# Short communication:
# The genotype of growth hormone gene that affects the birth weight and average daily gain in crossbred beef cattle

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[2]Departemen of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada. Jl. Fauna No. 3, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia
[3]Departemen of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada. Jl. Fauna No. 3, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia
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**Abstract.** *Hartatik T., Fathoni A, Bintara S., Ismaya, Panjono, Widyobroto B.P, Agus A, Budisatria I.G.S, Leroy P. 2020. Short communication: The genotype of growth hormone gene that affects the birth weight and average daily gain in crossbred beef cattle. Biodiversitas 21: 941-945.* Growth Hormone gene has been proposed to direct search for quantitative trait loci, and polymorphisms at these loci have been associated with several production traits in bovine. This research aimed to investigate the association of the genotype of partial growth hormone gene with birth weight and average daily gain in crossbred beef cattle. The materials of this research consist of 47 samples from four different breeds. Birth weight was measured just after calving delivery from the mother. The average daily gain was calculated from the period since birth weight to the second weight (90 days). Genotyping of the growth hormone gene was determined by the sequencing and PCR-RFLP method with the AluI restriction enzyme. The association between genotype and growth traits data was analyzed using analysis of variance (ANOVA) by RStudio version 1.1.383. The results showed the significantly different (P<0.001) in birth weight and average daily gain according to the different breed. Friesian Holstein breed showed the highest birth weight, followed by Belgian Blue Bull cross and others. The genotype valine/valine only occurs in Belgian blue bull cross and Wagyu bull cross cattle and showed moderate birth weight. Genotype affects birth weight significantly (P<0.001). The birth weight for genotype leucine/leucine, leucine/valine, and valine/valine was 34.82±18.13, 25.31±13.10, and 29.35±13.65, respectively. There was no significant difference in average daily genes according to the different genotypes. In conclusion, the growth hormone gene was probably one of the molecular genetics markers for excellent growth traits in different crossbred beef cattle.

**Keywords:** Average daily gain, birth weight, growth gene, polymorphism

## INTRODUCTION

The population of cattle in Indonesia was 16.6 million head in 2017 (Agriculture Ministry, 2017). The current human population of Indonesia is about 269 million and expected to increase every year. Therefore the meat consumption will follow to increase each year, and the government should provide a greater meat supply every year. The introgression of *Bos taurus* (exotic breeds) through artificial insemination is expected to increase meat production. Recently, Indonesia has developed other *Bos taurus* cattle breed i.e., Belgian Blue and Wagyu. Belgian Blue (BB) cattle are known as beef cattle with double muscle. The deletion of 11 nucleotides in the Myostatin gene of Belgian Blue caused the muscular hypertrophy or double-muscled (Kambadur et al. 1997). In the previous study, the deletion was inherited to its F1 generation on male and female cattle (Agung et al. 2016). While Wagyu cattle from Japan have excellent marbling quality. A study about the genetic diversity of Wagyu cattle has been conducted before using DNA markers, such as mtDNA and

SRY genes, which provided the information on population genetic construction and Wagyu cattle origins (Manen, 2017). One of the parameter growth traits is the average daily gain (ADG). With the development of technology using DNA-based marker analysis, the selection of beef cattle with a high performance of growth trait could be facilitated by using the growth hormone marker gene.

The polymorphism study in the growth hormone gene of five local cattle has been studied previously by Putra et al. (2016). The polymorphism of the growth hormone gene in exon 5 was recognized by the AluI restriction enzyme, which performs two alleles called L (leucine) and V (valine), which located in the 127th codon. Three breeds of local cattle (Bali, Madura, and Pesisir) only have an LL genotype (100% LL). Two crossbred (Simpo and Simpes) show two genotypes (LL and LV) in both cattle. The PCR-RFLP technique using the AluI restriction enzyme was one of the easier ways and more efficient to identify the variations of the nucleotide sequence in growth hormone gene fragments of the livestock. Our previous study was using this technique to determine the growth hormone gene

polymorphism in local cattle of Indonesia and the crossbred beef cattle using Limousin bull (Hartatik et al. 2013; Volkandari et al. 2013). The result shows the introduction of valine allele in crossbred beef cattle, such as two genotypes LL and LV were observed in Ongole grade cattle (96.15%LL, 3.85%LV), Limousin-Madura (81.46%LL, 18.52%LV), Limousin-Ongole grade cattle (78.57%LL, 21.43%LV), and Limousin (66.67%LL, 33.33%LV). Single nucleotide polymorphism of growth hormone gene in beef cattle in several countries also has been published by several researchers (Akcay et al. 2015; Akis Akad et al. 2012; Dario et al. 2005; Deepika and Salar 2013; Korkmazagaoglu and Akyuz 2013; Moravčíková et al. 2012; Reis et al. 2001; Sari et al. 2013, Sutarno 2010).

The new crossbred beef cattle have been developed recently. Belgian Blue Bulls semen and Wagyu Bulls semen were used artificial insemination to Brahman Cross cow. The purpose of the crossing was to improve the growth performance of the crossbred beef cattle. The candidate gene, which related to the growth traits, was one of the useful markers to be understood. The study of genotype in the growth hormone gene was subjected as a molecular genetic marker for growth performance in beef cattle. Thus, the study aimed to identify the association of growth hormone gene with birth weight and average daily gain in the crossbred of Belgian Blue Bulls and Wagyu Bulls.

## MATERIALS AND METHODS

### Data collection and DNA Extraction

Forty-seven samples were used in this research that consisted of Brahman cross (9), Belgian Blue Bull Cross (10), Wagyu Bull Cross (8), and Friesian Holstein (20). The bodyweight of cattle was measured on birth immediately and 90 days of age. The average daily gain was calculated from the period since birth weight to the second weight (90 days). The calves were raised together with their dam. The cows were fed with concentrate (2.6 kg), King grass (1.5 kg), rice straw (2.0 kg). Nutrient composition of concentrate for feeding was 89.52 DM, 13.75 CP, 67.87 TDN, 2.18 Ca, and 0.35P. Nutrient composition of concentrate for feeding until 90 days of age was 89.33 DM, 10.23 CP, 69.13 TDN, 2.18 Ca, and 0.35P.

Three milliliters of fresh blood samples were taken from the jugular vein. Blood samples were stored at vacutainer containing K3EDTA (anticoagulant). Genomic DNA was extracted using a DNA Extraction Kit (Geneaid). DNA samples were stored at -20°C until PCR-RFLP was performed.

### PCR-RFLP analysis

In this study, genotyping of growth hormone gene (211 bp) used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. A 211 bp fragment of growth hormone gene was amplified with a pair specific primer, according to Reis *et al.* (2001) (F: 5'GCTGCTCCTGAGGGCCCTTC 3'; R: 5' CATGACCCTCAGGTACGTCTCCG 3'). PCR amplification was performed in a total volume of 20 μL containing 10 μL PCR Mater mix (Kappa 2G Fast, BioSystem), 1 μL (10 pmol/μL) of each primer, 7 μL water-free nuclease and 1 μL DNA (30 ng/μl) template. PCR mixture was running by Thermal cycler with the following PCR program: initial denaturation of 5 minutes at 95°C, 35 cycles of 30 seconds at 94°C, 30 seconds at 65°C, 30 seconds at 72°C and final extension for 5 minutes at 72°C. PCR product was checked by 1% agarose gel and visualized under UV light.

Ten microliters of the PCR product growth hormone gene were digested using 0.2 μL AluI restriction enzyme (AG^CT) (10 U/μL) in total volume 20 μL. The mixture was digested at 37°C for 3 hours. Genotype was examined through a 2% agarose gel under UV light.

### Data analysis

Genotype and allele frequencies were directly calculated with the following formula (Warwick et al. 1983): (i) Alelle Frequency L = $\sum$ locus L/$\sum$ (locus L + locus V), (ii) Alelle Frequency V = $\sum$ locus V/$\sum$ (locus L + locus V), (iii) Genotype Frequency LL = $\sum$ locus LL/$\sum$ sample in population)x100%, (iv) Genotype Frequency LV = $\sum$ locus LV/$\sum$ sample in population) x 100%, (v) Genotype Frequency VV = $\sum$ locus VV/$\sum$ sample in population)x100%.

The data of birth weight and average daily gain were analyzed using the general linear model procedure in RStudio version 1.1.383. The factor was included breed, genotype, and the interaction between breed and genotype. The general linear model was performed to verify the association of the breed and genotype with birth weight and average daily gain as follows: $Y_{ij}=\mu+\tau_i +\varepsilon_{ij}$, where $Y_{ij}$=The analyzed trait, $\mu$=General mean, $\tau_i$ =$i^{th}$ breed or genotype effect, and $\varepsilon_{ij}$=Random error effect. The formula for the interaction between breed (4 breeds) and genotype (3 genotypes) with a 3 x 4 factorial model.

The Hardy-Weinberg equilibrium status for the allele and genotype frequencies was identified by Pearson's Chi-square test with the mathematical model according to Moonesinghe et al. 2010 and Kang and Shin 2004 :

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where, $X^2$ = Chi-square test value, $O_i$ = observed frequency, $E_i$ = expected frequency and n = the number of compared data. The Chi-square test values ($X^2$) were calculated using Pop-Gene 1.32 program (Yeh *et al.* 1997).

## RESULTS AND DISCUSSION

### Birth Weight (BW) and Average Daily Gain (ADG)

The variation of birth weight in this study was influenced by breed composition in the crossing program. The data show from highest to lowest BW (in kg): Friesian Holstein (39.08±4.81), Belgian Blue Bull Cross (31.80±5.55), Brahman Cross (24.44±4.61), and Wagyu Bull cross (21.63±1.99). The data of ADG show from

highest to lowest (in kg/day): Belgian Blue Bull Cross (0.76±0.11), Wagyu Bull cross (0.65±0.12), Friesian Holstein (0.50±0.09), and Brahman Cross (0.46±0.11). The average birth weight and ADG in all samples in this study were 31.76±16.76 and 0.57±0.15, respectively (Table 1). The result of birth weight and ADG was affected by breed composition (P<0.001). The normal body weight of Holstein's calves is approximately 40 kg at birth, but local veterinarians in Korea have reported calves weighing less than 20 kg at birth since 2013 (Ro et al. 2018). The utilization of Belgian blue Bull can improve birth weight performance and average daily gain (Table 1). The result of birth weight performance and average daily increase in this study was significantly different with P<0.001. The highest of ADG was found in Belgian Blue Bull Cross, it is probably related to double muscle trait in Belgian Blue breed and also the nutrition's need suitable for the growth. The pattern of Birth weight (A) and average daily gain (B) base on genotype was presented in Figure 1.

## Association of genotype with birth weight and average daily gain (ADG)

The result of genotyping in the growth hormone gene 211 bp was produced by three genotypes with the number frequency of genotype LL, LV, and VV as 31 (66%), 14 (30%), and 2 (4%), respectively. Allele Leucine (L) was a common allele in this population. However, there is no LL genotype in Wagyu Bull Cross cattle. Wagyu Bull Cross cattle only have Leucine/Valine genotype and Valine/Valine genotype (Table 2 and Table 3). The variation of body weight base on genotype was a significant difference (P<0.001), but there was no effect of genotype on ADG (Table 2). The result shows birth weight in the LL genotype was highest follow by the result of those in VV and LV genotype (Table 2, Figure 1.A). The new finding was V/V genotype in the new crossbred beef cattle in Indonesia (the offspring from Belgian Blue Bull cross and Wagyu Bull cross). The frequency of genotype L/V also increased in Wagyu Bull cross cattle. Genotype L/L was dominant in Belgian Blue Bull cross, Brahman Cross, and Friesian Holstein (Table 3). The chi-square tests also showed that the allelic and genotypic frequencies in four crossbred beef cattle were not deviated from HWE accept Wagyu Bull Cross (P < 0.05) (Table 4). These results give the sense that the frequency of allele and genotype in the population will be constant because of the absence of several factors, such as mutation, gene recombination, inbreeding, genetic drift and gene flow from other populations (Banos *et al.* 2008).

The finding in this study was consistent base on the results in Table 1 and Table 3, where the birth weight of Frisian Holstein in all populations and Frisian Holstein with LL genotype was 39.08±4.81 and 39.42 ± 4.96, respectively. Genotype LL in Frisian Holstein was 38% from the observed genotype in all samples (18 out of 47 samples). A similar result for genotype LV is found in Wagyu Bull Cross cattle as 50% from observed genotype samples (7 out of 14 samples). The allele frequency was varying for both breeds mentioned above.

Rahayu et al. (2019) suggested in the recent studies that the allele frequencies observed in most studies of polymorphisms were specific for different cattle breeds or different cattle populations. Comparing to the other sample, the percentage of LL genotype for Frisian Holstein calves in this research have a higher amount, so this condition probably influences the results of birth weight. Frisian Holstein, in this study, has the highest birth weight. The genotype of Friesian Holstein contains LL and LV genotype, with the percentage number 90% and 10%, respectively. The LL genotypes in dairy calves were observed to have a higher level of growth hormone (Serensen et al. 2002). The study of the growth hormone gene in three local cattle of Indonesia shows a 100% LL genotype. Crossbreeding of local cattle with the exotic breed was increasing the number of V alleles up to 70%. The introducing V allele could improve the performance of local cattle, but depend on the breed of local cattle (Putra et al. 2016).

**Table 1.** The average daily gain of four crossbred beef cattle

| Breed | N | BW (kg) | ADG (kg/day) |
|---|---|---|---|
| BX | 9 | 24.44[a]±4.61 | 0.46[a]±0.11 |
| BBB | 10 | 31.80[b]±5.55 | 0.76[c]±0.11 |
| WB | 8 | 21.63[a]±1.99 | 0.65[b]±0.12 |
| FH | 20 | 39.08[c]±4.81 | 0.50[a]±0.09 |
| TOTAL | 47 | 31.76±16.76 | 0.57±0.15 |

Note BW: birth weight; ADG: Average Daily Gain, BBB: Belgian Blue Bull Cross, BX: Brahman Cross, FH: Friesian Holstein, WB: Wagyu Bull Cross. [a,b,c] Superscript in the same column indicate the significantly different with P<0.001

**Table 2**. Genotype association with body weight and average daily gain

| Genotype | N | BW (kg) | ADG[ns] (kg/day) |
|---|---|---|---|
| LL | 31 | 34.82[b]±18.13 | 0.55±0.14 |
| LV | 14 | 25.31[a]±13.10 | 0.62±0.16 |
| VV | 2 | 29.35[ab]±13.65 | 0.64±0.04 |
| Total | 47 | 31.76±16.76 | 0.57±0.15 |

Note: [a,b] Superscript in the same column of body weight (BW) indicate the significantly different with P<0.001; [ns] Non-significant in ADG

**Table 3**. Data of birth weight and average daily gain base on genotype x breed interaction

| Genotype x breed | N | Birth weight [ns] (kg) | ADG [ns] (kg/day) |
|---|---|---|---|
| LL x BBB | 7 | 32.29 ± 4.89 | 0.73 ± 0.10 |
| LL x BX | 6 | 24.00 ± 5.69 | 0.46 ± 0.14 |
| LL x FH | 18 | 39.42 ± 4.96 | 0.51 ± 0.09 |
| LV x BBB | 2 | 26.50 ± 0.07 | 0.91 ± 0.07 |
| LV x BC | 3 | 25.33 ± 0.05 | 0.47 ± 0.05 |
| LV x FH | 2 | 36.00 ± 0.00 | 0.45 ± 0.13 |
| LV x WB | 7 | 21.90 ± 1.97 | 0.66 ± 0.05 |
| VV x BBB | 1 | 39.00 ± 0.00 | 0.66 ± 0.00 |
| VV x WB | 1 | 19.70 ± 0.00 | 0.61± 0.00 |
| Total | 47 | 31.76±16.76 | 0.57±0.15 |

Note: [ns] Non significant, BBB: Belgian Blue Bull Cross, BX: Brahman Cross, FH: Friesian Holstein, WB: Wagyu Bull Cross
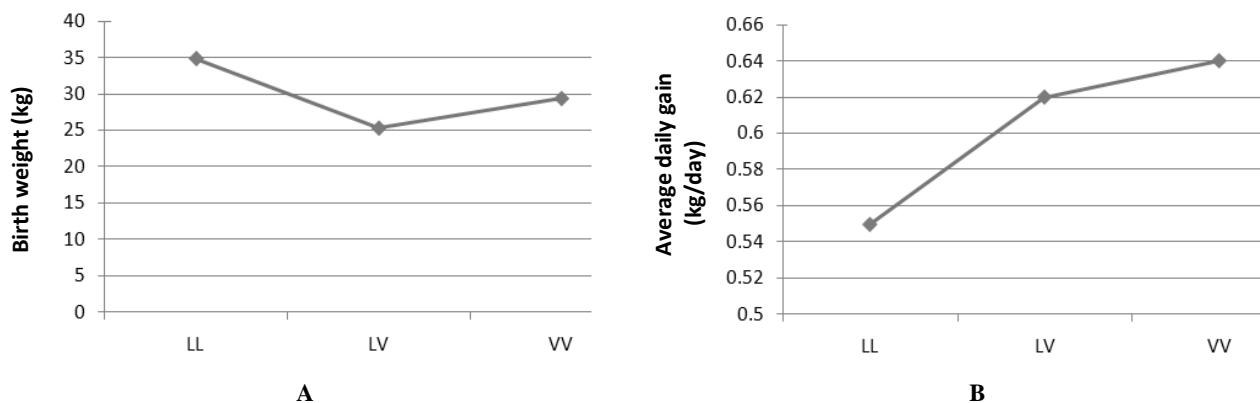
**Figure 1.** Birth weight (A) and average daily gain (B) base on genotype

**Table 4**. Chi-square test results for genotype frequencies in the population of four crossbred beef cattle

| Breed | Locus | Genotype | Observed number | Expected number | D | $X^2$ |
|-------|-------|----------|-----------------|-----------------|------|-------|
| BBB | GH | LL | 7 | 6.32 | 0.68 | 2.11 |
| | | LV | 2 | 3.37 | -1.37 | |
| | | VV | 1 | 0.32 | 0.68 | |
| BX | GH | LL | 6 | 6.18 | -0.18 | 0.23 |
| | | LV | 3 | 2.65 | 0.35 | |
| | | VV | 0 | 0.18 | -0.18 | |
| FH | GH | LL | 18 | 18.03 | -0.03 | 0.03 |
| | | LV | 2 | 1.95 | 0.05 | |
| | | VV | 0 | 0.03 | -0.03 | |
| WB | GH | LL | 0 | 1.40 | -1.40 | 4.08 |
| | | LV | 7 | 4.20 | 2.80 | |
| | | VV | 1 | 2.40 | -1.40 | |

Note: BW= birth weight; BBB= Belgian Blue Bull Cross, BX= Brahman Cross, FH= Friesian Holstein, WB=Wagyu Bull Cross

Birth weight in Friesian Holstein and Wagyu Bull cross was significantly different (P<0.05), and a similar result was shown that the genotype of growth hormone LL was also considerably different from those of LV. The growth hormone plays an essential role in not only body growth but also lactation. Therefore it is necessary for the cattle industry (Kasuya, 2016). The reports introduced above demonstrate that breed for milk production (Friesian Holstein) can support the development of an embryo during pregnancy, rather than breed for beef production. Thus, the growth performance was probably genetically influenced by breeds rather than by the genotype of the growth hormone gene.

In contrast, the research by Ishida et al. (2010) reported effective genetic polymorphisms of bovine growth hormone (bGH) gene associated with calf weight in Japanese Black cattle. The birth weights of calves with haplotype G-C-A are significantly lighter compare to the other haplotype from exon 5 of growth hormone gene (C-C-A, C-C-C, and G-T-A). Japanese Black cattle is one of four Japanese cattle call wagyu. In this study, the birth weight of the offspring from a Wagyu bull cross was lighter than three other observed breeds. Devesa et al. (2016) reported that the effect of growth hormone on growth performance probably could appear as a consequence of the metabolic induction of expression of other growth factors.

Therefore, the growth hormone in this research is not the only factor that affects growth performance in birth weight. This finding can be considered as part of the future experiment to observe the role of growth hormone gene in early development and pre-pubertal of crossbred beef cattle in Indonesia. The average daily gain from birth to 90 days old of age in this study was not different. However, it seems to increase gradually in V containing genotype (Figure 1.B).

Genotype and breed interaction does not affect birth weight and ADG (Table 3, Figure 2). The exciting result of the highest ADG was found at heterozygote LV of the Belgian Blue Bull cross in this study (0.91 ± 0.07). This result probably is the effect of heterosis in the first generation of crossbreeding. Furthermore, the ADG in all Belgian Blue Bull cross was higher than the average result (0.57±0.15).
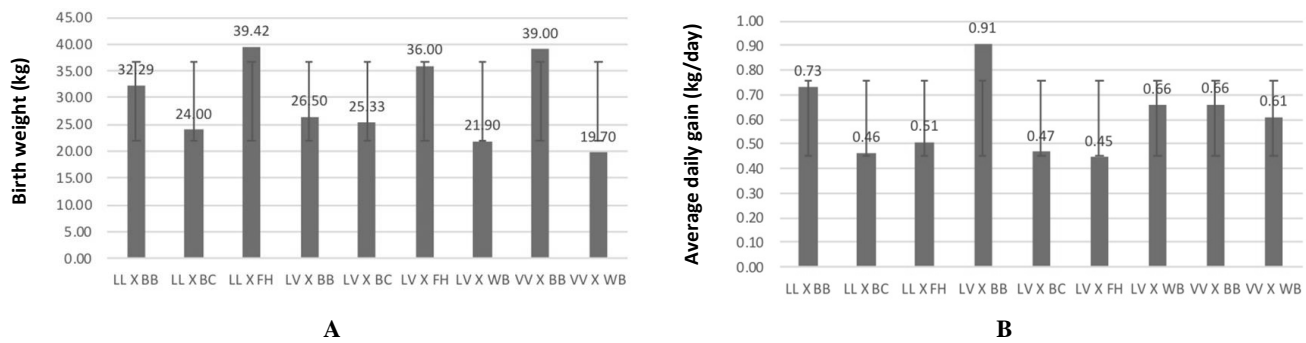
**Figure 2.** Birth weight (A) and average daily gain (B) based on genotype and breed interaction

In conclusion, the genotype of growth hormone was to influence the performance of birth weight. Allele valine (V) may improve the performance of ADG. Therefore, allele valine in this study is a potential molecular genetics marker that can be used for excellent growth trait selection in cattle.

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