Growth hormone locus polymorphism in Ongole Grade and Fries Holland Grade bulls and its implications in the digestibility of feed

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ABSTRAK

Penelitian ini bertujuan untuk menganalisis polimorfisme gen hormon pertumbuhan (GH) dan implikasinya terhadap daya cerna pakan berkualitas rendah dan tinggi. 40 sapi jantan dari Peranakan Ongole (Ongole Grade/ OG) dan 30 dari Peranakan Fries Holland (Fries Holland Grade/ FHG) digunakan sebagai subjek penelitian untuk mengukur pertambahan berat badan, komsumsi pakan, efisiensi pakan dan daya cerna terhadap pakan. Perlakuan berupa pakan sumber serat dari berbagai tanaman dengan berat 15% (P1: kualitas rendah) dan 22,5% dari total pakan (P2: kualitas tinggi). Gen GH sepanjang 329 bp diamplifikasi (F = 5'-CCCACGGGAGAGAATGAGGC–3'; dan R = 5'-TGAGGAACTGCAGGGGGA-3') dan dipotong menggunakan enzim restriksi Msp1. Tingkat polimorfisme antara sapi OG dan FHG mencapai 45%. Pertambahan berat badan sapi FHG menunjukkan lebih tinggi secara signifikan dibandingkan OG (P<0.05) pada minggu pertama hingga ketiga namun menjadi tidak berbeda pada minggu keempat. Dapat disimpulkan bahwa tingkat polimorfisme antara sapi jantan OG dan FHG adalah tinggi, serta sapi jantan FHG memiliki respon pertumbuhan dan efisiensi penggunaan pakan yang lebih tinggi dibandingkan OC.

Kata kunci : hormon pertumbuhan, keceraaan pakan, polimorfisme

ABSTRACT

This research aimed to analyze polymorphism of the growth hormone (GH) gene and its implication on digestibility of low and high quality feed. Forty bulls Ongole Grade (OG) and 30 bulls Fries Holland Grade (FHG) had been selected and subjected for an experiment to measure body weight gain, dry matter intake, feed efficiency and feed digestibility. The treatment was crude fibrous feed from various plants with a fibre weight of 15% (P1: low quality) and 22.5% (P2: high quality). The 389 bp of GH gene was amplified (F = 5'-CCCACGGGAGAGAATGAGGC–3'; dan R = 5'-TGAGGAACTGCAGGGGGA-3') and digested using Msp1 restriction enzyme. Polymorphism between OC and HFC bulls was 45%. The FHG bulls' weight gain were significantly higher than of OC (P<0.05) at first to third week, but become similar (P>0.05) at fourth week. It was concluded that the polymorphism between FHG and OC was high and HFC have a growth response and feed efficiency higher than those of OC.

Keywords : dry matter, feed digestibility, growth hormone, polymorphism.
INTRODUCTION

Challenges in the livestock sector are increasing, and development goals include the targets of consumption and improving the welfare of farmers. Beef products consumption per capita from 2011 to 2014 had tended to decrease, compared to poultry/broiler meat products which had tended to increase continuously every year. The consumption in 2011 was 0.417 kg per capita and had came the lowest (0.261 kg) in 2014 (General Directorate of Livestok, 2017). Furthermore, the beef contribution to fulfill national meat consumption also decreased each year (Lehtonen and Irz, 2013).

The domestic market requires 490,000 tons of meat in every year, equivalent to 1.4 million heads beef cattle with an average live-weight of 350 kg per head. Considerable effort is required to increase the production of meat, and it has to be at the same pace with the increase in population; this can be achieved by seeking the optimal level of production by local farms. Local farms can only provide 350,000 tons of meat, while the rest is fulfilled by imports in the form of fattened beef cattle and beef. It has been reported that imported livestock, such as Bali, Grati, Madura, and Ongole Grade, exhibit worse performance in the tropical environment than inferior cattle at the earliest possible stage, allowing the breeder to avoid rearing unproductive animals (Amiri et al., 2018). Therefore, Indonesian breeders, especially the traditional ones, rarely practice this procedure. Nowadays, new technology to aid early selection has emerged. This technology is based on the use of unique genetic markers which make it possible to distinguish the superior cattle from the inferior cattle at the earliest possible stage, allowing the breeder to avoid rearing unproductive animals (Amiri et al., 2018). The implementation of genetic marker technology in the selection process will increase the genetic quality in the cattle; indeed it has been demonstrated that Marker Assisted Selection (MAS) improves the genetic quality of cattle up to 30% (Wakchaure et al., 2015). This research aimed to develop an early selection method based on genetic markers which reflect the ability of the cattle to digest low-quality feed.

MATERIALS AND METHODS

Feed treatments and variables

Forty Ongole Grade (OG) and thirty Fries Holland Grade (FHG) bulls were subjected for this research. The treatment was crude fibrous feed containing dry straw and leaves waste from paddy (Oryza sativa), maize (Zea mays), cassava (Manihot utilissima), and elephant grass (Pennisetum purpureum). In prior to treatment, proximate analysis was conducted. The treatment consisted of two types of crude fibrous feed, i.e. fibre with weight of 15% (P1: low quality) and 22.5% (P2: high quality). Those types of feed had been given regularly everyday for a month. Observation was conducted every week including weight gain, dry matter consumption, and efficiency but the digestibility was measured at the first and fourth week.
**Polymorphism Analysis**

DNA was isolated from blood collected from the jugular vein from each subject by using a venoject. The amplification target was growth hormone (GH) fragment in the 3’ intron region and the DGAT1 gene in exon VIII. This procedure was using primers F = 5’–CCCACGGGCAAGAATGAGGC-3’ and R = 5’–TGAGGAACCTGCGGGCCCA– 3’, with length 329 bp. A thermal cycler was used to perform amplification with configurations. Amplicon was followed by 2% agarose gel electrophoresis to validate the amplification result. Each PCR product was digested with Msp1 restriction enzyme (GH gene) at 37 ºC for 3 hours (Putra et al., 2014). The digested result was checked by 2% agarose gel electrophoresis. The GH gene polymorphism was determined by the formula (Nurdianawati et al., 2016):

\[
\text{PIC}_i = 1 - \sum p_{ij}^2
\]

Where: PIC: polymorphic information content of i locus; \( \sum p_{ij}^2 \) is j allele frequency of i locus.

The formula below was used to calculate allele frequency:

\[
p = \frac{2(AA) + (AB)}{2N}
\]

\[
q = \frac{2(BB) + (AB)}{2N}
\]

Where p is the frequency of A allele, and q is the frequency of B allele. AA is the number of AA genotype, BB is the number of BB genotype, and AB is the number of AB genotype. N is the total number of individuals (Jakaria and Noor, 2011).

**RESULTS**

**Daily Weight Gain**

The daily weight gains of both breeds from the beginning of the study until the first week were significantly different, either from the first week to the second week (P<0.05). Daily weight gains for different treatments within each of the breeds were basically the same from the second to third weeks although different breeds still demonstrated significantly different (p<0.05) daily weight gains compared to each other. FHG and OG bulls exhibited the same daily growth while comparing feed treatments still have a significant (P<0.05) result (Table 1). The interaction between breed and treatment was nearly significant (P<0.05). Nevertheless, the mean value of daily growth was affected by breed and treatment separately, while breed and treatment were not affecting each other (Table 2).

| Breed       | High-quality feeds | Low-quality feeds | Mean *) |
|-------------|--------------------|------------------|---------|
| First week  |                    |                  |         |
| OG, kg      | -3.23              | 4.33             | 0.52\(^a\) |
| FHG, kg     | 7.33               | 12.33            | 9.83\(^b\) |
| Mean **)    | 1.27\(^a\)         | 7.76\(^b\)       |         |
| OG, kg      | -2.15              | 9.67             | -0.05\(^a\) |
| Second week |                    |                  |         |
| FHG, kg     | 6.80               | 9.67             | 8.23\(^b\) |
| Mean **)    | 1.69\(^a\)         | 5.31\(^b\)       |         |
| OG, kg      | 5.89               | 0.15             | 2.98\(^a\) |
| Third week  |                    |                  |         |
| FHG, kg     | 12.80              | 13.87            | 13.34\(^b\) |
| Mean **)    | 8.90\(^a\)         | 6.11\(^a\)       |         |
| OG, kg      | 0.19\(^a\)         | 0.67\(^a\)       | 0.43    |
| Fourth week |                    |                  |         |
| FHG, kg     | 0.53\(^b\)         | 0.55\(^a\)       | 0.54    |
| Mean **)    | 0.31\(^a\)         | 0.62\(^b\)       |         |

OG : Ongole Grade; FHG: Fries Holland Grade; *) mean based on breed type; **) mean based on each treatment; values followed by different superscript at the same column (*) or row (**) show significantly different at 5%.

Table 1. The Influence of Breed Types, Treatments, and the Interactions on Weight Gain (kg) at the First Week

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Dry Matter Consumption
Table 3 showed that FHG bulls consumed significantly (P<0.05) more dry matter than OG bulls (10.14 kg and 4.65 kg per day, respectively). This may be correlated with the fact that the mean starting weight for the different breeds was significantly different (P<0.05) in the first place (FHG weighed 349 kg, whereas OG weighed only 265 kg) (Data not showed).

Feeding efficiency
Feeding efficiency was calculated by dividing mean daily growth by dry matter consumption. Breed strongly affected the feeding efficiency (P<0.05), but feeding treatment did not have a significant effect (Table 4). This experiment produced different results compared with other similar studies. The current understanding is that OG bulls should have high adaptability to low-quality feed compared with other bull breeds.

Growth Hormone (GH) Polymorphism
Polymorphic information content (PIC) for

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**Table 2. The Influence of Breed Types, Treatments, and the Interactions on Weight Gain (kg) (Mean for All Week)**

| Breeds  | High-quality feed | Low-quality feed | Mean * |
|---------|-------------------|------------------|--------|
| OG      | 0.08              | 0.27             | 0.17a  |
| FHG     | 0.56              | 0.71             | 0.63b  |
| Mean ** | 0.28a             | 0.46b            |        |

OG ; Ongole Grade; FHG: Fries Holland Grade; *) mean based on breed type; **) mean based on each treatment; values followed by different superscript at the same column (*) or row (**) show significantly different at 5%

**Table 3. Breed and Treatment Effect on Dry Matter Consumption (%)**

| Feed quality      | Dry matter consumption (%) |
|-------------------|----------------------------|
|                   | OG Breed* | FHG Breed* |
| High-quality feed | 51.1b     | 33.9a      |
| Low-quality feed  | 37.4a     | 48.3b      |

*) The both value followed by a different superscript at the same column showed significantly different at 5%

**Table 4. Feeding efficiency on FHG and OG bulls**

| Feed quality      | Feeding efficiency |
|-------------------|--------------------|
|                   | OG | FHG | Mean **) |
| High-quality feed | 0.011 | 0.068 | 0.035±1.1 a |
| Low-quality feed  | 0.052 | 0.081 | 0.064±0.9 b |
| Mean *)           | 0.031a | 0.074b |

*) mean based on each treatment; **) mean based on breed type; both values followed by a different superscript at the same row (*) or column (**) showed significantly different at 1%.
the GH locus was determined as 0.455 or 45.5 % (A allele frequency or p was 0.35 and B allele frequency or q was 0.65) (Table 5). These numbers indicate a relatively high degree of polymorphism.

**GH Genotype Interaction on Dry Matter Feed Consumption and Digestibility**

GH genotype and interaction between genotype and breed did not significantly affect dry matter consumption. On the contrary, GH genotype affected the consumption significantly (P<0.05), even though the interaction between treatment and genotype did not produce a significant difference to dry matter consumption (Table 6). Based on our results, the GH-AA genotype has the potential to be a genetic marker since, for both kinds of feed, the bulls with GH-AA consumed a higher dry matter than both GH-AB and GH-BB bulls. The interaction between breed and genotype have a significant result (P<0.05) with respect to dry matter consumption. Treatment and GH genotype also interact significantly (P<0.05) with dry matter digestibility (Figure 1).

**Influence of Breed, Feed type, and GH Genotype on Weight Gain**

Breed had a significant effect (P<0.05) on mean daily weight gain, but feed treatment did not have any significant effect on daily weight gain. On the other hand, GH genotype nearly had a significant effect (P<0.05) on daily weight gain. The interaction between breed and genotype or between treatment and genotype was not significant for mean daily weight gain from the 1st weighing to the 4th weighing (Table 7).

**DISCUSSION**

Based on this research, Ongole Grade (OG) bulls are almost less heavy compared with Fries Holland Grade (FHG). These data are related to the dry matter consumption which is proportional to its body weight. Faverdin et al. (2017) also exhibited that body size and body frame is positively related to the feeding consumption and milk production in dairy cattle. The data in this study; however, contradict the current understanding that OG bulls have the best temperature adaptability due to their low basal metabolism rate. In addition, OG bulls are able to convert high crude fiber feed into high-quality beef via its symbionts in the omanus. Therefore, they have the ability to utilize high crude protein better than other breeds.

There was a tendency for OG bulls to gain a lower amount of weight on a daily basis than HFC bulls. Low-quality feed had a significant effect on body weight first and second week but high-quality feed caused the bulls to gain significant weight by the third week. The bulls’ adaptation to feeding during the first days may account for these results. OG bulls showed better growth after high-quality feed treatment after 3 weeks. This

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**Table 5. Genotype Frequency of Growth Hormone**

| Genotype | Frequency (%) |
|----------|--------------|
| AA       | 12.25        |
| AB       | 45.5         |
| BB       | 42.25        |

**Table 6. The Influence of Breed, Feet Type and GH Genotype on Feed Consumption**

| Genotype | Breed type | Feed quality | Mean * |
|----------|------------|--------------|--------|
|          | OG         | FHG          |        |
|          | High       | Low          |        |
| AA       | 6.07       | 10.25        | 7.81±1.8 b |
|          | 8.79       | 10.91        | 9.58±2.7 b |
| AB       | 1.51       | 8.76         | 5.74±2.3 a |
|          | 6.27       | 5.29         | 5.74±1.7 a |
| BB       | 5.65       | 9.54         | 7.27±1.2 b |
|          | 5.74       | 6.74         | 6.41±3.1 a |
| Mean **  | 5.23 a     | 10.52 b      | 7.70a  |
|          |            |              | 7.13a  |

The number followed by a different superscript in the same column (*) and row (**) is significantly different at 5%, OG = Ongole Grade , FHG = Fries Holland Grade
indicated that OG bulls were starting to convert feed into beef after 3 weeks of treatment.

The GH-BB genotype can be considered as a genetic marker for both breeds. In addition, GH-AB and GH-BB can be utilized as genetic markers of dry matter digestibility in OG bulls. Furthermore, GH-AA can be considered as well, but only in HFC breeds in addition to GH-BB genotype. This genetic variation is due to the different ancestry for each breed (Othman et al., 2015).

**CONCLUSION**

There were a potency of GH-AA and GH-BB as genetic markers for growth in both the OG and FHG breeds with high polymorphism, regardless of the feed quality. FHG bulls grew better, consumed higher dry matter, and fed more efficiently than OG bulls. The low-quality feed was digested more efficiently, thus producing higher daily weight gain. It was concluded that bulls with GH-AA and GH-BB genotypes have better mean daily growth than the GH-AB genotype; so both genes have the potential to serve as genetic markers for the growth of FHG and OG breed bulls, regardless of feed quality.

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