Production of Progenies by Different Growth Hormone Genotypes (*GH-MspI*) of Their Parents Using PCR-RFLP in Ongole-crossbred Cattle

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Abstract. The objective of this study was to identify different growth hormone (GH) genotypes using *MspI* enzyme-restriction in parental cows and bulls mated by artificial insemination influencing body weight and average daily gain of their progenies. Total of 74 blood samples of Ongole-crossbred cows and their female progenies and 2 blood samples of bulls of Ongole breed were used in this study. Blood samples were screened for the presence of GH gene using PCR-RFLP method involving *Msp1*enzyme-restriction on 1.2 % agarose gel. To eliminate different age effects of the progenies, body weight data were adjusted for the 50 and 345days old of ages for the first and second weighing, respectively. Data were analyzed using statistical program in Excel XP. The results showed that various genetic factors of growth hormone *Msp1* restricted enzyme had significant influence on growth performance and average daily gain of Ongole-crossbred progenies during 50 to 345 days of age. The heterozygous genotypes of the growth hormone *Msp1+/-* restricted enzyme excelled over their homozygous genotypes in respects of body weight gain. Therefore, the $Msp1^{+/+}$, $Msp1^{+/-}$ and $Msp1^{-/-}$ genotypes can be used as the candidate genes in Ongole crossbred cattle to improve their body weight.

Keywords: Ongole-crossbred cattle, body weight gain, growth hormone, *Msp1* gene.

Abstrak. Tujuan dari penelitian ini adalah untuk mengidentifikasi genotipe hormon pertumbuhan yang berbeda (GH) menggunakan pembatasan enzim MspI pada sapi induk dan pejantan yang dikawinkan melalui inseminasi buatan yang mempengaruhi bobot badan dan rata-rata pertambahan bobot badan harian keturunannya. Total 74 sampel darah sapi betina persilangan Ongole dan keturunan betinanya serta 2 sampel darah sapi pejantan bangsa Ongole digunakan dalam penelitian ini. Sampel darah diperiksa untuk kehadiran gen GH menggunakan metode PCR-RFLP yang melibatkan pembatasan enzim Msp1 pada gel agarosa 1,2 %. Untuk menghilangkan efek umur yang berbeda dari keturunan, data bobot badan disesuaikan ke arah 50 dan 345 hari untuk masing-masing penimbangan pertama dan kedua. Data dianalisis dengan menggunakan program statistik pada Excel XP. Hasil penelitian menunjukkan bahwa berbagai faktor genetik hormon pertumbuhan enzim terbatas Msp1 memiliki pengaruh yang signifikan terhadap kinerja pertumbuhan dan rata-rata pertambahan bobot badan harian keturunan persilangan Ongole pada umur 50-345 hari. Genotipe heterozigot dari hormon pertumbuhan enzim terbatas Msp1 + / - mengungguli genotipe homozigot mereka dalam hal penambahan bobot badan tubuh. Oleh karena itu, genotipe Msp1 + / + , Msp1 + / - dan Msp1 - / - dapat digunakan sebagai gen bakal pada sapi persilangan Ongole untuk meningkatkan bobot badan mereka.

Kata kunci: Sapi persilangan Ongole, pertambahan bobot badan, hormon pertumbuhan, gen Msp1.

Introduction

The Indonesian cattle breeds are supposed to be of unknown compositions of mixed species origin. The Ongole crossbred cattle (OCC) 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 (Mohamad et al., 2009). 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. The OCC animal plays a role for increasing income of smallholder animal agriculture in North Sulawesi, Indonesia.

Nowadays, selection for better performance of such important Indonesian local indigenous breed has found more priority in advance of

genetically molecular biotechnology. Growth hormone (GH) in beef cattle plays a vital role in post-natal growth and general metabolism (Zhao el al., 2004; Kish, 2008). 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 (Zhao el al., 2004; Pawar et al., 2007). With the development of molecular biology and biotechnology, scientists are able to achieve more accurate and efficient selection goal by marker-assisted selection (MAS). In general, validating the genetic markers of growth traits is the initial and crucial step to establish a MAS system (Allan et al., 2007).

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 growth and development, tissue growth, lactation, reproduction, as well as protein, lipid and carbohydrate metabolism (Ayuk and Sheppard, 2006). 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* (Beauchemin et al., 2006). It has been reported that the restriction fragment length polymorphisms (RFLP) of GH were associated with body weight in Grati dairy cows (Maylinda, 2011).

The studies of GH gene *MspI* locus have been reported in Ongole crossbred cattle (Sutarno et al., 2005; Sutarno, 2010), Brahman cattle (Beauchemin et al., 2006), Indian Zebu cattle (Shodi et al., 2007), West coastal Sumatera cattle (Jakaria et al., 2007), and Grati dairy cows (Maylinda, 2011). Their studies indicated that *Msp1+/+* and *Msp1+/-* genotypes can be used as the candidate genes in cattle selection for breeding program of beef cattle. Moreover, these genotypes had a stronger correlation to the higher body weight than *Msp1-/-* genotype in Grati dairy cows (Maylinda, 2011). 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 (Jakaria et al., 2007). The objective of this study was to identify the genetic factors of different growth hormone (GH) genotypes using *MspI* enzyme-restriction in parental bulls and cows influencing growth traits including body weight (BW) and average daily gain (ADG) of their Ongole-crossbred progenies bred by the artificial insemination technique in North Sulawesi province, Indonesia.

Materials and Methods

Animals and sample collection. The total of 74 animals comprising 37cows at the age of 4 to 5 years old and their 37 female progenies of Ongole crossbred cattle in North Sulawesi province at the ages ranging from 5 days to 50 days old for the first weighing and 295 days to 345 days old for the second weighing were used in this study. All cows were reared under private areas belong to farmers with unknown ancestors. Progenies were born from those cows mated by artificial insemination (AI) technique using germ plasmas (semen) of the two Ongole bulls called “Kirsta” and “Tunggul” from “the artificial insemination bull germ plasma center” (BBIB) at Singosari, East Java province, Indonesia. Prior to blood collection, body weights of animals were determined by using a digital weighing scale. The parameter of the animal body weight were measured using digital weighing scale when animals were standing as described in Ozkaya and Bozkurt (2008).

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. Blood samples of those cows, their progenies and two Ongole bulls as source of germ plasmas (mated by artificial insemination) were collected during July 2011 from Jugular vein of animals in 10 ml EDTA (10%) tubes during the *MspI* selection experiment and stored in the refrigerator (4^{o}C) until ready for DNA isolation. Genomic DNA from whole blood of Ongole crossbred cows, bulls and their calves 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 (Sulandari and Zein, 2003) involving restriction of *Msp1*enzyme produced by The Vivantis Company Inc. (Product No. RE1302, February 2012). Amplification of the fragment of 329 bp at intron 3 (Gordon et al., 1983; Dybus, 2002) was done with PCR using the primers consisted of forward primer GH5: 5'---CCCACGGGCAAGAATGAGGC---3'; and reverse primer GH6: 5'---TGAGGAACTGCAGGGGCCCA---3' (Gordon et al., 1983; Mitra et al., 1995) produced by Laboratory "The Midland Certified Reagent Company Inc. Texas, USA, Product Lot Number: 280511-03B (November 2011)". The reaction mixture of PCR was performed by using 1x Taq pol 25 µl of master mix (Axygen Biosciences, CA, USA).

Composition PCR kit Reaction (Solis Biodyne) (master mix containing $MgCl_2$ 1.5 µM 1x reaction) consist of 5 µl Firepol Master Mix (Ready-to-Load), 1 µl Primer GH5 (10 pmol/µl), 1 µl Primer GH6 (10 pmol/µl), 0.75 µl $MgCl_2$ (50 µM),14,5 µl H_2O (Mili Q water), and 2 µl Sample of DNA (total volume of 25 µl). Final concentrations of 25 µl PCR for reaction component consisted of Taq Polymerase 1.2 U, Reaction Buffer B 1x, dNTPs 200 µM (each of dATP, dCTP, dCTP, dTTP), Primer (forward) GH5 0.4 µM, Primer (reverse) GH6 0.4 µM, and $MgCl_2$ 3.0 mM.

The mixture was placed in thermal cycler PCR machine (Biometra T personal type) with the conditions of the thermal cycler were as follows: the initial denaturation temperature step at 94 ^{o}C for 5 min for 1 cycle followed by 35 cycles of denaturation at 94 ^{o}C for 30 sec, annealing at 60^{o}C for 30 sec, elongation at 72 ^{o}C for 30 s and a final extension at 72 ^{o}C for 1 min. To digest this fragment, a protocol of restricted fragment length polymorphism (RFLP) with restriction enzyme *MspI* was used to recognize the particular site of CC↓GG. The PCR product of GH gene was digested at 37^{o} C for 3 hours by *MspI* enzyme. Reaction consisted of 2 µl Buffer V2 10X, 7.5 µl H_2O, 0.5 µl Enzyme Msp I (20 U/µl), and 10 µl PCR product.

The digested products were separated by horizontal electrophoresis (100 volts, 30 min) in 1.2% agarose gels in 1x buffer TBE (Tris, Boric acid, EDTA). Agarose gels were made by weighing 1.2 g agarose powder and placed into 100 ml Buffer TBE (Tris-Boric-EDTA) 1x. Agarose solution was boiled on the hot plate. The warm agarose was poured into comb printing tools to form several wells. A compact agarose gel was moved into electrophoresis tool immersed with Buffer TBE 1x. Loading samples were done by dropping 9 µl PCR product of digested DNA mixed with 1 µl loading dye into each well of agarose gel and into control well of DNA ladder 100 bp (loading dye was included in master mix Firepol).Following the end of PCR and RFLP process, the products were then subsequently electrophorated using 1.2% agarose gel. Each sample of the digested DNA of 10 µl was added by 2 µl of loading dye. The mixture was dropped in artificial hole of agarose gel to run the process of electrophoresis. The products of electrophoresis were immersed in the 10% ethidium bromide during 20 minutes to identify

polymorphism of alleles based on the length of the band. The picture of DNA band products was visually taken on the UV-Transiluminator using camera to be compared with DNA Ladder (Marker) for allele and genotype identification. The cut fragment length of DNA band after *Msp1* enzyme digestion of 224 bp and 103 bp was identified as normal allele (*Msp1+*) and *Msp1+/+* genotype. The cut fragment length of DNA band after *Msp1* enzyme digestion of 327 bp, 224 bp and 103 bp was identified as allele *Msp1+* and allele Msp1- and *Msp1+/-* genotype. While, the uncut fragment length of DNA band after *Msp1* enzyme digestion of 327 bp was identified as mutant allele (*Msp1-*) and *Msp1-/-* genotype (Figure 1).

Data analysis. PCR-RFLP data were analyzed by allele frequency (Nei, 1987). The allele frequency was calculated by the methods as follows:

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

Where, 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 (χ^2) (Byrkit, 1987) as follows:

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

Where, χ^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.

Comparison of the means of body measurement variables within animal genotype was tested using *t* test (Mendenhall, 1987) as follows:

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

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

Where, $\bar{x}_1$ and $\bar{x}_2$ are mean of genotype 1 and 2, n_1 and n_2 are sum of the animals with genotype 1 and with genotype 2.

The data of the parental cows within 5 years old age were used in this study. The female progenies used in this study comprised 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 these progenies were corrected by adjusting for the 50 days old of age for the first weighing and for the 345 days old of age for the second weighing for elimination of different age effects on animals using the formula (Jakaria et al., 2007) as follows:

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

Data were analyzed using software of the statistical program function in Excel XP (2007).

Results and Discussion

Polymorphism detection. Polymorphism detection was performed in 37 Ongole crossbred cows, 2 Ongole breed bulls and 37 from their progenies. The digestion of 327 bp PCR product for growth hormone (GH) gene with restriction endonucleases *Msp1* enzyme differentiated alleles marked *Msp1+* and *Msp1-*. The *Msp1* digestion of the PCR products produced digestion fragments of 104 bp and

223 bp for allele *Msp1+* and of 327 bp for allele *Msp1-*. The size of fragment for allele *Msp1-* was 327 bp after restriction digestion (Figure 1).

The population of Ongole crossbred cows and their offspring were detected and had three genotypes. The homozygous genotype *Msp1+/+* (224 bp and 104 bp) was detected in 11 animals. The heterozygous genotype *Msp1+/-* (327 bp, 224 bp, 104 bp) was detected in 29 animals. The homozygous genotype *Msp1-/-* (327 bp) was detected in 34 animals. Moreover, the homozygous genotype *Msp1+/+* (224 bp and 104 bp) was detected in one Ongole bull called "Krista" (Genotypic code of Kr_+/+), while the homozygous genotype *Msp1-/-* (327 bp) was detected in one Ongole bull called "Tunggul" (Genotypic code of Tu_-/-). These three genotypes were the same with research reported by Zhou et al. (2005) to show that amplification of PCR-RFLP for GH gene using *Msp1* enzyme restriction in Beijing Holstein produce three genotypic animals. This enzyme recognized only the restriction site of four nucleotides for C*CGG.

Differentiation of the *Msp1+* and *Msp1-* alleles was characterized by number of restricted fragment. The *Msp1+* allele had two fragments with length of each fragment was 104 bp and 223 bp. Meanwhile, the *Msp1-* allele had only one fragment with its length of 327 bp. The difference of these two fragments of *Msp1+* and *Msp1-* alleles was caused by mutation of Cytosine (C) to Thymine (T) (Rifa'i, 2010). This was in agreement with study reported by Nei (1987) that the GH gene had high variability due to mutation. Mutation occurred on DNA level due to nucleotide changes, either transition or insertion (Cambell and Reece, 2008). 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 (Rifa'i, 2010).

Gene variation of GH locus for *Msp1* in cattle was detected in the position of intron 3 (Rifa'i, 2010) at the sequence position of 1547 based on nucleotide sequence from GenBank, number: M57764.1 in Gordon et al. (1983). The intron area was the internal space in which protein code in gene sequence was disappearing during transcription due to mutation effect occurring in GH locus of *Msp1* in term of silent mutation (Cambell and Reece, 2008; Rifa'i, 2010). Silent mutation did not occur at site of active protein and did not cause the amino acid change, because several amino acids were encoded by different codons (Cambell and Reece, 2008). From the total of 37 parental cows in this study, the 21 cows were mated by AI technique using sperms of Ongole breed bull called "Tunggul" (Tu_-/-), and the 16 cows were also mated by AI technique using sperms of Ongole breed bull called "Krista" (Kr_+/+). The selected growth hormone locus using alleles of Msp1+ and Msp1- enzyme restriction in Ongole-crossbred parental cows and bulls was inherited to their progenies following Mendelian mode inheritance (Paputungan et al., 2012).

The *Msp1* genotype frequencies of animal population using AI technique. The frequencies of cow (G0) and progeny (G1) genotypes (GH-*Msp1*) determined in the population mated by each genotype of bull (G0) had been reported by Paputungan et al. (2012). It was found that genotype and allele frequencies of GH-*Msp1* were not under genetic equilibrium (P>0.05). Maylinda (2011) reported that Grati dairy cow population was in genetic equilibrium. This was supported by the fact that a population property of gene pool for GH-*Msp1* under the Hardy-Weinberg equilibrium pattern was a function of both allele frequencies and biological interactions among genes (Carter et al., 2005). This in equilibrium of genotypic frequencies of GH*Msp1*caused the instability of genotypic frequencies of GH gene from G0

generation to the next generation (G1) due to the breeding of selected genotypic bulls and parental cows mated by artificial insemination without random mating system for animal population in North Sulawesi province. The factor affecting genetic equilibrium was selection program with non random mating system, such as the artificial insemination mating system (Rifa'i, 2010).

Genetic analysis of progeny production with different GH*Msp1* genotypes. Breeding program must be continued as the first step to increase the frequency of the favorable allele in breeding station (Jawasreh et al., 2012). In North Sulawesi, the AI service center applied the straw containing spermatozoa germ plasma of Ongole bull from germ plasma center Singosari, East Java.

Carter et al. (2005) reported the analysis of gene interaction and found that it might be two or more genes can interact to express a particular phenotype.

The means for progeny body weights (BW) at 50 and 345 days of age and average daily gain (ADG) during 50 to 345 days of age were presented in Table 1. The overall means for BW50d, BW345d and ADG50-345 of the progenies were 49.62± 6.23 kg, 171.62±12.98 kg, and 0.417 ± 0.053 kg, respectively. The random effects of sire had no significant (P>0.05) influence on all growth traits under this study. The heterozygous genotype of the *Msp*1$^{+/-}$ within parental cows gave significant (P<0.05) influence on BW345d and ADG50-345d of the progenies compared with those of the *Msp*1$^{+/+}$ genotype cows (Table 1).

In the present study, there were significant differences in BW and ADG between progenies born by the cows with homozygous genotypes and those with heterozygous genotypes mated by both homozygous genotypes of sires (Krista and Tunggul). The cows with heterozygous genotype of *Msp1*$^{+/-}$ had highly significant (P<0.05) influence on all growth traits of the progenies considered in this study (Table 1). In the interaction affect, the cows with homozygous and heterozygous genotypes of *Msp1* restricted enzyme mated by sires with the opposite genotypes of *Msp1* restricted enzyme produced higher growth trait progenies compared with those produced by the parents with same homozygous genotypes. This study revealed that there was definite pattern of outstanding progenies in growth traits produced by mating of different growth hormone (*Msp1* restricted enzyme) genotypes of both parental cows and sires.

The growth of animals was under the hormonal control of GH, growth hormone receptor (GHR) and insulin-like growth factor 1(IGF-1) (Reyna et al., 2010).

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 (Kish, 2008). This indicated that the level of blood GH reflects the GH genotype. This study revealed that superior animals differ genetically from inferior animals mainly in their regulation of nutrient utilization and the GH release (Rejduch, 2008). The heterozygous *Msp1*$^{+/-}$ genotype would indicate a trend of heterosis effect. This genotype was more responsible for the animal body weight (BW) and average daily gain (ADG). This is in agreement with some reports (Fahmi, 2004; Marson et al., 2005; Javanmard et al., 2005) who stated that heterosis effect wasa productive trait advantage of outstanding progeny inherited from crossing of both parents producing lower productive trait average compared with that of their progeny.This study revealed that the animals with the heterozygous *Msp1*$^{+/-}$ genotype performed the outstanding average of BW and ADG compared with the average of those in both homozygous *Msp1*$^{+/+}$ and *Msp1*$^{-/-}$ genotypes of animals.

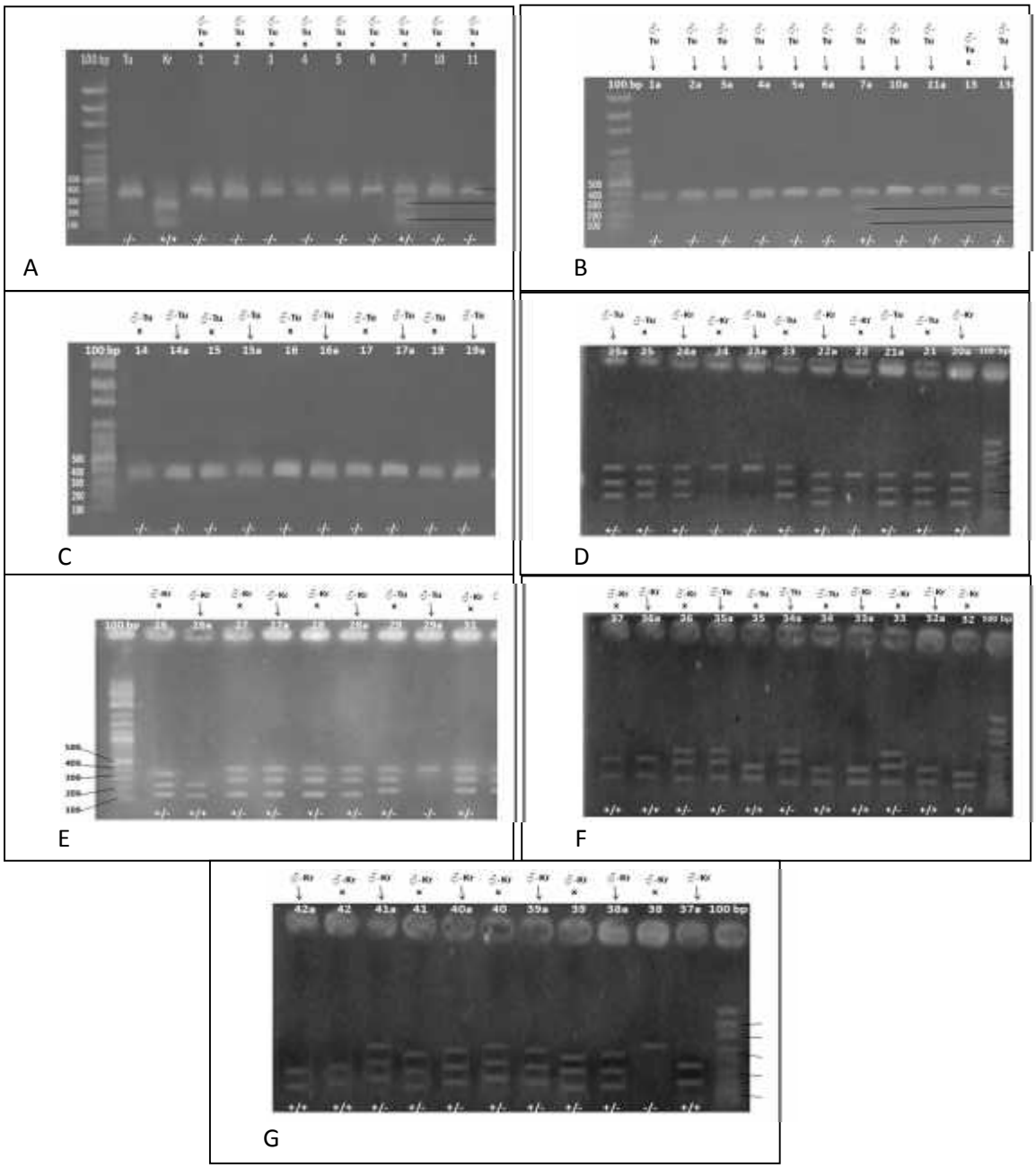

Figure 1. Genotyping results of *Msp* 1 enzyme restriction in GH locus detected through agarose gel electrophoresis. (A) Tu=Tunggul (Ongole bull, source of sperms for AI), Kr=Krista (Ongole bull, source of sperms for AI), Cows (1,2,3,4,5,6,7,10,11) mated by AI method using sperms of Tu; (B) Cow (13) 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 (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.

Table 1. The means and standard errors of production traits in the Ongole crossbred female progenies bred by artificial insemination technique

Sources	No. of progenies	Weight at 50 days old (kg)	Weight at 345 days old (kg)	ADG 50-345 days(kg)
Overall mean	37	49.62± 6.23	171.62±12.98	0.417 ± 0.053
Sire effect				
Krista ($Kr^{+/+}$)	16	48.94± 6.35^{ns}	172.69±10.76^{ns}	0.419 ± 0.026^{ns}
Tunggul ($Tu^{-/-}$)	21	50.14± 6.25^{ns}	170.81±14.66^{ns}	0.409 ± 0.058^{ns}
Cow genotype effect				
Msp1$^{+/+}$	5	46.40± 7.30 ^{ns}	165.80± 8.55 ^{a}	0.405 ± 0.011^{a}
Msp1$^{+/-}$	14	50.71± 5.93 ^{ns}	173.71± 8.27 ^{b}	0.417 ± 0.022^{b}
Msp1$^{-/-}$	18	49.67± 6.23 ^{ns}	171.61±16.57 ^{ab}	0.414 ± 0.074^{ab}
Interaction effect				
$Kr^{+/+}$x *Msp1*$^{+/+}$	3	45.00± 5.20 ^{a}	168.00± 4.58^{a}	0.398 ± 0.008^{a}
$Tu^{-/-}$x*Msp1*$^{+/+}$	2	48.50± 12.03 ^{ab}	172.50± 9.19^{ab}	0.415 ± 0.002^{b}
$Kr^{+/+}$ x *Msp1*$^{+/-}$	9	49.89± 7.03 ^{ab}	174.78± 7.05^{b}	0.416 ± 0.019^{b}
$Tu^{-/-}$ x *Msp1*$^{+/-}$	5	52.20± 3.27 ^{b}	178.20± 4.97^{b}	0.420± 0.030^{b}
$Kr^{+/+}$ x *Msp1*$^{-/-}$	4	49.75± 5.68 ^{ab}	177.25± 4.66^{b}	0.445 ± 0.030^{b}
$Tu^{-/-}$ x *Msp1*$^{-/-}$	14	49.64± 6.58 ^{ab}	166.00± 6.83^{a}	0.412 ± 0.082^{ab}

Values bearing different superscript at the same column differ significantly (P<0.05)

Conclusion

The present study showed that the various genetic factors of growth hormone *Msp1* restricted enzyme had significant influence on the growth performance and average daily gain of Ongole-crossbred progenies during 50 to 345 days of age. The heterozygous genotypes of the growth hormone *Msp1+/-* restricted enzyme excelled over their homozygous genotypes in respects of body weight gain. Therefore, the *Msp1*$^{+/+}$, *Msp1*$^{+/-}$ and *Msp1*$^{-/-}$ genotypes can be used as the candidate genes in Ongole crossbred cattle to improve animal body weight. The AI technique should be maintained for breeding in increasing the favorable*Msp1+/-* heterozygous genotype in large population.

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