GH, GHRH, and PIT Genes Polymorphisms of Local Swamp Buffalo in Pandeglang District, Banten Province

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Abstract. The molecular selection of the genes influencing growth qualities can be used to genetically improve the production of native swamp buffaloes. Local swamp buffalo (60 hds.) maintained by smallholders in Pandeglang Regency, Banten Province, were examined for the genetic polymorphism of the growing family (GH, GHRH, and PIT1) genes. By using restriction enzymes of MspI (GH gene), HaeIII (GHRH gene), and HinfI (PIT-1 gene), the genotyping of GH g.1547T>C, GHRH g.4666G>C, and PIT-1g.1256G>A SNPs was found by polymerase chain reaction-restriction fragment polymorphism (PCR-RFLP). Genotyping on the respective GH_g.1547T>C, GHRH_g.4666G>C, and PIT-1_g.1256G>A loci, however, resulted in only one type of genotype i.e. CC, GG, and AA with one type of allele i.e. C, G, and A successively. Therefore, the frequencies were 100% or monomorphic for those genotypes and alleles of each growth gene. Also, the PIC values of each locus were 0.00, as were the heterozygosity observation (Ho) and expectation (He) values. It is possible to suggest increasing the genotype frequency of the SNPs of the relevant growth genes that are favorably correlated with the growth traits and associated economic traits of the observed local swamp buffalo in this area.

Keywords: Swamp buffalo · growth family genes · PCR-RFLP · SNP

1 Introduction

Swamp buffalo is one of the local animal genetic resources that have many functions for the Indonesian community. Some of its functions are sources of red meat, labor, savings, religious, traditional events, and organic fertilizer. The population of local swamp buffalo spread in various areas due to their good adaptability. Although, the increasing buffalo meat demand and the changing natural habitat have caused population declines in some areas. The total number of buffalo in the country over the past four years, 2016–2020, was 1,355, 1,322, 1,134, and 1,154 thousand hds [1]. The local swamp buffalo population in Banten Province was around 59,290 hds. relatively low compared to those in the four most populous provinces, providing East Nusa Tenggara (179,708 hds.), South Sulawesi (118,472 hds.), West Nusa Tenggara (115,178 hds.), and North Sumatra (97,218 hds.). Pandeglang Regency is one of the local swamp buffalo breeding areas in Banten Province.

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Based on Pandeglang Regency Statistics Data [2] reported that the local swamp buffalo population in Pandeglang Regency was around 13,298 hds., the third largest population after Lebak (19,620 hds.) and Serang (16,394 hds.) Regencies.

In Pandeglang, buffalo play numerous significant functions for farmers and the local population. However, the conditions of rising red meat demand, moderately intensive management, and habitat function conversion result in these animals' population loss and genetic degradation, as shown in some places in Indonesia [3]. Increasing productivity through mating and selection programs should be pursued. Molecular selection technology could be an option to assist quantitative selection in the efforts to improve the genetic productivity of animals. Molecular markers can give a general picture of the genetic makeup of a population by, among other things, identifying the key genes affecting crucial characteristics like growth traits. Improving the productivity of local swamp buffalo growth traits requires the exploration of genetic polymorphisms in growth gene families including among others GH (Growth Hormone) [4–6], GHRH (Growth Hormone Releasing Hormone) [6, 7]; and PIT-1 (Pituitary-Specific Transcription Factor 1) [8, 9].

Growth hormone or GH is crucial for bodily metabolism and growth, performing a substantial part in the lactation, reproduction, and growth processes [4, 9]. The bovine GH gene is located on chromosome 19 in the area of q26-qter bases and has an approximate size of 1800 bp consisting of 5 exons and 4 introns [10]. Growth Hormone Releasing Hormone or GHRH is a hormone from the hypothalamus that stimulates produced by the hypothalamus and release of growth hormone [7]. The extra pituitary cells or tissues are directly impacted by the GHRH gene and its compounds [11]. PIT-1 is a POU domain member that is crucial for cell proliferation and differentiation [12]. High levels of genetic diversity of these bovine GH, GHRH, and PIT-1 genes have been observed in several previous studies [7, 8, 13–16]. The genetic variability of these three genes has, nevertheless, been relatively low in buffalo [5, 8, 9, 17, 18], although several other studies have obtained genetic variations to be quite high [19, 20]. Single nucleotide polymorphisms (SNPs) are DNA sequence differences that happen when one of those four nucleotides of Adenine (A), thymine (T), cytosine (C), or guanine (G) changes in the genome [9]. Because of to their vast distribution and high frequency throughout the genome, single nucleotide polymorphisms (SNPs) are excellent genetic markers that enable the selection of people who have economically valuable qualities (growth, reproduction, and production). Less is now understood about the genetic variants of the expanding family genes in the nearby swamp buffalo. Determine the degree of genetic diversity, prevent the loss of genetic diversity, and develop selective breeding plans using polymorphism evaluation [21].

Using PCR-RFLP or the polymerase chain reaction-restriction fragment length polymorphism technique, this study attempts to study diversity of the growth genes (GH, GHRH, and PIT-1 genes) in swamp buffalo kept by farmers in Pandeglang District, Banten Province.

2 Materials and Methods

2.1 Research Period and Location

This field research was conducted in 2013–2014 in Pandeglang District, Banten Province. Pandeglang has boundaries in the north with Serang Regency, the east with Lebak Regency, the south with the Indian Ocean, and the west with Sunda Strait. Pandeglang has an area of about 2,746.89 km^2. Topography in central and southern regions is generally a plain with relatively low mountain heights, while the northern area is highland. Climate is a tropical climate as in other parts of Indonesia with a relative humidity level of around 81% and an air temperature between 20–31 °C.

Molecular research was done at Animal Molecular Genetics Laboratory, Division of Animal Breeding and Genetics, Dep. of Anim. Production Sci. and Tech., Fac. of Animal Husbandry, Bogor Agricultural University, West Java, Indonesia.

2.2 Materials

A number of 60 swamp buffalo from Pandeglang District were used as samples, including males (18) and females (4). Animals were commonly raised by farmers with semi-intensive management on a small scale. Buffaloes were grazed during the day and penned at night. At the time of grazing, animals feed for some kind of forage and other feed sources around the garden, post-harvesting rice fields, or open land. Mating takes place naturally between females and males.

2.3 Method

Genetic variants of the GH, GHRH, and PIT1 genes were assessed using a modified standard approach [22]. DNA extraction, gene amplification, electrophoresis, and restriction of base fragments from PCR results were all successfully completed. A previous work by Anggraeni et al. [3] outlined the precise steps of these several procedures.

DNA Extraction
Phenol-chloroform method was employed for DNA extraction, as reported by Sambrook [22]. 40 l of 5 M NaCl, 400 l of phenol, and 400 l CIAA were added to cells to purify them. DNA products were frozen after being centrifuged to remove the phenol and then dissolved in TE 80%.

Amplification (PCR) and RFLP
Forward and reverse primers, dNTPs, MgCl2, 10xBuffer, Taq polymerase, and distilled water were the chemicals used in the PCR operations. The primers for the GH gene intron 3 at the GH g.1547T>C locus [23], the GHRH gene intron 2 at the g.4666G>C location [24], and the PIT-1 gene in exon 6 at the g.1256G>A locus [25] were utilized for the amplification process. For the DNA extraction procedure for the GH gene g.1547T>C locus, GHRH gene g.4666G>C locus, and PIT-1 gene g.1256G>A locus, information on the size of the PCR product, the annealing temperature, the primer base pairs, and the references were described in accordance with Mitra et al. [23], Moody et al. [24],

and Woollard et al. [25], respectively. For the GH gene, HaeIII for the GHRH gene, and HinfI for the GH gene, the restriction enzymes.

Electrophoresis
Agarose gel, 0.5 x TBE, ethidium bromide, loading dye, and a 100 bp marker were used in the electrophoresis procedure. Restriction enzymes of MspI, HaeIII, and HinfI, buffer, and pure water were required for RFLP.

Data analysis
Each SNP of the GH, GHRH, and Pit1 genes was genotyped using the POPGENE32 software version 1.32 to calculate the genotype frequency, allele frequency, heterozygosity observation (Ho), and heterozygosity expectation (He) values. To calculate genotype frequency, the number of a certain genotype to the whole population was compared.

3 Results and Discussion

3.1 Allele and Genotype Frequency

The GH, GHRH, and PIT-1 genes from the local swamp buffalo were amplified, and the resulting PCR products had lengths of 327 bp, 451 bp, and 611 bp, respectively. Exon 3's 33 base pairs and intron 3's 294 base pairs were covered by the GH gene's PCR product (GenBank M57764.1). The PCR results from the GHRH gene had 86 bp in exon 2, 266 bp in intron 2, and 99 bp in exon 3. (GenBank AF242855). The PIT-1 gene's amplicon has 43 bp and 408 bp-long intron and exon, respectively (GenBank Y 15995).

The PCR products of the GH, GHRH, and PIT-1 genes were effectively cleaved by the associated restriction enzymes MspI, HaeIII, and HinfI. The C*CGG restriction site of the GH gene was discovered by the MspI restriction enzyme, the GG*CC restriction site of the GHRH gene by the HaeIII enzyme, and the G*ANTC restriction site of the PIT1 gene by the HinfI enzyme. The genotype and allele frequencies of the local swamp buffalo, as indicated by the GH g.1547T>C, GHRH g.4666G>C, and PIT-1g.1256G>A loci, are evaluated in Table 1.

Table 1. The GH, GHRH, and PIT-1 genes' genotypes and allele frequencies in the local swamp buffalo in Pandeglang District

Genes/Locus	Number of Genotypes			Genotype Frequency			Allele Frequency	
GH gene	TT	TC	CC	TT	TC	CC	T	C
g.1547T>C locus	0	0	60	0.00	0.00	1.00	0.00	1.00
GHRH gene	GG	GC	CC	GG	GC	CC	G	C
g.4666G>C locus	0	0	0	1.00	0.00	0.00	1.00	0.00
PIT-1 gene	GG	GA	AA	GG	GA	AA	G	A
g.1256G>A locus	0	0	60	0.00	0.00	1.00	0.00	1.00

3.2 GH_ g.1547T>C SNP

The single genotype that appeared after genotyping the GH gene at the g.1547T>C locus was the CC (AA/++) genotype. The MspI restriction enzyme cut at the C*CGG site in intron 3 to yield two base segments, 223 bp, and 104 bp, resulting in the CC (AA/++) genotype of the GH gene. One base fragment was found for the same length as the PCR result by 327 bases, allowing for the possibility of the TT (BB/—) genotype [23]. The TT genotype, however, was not identified in this study. The polymorphism at the 1547th nucleotide of the GH gene, known as a transition mutation, transformed thymine (T) to cytosine (C) [26]. All of the buffaloes solely had the CC genotype due to a nucleotide change at the GH g.1547T>C gene.

Only the CC (AA/++) genotype was produced by all of the observed buffaloes genotyped for the locus GH g.1547T>C, thus the frequency of the C allele was also 1.00 (100%). This C allele frequency above 0.99 caused all of these animals for GH g.1547T>C SNPs to be monomorphic [27]. The same genotype was found in swamp buffalo in Aceh for possessing only the CC (++) genotype [18]. In contrast, the GH g.1547T>C SNP only resulted in the TT (BB/—) genotype and the T (B/-) allele for that buffalo from North Tapanuli District in North Sumatra [3]. According to these findings, the buffalo in North Tapanuli only possessed the wild T allele while the buffalo in Pandeglang only have the mutant C allele against to the buffalo from Aceh.

As reported by Sodhi et al. [28] conducted studies on 17 different types of Indian zebu cattle (750-hds) in two distinct regions. Similar PCR products (329 bp) of the bGH gene in intron 3 were generated by genotyping findings. The frequencies of the two types of alleles, T (B/-) and C (A/+) were from 0.06 to 0.33, while the frequencies of the two genotypes, TT (BB/—) and TC (AB/+-), were from 0.67 to 0.94 None of these breeds of cattle possessed the genotype CC (AA/+/+). When compared to Bos taurus breeds, these Bos indicus breeds' high frequencies of the TT (BB/—) and TC (AB/+-) genotypes were confirmed as a distinguishing characteristic.

3.3 GHRH_g.4666G>C SNP

Whenever the g.4666G>C locus of the GHRH gene intron 2 was genotyped, the results revealed that all of the examined buffaloes had just the GG (AA) genotype. Two cutting sites were discovered by the HaeIII enzyme (GG * CC) at nucleotides 4666 * 4667 in intron 2 and 4760 * 4761 in exon 3, resulting in three fragments of 312 bp, 94 bp, and 45 bp, indicating the presence of the GG (AA) genotype. The four fragments of 194 bp, 118 bp, 94 bp, and 45 bp produced by the three cutting sites at nucleotides 4472*4473 (intron 2), 4666 * 4667 (intron 2), and 4760 * 4761 (in exon 3) could be applied to confirm the CC (BB) genotype [25]. The findings demonstrated that none of a base mutation at the GHRH g.4666G>C gene was present in the animals, causing of them with the GG (AA) genotype (100%) and only the G allele (100%). In a prior investigation, At the g.4666G>C locus, the CC (BB) genotype was the only one detected in buffalo from the North Tapanuli Regency [3]. However, a different study of Aceh buffalo produced three genotypes, GG, GC, and CC, with corresponding genotype frequencies of 0.13, 0.80, and 0.07 [18]. Further, the genotype polymorphism of the g.4666G>C locus was informed to have no effect ($P > 0.05$) on buffalo body sizes.

A study by Czerniawska-Piątkowska [29] on the GHRH g.4666G>C SNP in Polish HF cattle found significant genotype frequencies of CC (BB) (0.631 and 0.704), followed by heterozygous GC (AB) genotype frequencies. Therefore, HF cattle with the C (B) allele had frequencies (0.673 and 0.794) that were higher than those with the G (A) allele (0.206 and 0.175). The analysis of the association study of this SNP on milk quality revealed that cows with the GG (AA) genotype produced higher milk protein (3,43%) and milk fat (4.26%) than those with the CC (BB) genotype. Additionally, earlier research discovered that GG (AA) cows produced milk of greater quality [7, 30]. The observed buffalo had only the GG (AA) genotype that seemingly was positively associated with milk quantity and quality. However, it is necessary to study relating the effect of this SNP on growth characteristics in these animals.

3.4 PIT-1_g.1256G>A SNP

Based on the results of the PIT-1 gene genotyping at the g.1256G>A locus in intron 6, each of the observed buffalo had the AA genotype. The restriction site on the G*ANTC base segment of the HinfI enzyme at nucleotides 1256*1257 (exon 6) produced two fragments, 207 bp and 244 bp, which were responsible for the AA genotype. As contrary, the GG genotype was possible for the appearance of only one base fragment 451 bp [25]. Individuals exhibiting the AA genotype (A) in exon 6 of the PIT-1 gene, position 357 or the g.1256G>A locus, had a transitory mutation from guanine (G) to adenine (A) [8]. However, this mutation is referred to be a silent mutation because it did not affect amino acids [30]. Hence, all of the animals used in this investigation had the AA genotype (100%) and not the GG one.

PIT-1g.1256G>A SNP genetic polymorphisms in cattle and buffalo have also been reported in some earlier studies [17, 31]. The name of the genotypes used for the PIT-1_g.1256G>A locus was based on the form of a base mutation from guanine to adenine (G>A) that differed from the genotype naming from the previous studies. In this study, wild-type nucleotides were designated as having the GG genotype, in earlier investigations, they had the genotype AA, and those with base mutations had the AA (BB) genotype. The findings of this study agreed with those of the studies of Hasanain et al. [17] in local Egyptian buffalo and [9] for finding just the AA (BB) genotype or the A (B) allele. Local buffalo and some Iranian cattle (17 breeds) were found to have the PIT-1 gene at the same site. In Iranian cattle, the genotype frequencies of AA (BB), GG (AA) and AB (AB) range from 0.000 to 0.921, respectively [8].

The G (A) genotype of the swamp buffalo appears to be better suited for milk production and milk quality than the AA genotype, as shown by our study results. This may be consistent with the nearby swamp buffalo, whose primary purpose is sole as a meat type rather than a milk type.

3.5 PIT-1_g.1256G>A SNP

The PIT-1 gene genotyping at locus g.1256G>A in intron 6 revealed that each of the observed buffalo had the AA genotype. The HinfI enzyme restriction on the G*ANTC bases in nucleotide 1256*1257 (exon 6) of the AA genotype resulted in two fragments, comprising 207 bp and 244 bp. The GG genotype, by contrast, had a fragment length of

451 bp [25]. The PIT-1g1256 locus in exon 6 of the AA buffalo contained a mutation base that represented a change from guanine (G) to adenine (A) [8]. Nevertheless, this alteration was referred to as a silent mutation because of no altering amino acids [32]. Buffalo observations only result in the AA genotype instead of the GG genotype.

Other studies also revealed genetic variations in the PIT-1 gene at the g1256 locus in cattle and buffaloes [8, 31]. The genotype nomenclature used in those earlier research was distinct from the genotype identification at the PIT-1g.1256G>A locus, which would be predicated on the occurrence of the base mutation (G>A). Instead of the AA genotype from the other research, the non-mutation (wild type) nucleotide in this study was identified as the GG genotype. By contrast, animals with base mutations were described as having an AA genotype rather than a BB genotype. According to Javanmard et al. [8], the PIT-1 gene at the same locus in some local Iranian cattle breeds (17 breeds) was entirely polymorphic. For cattle, the frequency of the AA (BB) genotype ranges from 0.000 to 0.375, and for buffaloes, the frequencies of the A (B) and G (A) genotypes decrease by 0.233 and 0.766 respectively.

The observed swamp buffalo had the AA (BB) genotype which was not associated with advantages for milk production and milk quality. This probably corresponded to the swamp buffalo for their main functions more as a meat type than a milk type. The G (A) allele seemingly had a better effect on milk production and quality.

3.6 Heterozygosity Value and Degree of Polymorphism (PIC)

By computing the values of observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content, the level of polymorphism of a gene can be identified (PIC). Only CC, GG, and AA genotypes and C, G, and A alleles were found in tests for the GH g.1547T>C, GHRH g.4666G>C, and PIT-1g.1256G>A loci. For these three loci, no genetic diversity was found, which would be consistent with the earlier findings.

High genetic polymorphisms in cattle and buffalo have been reported in some studies, but none of these polymorphisms have been associated with genotype or allele frequency values of 1.00, nor with any observed heterozygosity (Ho) or PIC values of 0.00. As pointed out by Hildebrand et al. [33] that One gene contained just two alleles, giving it a maximum PIC value of 0.375. In contrast, getting just one allele resulted in a PIC value of 0.000 while getting an unlimited number of alleles resulted in a PIC value of 1. In 17 native Iranian breeds of cattle and buffalo, the PIT-1g. gene was found with heterozygosity values ranging from 0.1454–0.4730 for cattle and Ho = 0.3578 for buffalo. Crossbreeding and the introduction of new genetic variants have been proposed to boost gene diversity and sustain conservation.

4 Conclusions

SNPs of the GHRH g.4666G>C, PIT-1g.1256G>. and GH g.1547T>C. SNPs were monomorphic in the observed swamp buffalo. Particularly for SNPs strongly connected with growth and economic qualities, these three SNPs may be advised for bringing new swamp buffalo blood from outsides.

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