (BW) adjusted to 50 days old in each genotype of the Ongole crossbred female progenies reproduced by AI technique

Genotype Frequency	CG (cm) at 50 days old	BL (cm) at 50 days old	BW (kg) at 50 days old
Born by Superior cow group (SCG):	Born by SCG:	Born by SCG:	Born by SCG:
***Msp1*$^{+/+}$ = 4**	96.00 ±10.82 ^{a}	61.50 ± 2.65 ^{a}	50.75 ±11.81 ^{b}
***Msp1*$^{+/-}$ = 12**	106.00 ± 6.18 ^{b}	60.67 ± 2.96 ^{ab}	51.25 ± 3.86 ^{bc}
***Msp1*$^{-/-}$ = 4**	103.50 ± 0.71 ^{b}	58.75 ± 3.86 ^{b}	53.25 ± 9.32 ^{c}
Subtotal = 20	103.94 ± 7.48 ^{y}	60.45 ± 3.07 ^{y}	51.55± 6.70 ^{y}
Born by Inferior cow group (ICG):	Born by ICG:	Born by ICG:	Born by ICG:
***Msp1*$^{+/+}$ = 2**	98.50 ± 6.14 ^{a}	71.00 ± 1.41 ^{c}	46.50± 6.36 ^{a}
***Msp1*$^{+/-}$ = 3**	96.42 ± 5.86 ^{a}	68.33 ± 0.58 ^{c}	48.42± 4.64 ^{ab}
***Msp1*$^{-/-}$ = 12**	102.75 ± 4.19 ^{b}	65.50 ± 6.36 ^{b}	43.67± 4.73 ^{a}
Subtotal = 17	97.85 ± 5.99 ^{z}	66.65 ± 5.64 ^{z}	47.35± 4.86 ^{z}
Total = 37	100.65 ± 7.30	63.30 ± 5.38	49.62 ± 6.23

The values bearing different superscript in the same column differ significantly ($p<0.05$).

Table 4. PCR-RFLP product and mean of chest girth (CG), body length (BL), body weight (BW) and average daily gain (ADG) adjusted to 345 days old in each genotype of the Ongole crossbred female progenies reproduced by AI technique

Genotype Frequency	CG (cm) at 345 days old	BL (cm) at 345 days old	BW (kg) at 345 days old	ADG (kg) at 50-345 days
Born by Superior cow group (SCG):	Born by SCG:			
***Msp1*$^{+/+}$ = 4**	137.25 ± 2.22 ^{a}	Born by SCG:	Born by SCG:	Born by SCG:
***Msp1*$^{+/-}$ = 12**	138.83 ± 1.70^{ab}	97.25 ± 4.35^{a}	176.75 ± 8.85^{a}	0.400 ± 0.011^{a}
***Msp1*$^{-/-}$ = 4**	140.50 ± 1.91 ^{b}	97.25 ± 3.44^{a}	177.08 ± 8.97^{a}	0.425 ± 0.029 ^{b}
Subtotal = 20	138.85 ± 2.03^{b}	96.25 ± 1.89^{a}	175.75 ± 5.74^{a}	0.371 ± 0.011 ^{a}
		97.05 ± 3.25^{a}	176.75 ± 8.02 ^{a}	0.409 ± 0.053 ^{b}
Born by Inferior cow group (ICG):	Born by ICG:			
***Msp1*$^{+/+}$ = 2**	137.00 ± 1.41 ^{a}	Born by ICG:	Born by ICG:	Born by ICG:
***Msp1*$^{+/-}$ = 3**	141.00 ± 3.00 ^{b}	96.08 ± 0.71 ^{a}	166.00± 4.24 ^{b}	0.402 ± 0.007 ^{a}
***Msp1*$^{-/-}$ = 12**	140.42 ± 4.08^{b}	98.08 ± 2.54 ^{b}	167.00± 5.29 ^{b}	0.428 ± 0.012 ^{b}
Subtotal = 17	140.12 ± 3.76^{b}	95.50 ± 2.54 ^{a}	164.33± 4.00 ^{b}	0.428 ± 0.062 ^{b}
Total = 37	139.43 ± 2.98	97.41 ± 2.40 ^{a}	165.00± 4.80 ^{b}	0.425 ± 0.053 ^{b}
		97.22 ± 2.86	171.35 ± 8.91	0.416 ± 0.053

The values bearing different superscript in the same column differ significantly ($p<0.05$).

Polymorphism of Growth Hormone Msp1 Enzyme-Restriction Associated with Production Performance of Ongole-Crossbred Cattle Mated by Artificial Insemination Technique

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