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Confirmation of Existing Insulin-like Growth Factor-1 Gene Associated with Growth and Milk-Production Traits and Genetic Diversity in Buffalo

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Abstract

Insulin-like growth factor-1 (IGF-1) gene plays an important role in the endocrine system of animals by regulating nutrient metabolism, growth, and milk production. There have been extensive molecular genetics research studies on cattle but less studies have focused on buffalo (Bubalus bubalis). This study aimed to confirm the association of IGF-1 gene in swamp or river buffalo (B. bubalis spp.) with growth and milk production traits. DNA samples were obtained from 12 buffalos (eight swamp buffalo and four river buffalo). One Bali cattle (Bos javanicus) was included as an outgroup (control). The eight swamp buffalo originated from East Nusa Tenggara (n = 1), Baluran, East Java (n = 4), and Banyuwangi, East Java (n = 3), while the four river buffalo originated from Sei Putih, Medan of North Sumatera. All DNA samples were amplified using an IGF-1 primer for 30 cycles, and amplicons were visualized on 1% agarose gel. Five of the 13 samples were sequenced to determine nucleotide sequence variation between the swamp and river buffalo. The results revealed that the size (225–231 bp) of all the fragments was in accordance with that of IGF-1. There was not found genetic variation among the buffalo samples. The results indicate that buffalo samples bear growth and milk production traits.

Introduction

Genetic improvement is important for increasing animal productivity. In the past, farmers practiced conventional breeding selection methods with the aim of producing animals with superior traits. These selection methods required large sample numbers, and they were time consuming and laborious. Recently, improved technologies have been developed based on genetic markers that allow rapid and accurate selection of important and unique traits with low heritability [1]. These technologies represent a breakthrough for the animal industry.

In Indonesia, cattle (meat and milk products) rather than buffalo supplies the high demand for animal protein for human consumption [2]. Moreover, it was stated that up
to 2015, the cattle population in Indonesia (15,494,288) greatly exceeded that of buffalo (1,381,331). By 2035, human population of Indonesia is projected to reach almost 300 million [3]. Given the government’s aim of food security (food self-sufficiency and food sovereignty), a second layer of animal production other than cattle should be considered as animal protein sources [4]. Research on buffalo, with a focus on meat and milk production as an animal protein source is recommended to fulfill Indonesian food needs.

By screening of microsatellite markers [5,6] and single nucleotide polymorphisms (SNPs) [7,8] of IGF-1 associated with growth and milk production in dairy cattle were previously reported. Other studies also reported an IGF-1 gene polymorphism linked to milk production and growth in Polish Holstein-Friesian dairy cattle [8,9].

There have been few studies of IGF-1 gene expression in buffalo [11-13]. Trait information at the genetic level is needed for animal selection breeding programs. The use of buffalo as an alternative to cattle as a source of meat and milk production has potential to support food safety in Indonesia. In this study, the presence of the IGF-1 gene in buffalo was determined, and sequence variation in IGF-1 in different types of buffalo (river and swamp) was analyzed.

Methods

Samples and DNA collection. Samples were obtained from 12 buffalo (Bubalus bubalis) species (eight swamp buffalo and four river buffalo). The swamp buffalo originated from East Nusa Tenggara (n = 1), Baluran, East Java (n = 4), and Banyuwangi, East Java (n = 3). The four river buffalo originated from Sei Putih, Medan of North Sumatera. One member of the Bali cattle (Bos javanicus) family was included as an outgroup. The base of the tail was punctured, and blood was collected in a vacutainer tube containing 0.5% EDTA. DNA samples were extracted from fresh blood using a previously described DNA extraction method based on a high concentration of NaCl [14].

Polymerase Chain Reaction (PCR) and visualization. PCR amplification of all 13 DNA samples was performed using the forward primer 5'-GCTTGGATGGACCATT TTG-3' and reverse primer 5'-CACTTGAGGGGCAAATG ATT-3. The primer pair was generated from the reference sequence AH009378.1 [15], and the expected amplicon size was between 225 and 231 bp. The PCR reaction was carried out in a total volume of 20 µL containing 2 µL of genomic DNA (50 ng/µL), 2 µL of each primer (10 pmol/µL each of the forward and reverse primers), PCR master mix (Bioneer, Korea) containing 1 U top poly-merase, 250 µM dNTP, 10 mM Tris-HCl, 30 mM KCl, and 1.5 mM MgCl2), with 14 µL of Nuclease free water (Thermo-Fisher Scientific, Lithuania) added. The PCR conditions were 2 min at 94 °C, 1 min at 94 °C, 30 sec. at 58 °C, 1 min at 72 °C, and 30 cycles for 4 min at 72 °C [16]. The PCR was carried out in a Mastercyler Gradient (Eppendorf, Germany). The PCR products were analyzed on a 1% agarose gel, stained with ethidium bromide, and then visualized using a UV trans-illuminator (Major Science, California, USA).

Sequence analysis. Six of the 13 samples were subjected to sequence analysis by sending the PCR products to the sequence services (1st BASE, Malaysia). The sequences were identified using the BLAST program to confirm the gene identity. To determine variation of nucleotide sequences between the buffalo (river and swamp buffalo) and cattle, the sequence data results were aligned using the BioEdit software version 7.0 program [17]. The similarity of IGF-1 sequences was analyzed by BLAST (https://blast.ncbi.nlm.nih.gov). A phylogenetic tree was constructed using the neighbor-joining method, with 1000× bootstraps and run using MEGA 5.0 software [18].

Results and Discussion

The results of the DNA amplification revealed a PCR band of 225–231 bp in all DNA samples of the 12 buffalo (swamp and river) and in one sample of Bali cattle (Figure 1). The IGF-1 size (225–231 bp.) was similar to that found in a previous study of cattle [15].

The targeted fragments spanned from nucleotide position of 792 up to 1021 in the reference sequence of AH009378.1 for IGF-1. The IGF-1 gene of buffalo was located in 4q31 of chromosome 4 [19,20]. The findings indicated that both swamp and river buffalo possess the IGF-1 gene. Previous studies concluded that the IGF-1 gene was associated with milk and growth traits [9,15,21]. Swamp buffalo was best suited to meat production, whereas the river buffalo more suitable for milk production [22]. The IGF-1 gene appears to have roles in meat, growth, and milk production in Buffalo [23]. Milk production is a quantitative trait, which is usually encoded by multiple genes [24,25]. Previous studies reported that the IGF-1

![Figure 1. PCR Products of IGF-1 (1–8 = Swamp Buffalo; 9–12 = River Buffalo; 13 = Bali Cattle; M = DNA Marker)](image)
gene influenced multiple births in cattle and milk production traits in dairy cows, with the IGF-1 concentration increased both in serum and follicle fluid of ovaries [9,26]. Research also demonstrated a direct link between milk production and double ovulation rates in cattle [27]. Furthermore, research showed that multiple genes other than IGF-1 regulated economically important traits in cattle [1].

The results of the sequence alignment analysis are presented in Figure 2. A nucleotide deletion sequence was found when aligned to the reference sequence of *Bos taurus* AH009378.1. The nucleotide deletion was observed at nucleotide position 4 (A) and 73 (G). A nucleotide substitution was also detected at position 30 (A>T) both in buffalo and cattle (Figure 2).

Figure 3 presents the results of the peak nucleotide analysis of the *IGF-1* gene in buffalo and cattle (see arrows). At nucleotide position 90, a nucleotide base difference was detected, with a T nucleotide present in Bali cattle (*Bos javanicus*) and *Bos taurus* (GenBank NCBI) and a C nucleotide present in buffalo (swamp and river).

Based on the results of the sequence analyses (Figs. 2 and 3), there was no genetic variation among the buffalo. Nucleotide differences and similarities in *IGF-1* sequences of buffalo samples as compared with those of Bali cattle and *Bos taurus* are summarized in Table 1.
Table 1. Location of Genetic Variation

| Samples                          | Base position | Similarity (%) |
|---------------------------------|---------------|----------------|
| Bos taurus (AH009378.1)         | A A G T       | 100            |
| Bali cattle                     | - A>T - T    | 97             |
| Swamp buffalo Banyuwangi (3 L)  | - A>T - T>G  | 96             |
| Swamp buffalo Baluran (12 L)    | - A>T - T>G  | 96             |
| Swamp buffalo Baluran (2 L)     | - A>T - T>G  | 96             |
| River buffalo Medan (11 M)      | - A>T - T>G  | 96             |
| River buffalo Medan (27 M)      | - A>T - T>G  | 95             |

There was no sequence variation in the IGF-1 regions of the swamp and river buffalo, with 100% sequence similarity (Table 1). However, there were several differences in the nucleotide sequence of buffalo as compared with those of the reference sequence of Bos taurus AH009378.1. There was a genetic relationship between river and swamp buffalo (B. bubalis spp.), as shown in Figure 4.

Previous studies of IGF-1 microsatellites in Chancim cattle indicated that IGF-1 controlled birth weight [16,21] and weaning weight [21]. Another study concluded that a DNA polymorphism at the IGF-1 locus was an effective marker for growth traits [27]. Researchers also reported that an allele at 225 bp of the IGF-1 gene was positively associated with weaning weight in Nelore cattle [21]. A study of polymorphism in the 5'-UTR of the IGF-1 gene in three cattle breeds in China (Qianchuan, Nanyang, and Chinese Holstein) reported that there was allele A more dominant than C. The allele C was positively associated with body size of Nanyang cattle and with milk fat production and milk protein of Chinese Holstein cattle [28].

There have been only a few molecular studies of IGF-1 in buffalo [11-13]. One previous study reported that there was a G → A substitution in intron 3 of the IGF-1 gene of swamp buffalo (Bubalus b. bubalis kerebau) [2]. The same study detected a nucleotide substitution by EcoI30I restriction enzyme analysis [11].

Conclusions

Based on the molecular analysis in the present study, it can be concluded that both swamp and river buffalo possess the IGF-1 gene. It can also be concluded that no genetic variation exists between swamp and river buffalo and that river and swamp buffalo (B. bubalis spp.) belong to the same group with respect to genetic relatedness. Comparison of the IGF-1 sequence fragment of buffalo to that of a reference sequence of Bos taurus revealed a nucleotide deletion and substitution. Further studies are needed to determine other than IGF-1 gene expression for milk and meat of buffalo in Indonesia.

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