

## Genetic Polymorphism of Growth Hormone Genes in Indonesian Local Buffalo

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### Introduction

Growth hormone (GH) is an anabolic hormone synthesized and secreted by lobe somatotroph cells in the anterior pituitary (Ayuk and Sheppard, 2006). The GH gene has important roles in prenatal growth, postnatal development, growth of tissue, lactation and reproduction. It also has key roles in metabolisms of protein, lipid and carbohydrate (Thidar Myint *et al.*, 2008). GH activation in the cells depends on its receptor, known as growth hormone receptor (GHR). GHR, as a transmembrane protein, binds GH with high affinity and specificity. Changes in GHR function, therefore, can be affected by the ability of GH binding and activity in targetted tissues (Di Stasio *et al.*, 2005). Another imporant gene affecting animal growth is the *pituitary transcription factor* (Pit-1). The Pit-1 gene is known as specific transcription factor, playing a key role in pituitary development and hormone expression in mammals.

Information on the genetic diversity of the growth hormone family genes in local swamp buffalo in Indonesia is still very limited. This study aimed to evaluate the genetic polymorphism of Pit-1, GHRH, GH and GHR genes in the Indonesian local swamp buffalo.

### Materials and Methods

A total of 631 head of local swamp buffalo were used as samples in this study. These buffaloes originated from seven provinces or regions, i.e. from Aceh (83), North Sumatera (65), Riau (19), Banten (209), Central Java (75), West Nusa Tenggara (103), and South Sulawesi (107). DNA samples were extracted from blood and meat specimens using the phenol chloroform method (Sambrook and Russell, 1991) modified by Andreas *et al.*, (2010).

Amplification and genotyping of GHRH, Pit-1, GH and GHR genes were according to references, i.e. Moody *et al.*, (1995) for GHRH, Wollard *et al.*, (1994) for Pit1, Mitra *et al.*, (1995) and Balogh *et al.*, (2008) for GH, and Andreas *et al.*, (2010) for GH gene. The data were analyzed for genotype frequency, allele frequency, heterozygosity, and polymorphic information content (PIC).

### Results and Discussion

As shown in Table 1, no polymorphism was detected for GH|*AluI*, GHR|*AluI*, and Pit-1|*hinfI* loci. All buffaloes observed had only one type of allele, i.e. the LL genotype at the GH|*AluI* locus, the AA genotype at the GHR|*AluI* locus, and the BB genotype at the Pit-1|*hinfI* locus. Polymorphism was found only in the GH|*MspI* and GHRH|*HaeIII* loci with allele frequencies

of 0.94 and 0.06 for GH|*MspI* (+) and GH|*MspI*(-), respectively, and 0.16 and 0.84 for GHRH|*HaeIII* (A) and GHRH|*HaeIII* (B), respectively.

Table 2 shows that most of the local buffalo populations which were polymorphic for the GHRH|*HaeIII* and GH|*MspI* loci had values of  $H_o < H_e$ . The exception was for the GH|*MspI* locus in North Sumatera buffalo, which had  $H_o > H_e$ . This indicates that the two loci had low levels of heterozygosity. The PIC values for the two loci for the buffalo from West Nusa Tenggara and Central Java were higher than those of the other provinces. The genetic diversity of Indonesian local swamp buffalo, therefore, is generally very low. This might be caused by the limited attention given to buffalo breeding programs.

**Table 1.** Frequency of alleles at Pit-1.GHRH.GH and GHR loci

Original province (n)	GHRH  <i>HaeIII</i>		GH  <i>MspI</i>		GH  <i>AluI</i>		GHR  <i>AluI</i>		Pit1  <i>HinfI</i>	
	A	B	(+)	(-)	L	V	A	B	A	B
Aceh (83)	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
North Sumatera (65)	0.15	0.85	0.89	0.11	0.00	1.00	0.00	1.00	0.00	1.00
Riau (19)	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
Banten (209)	0.13	0.87	0.99	0.01	0.00	1.00	0.00	1.00	0.00	1.00
Central Java (75)	0.31	0.69	0.85	0.15	0.00	1.00	0.00	1.00	0.00	1.00
West Nusa Tenggara (103)	0.32	0.68	0.88	0.12	0.00	1.00	0.00	1.00	0.00	1.00
South Sulawesi (107)	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
Overall	0.16	0.84	0.94	0.06	0.00	1.00	0.00	1.00	0.00	1.00

**Table 2.** Expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosities, and polymorphic information content (PIC) for the GHRH|*HaeIII* GH|*MspI* loci

Original province (n)	GHRH  <i>HaeIII</i>				GH  <i>MspI</i>			
	$H_o$	$H_e$	PIC	F Statistics	$H_o$	$H_e$	PIC	F Statistics
Aceh (83)	0.00	0.00	0.00		0.00	0.00	0.00	
North Sumatera (65)	0.02	0.26	0.24		0.22	0.19	0.18	
Riau (19)	0.00	0.00	0.00	Fis = 0.001	0.00	0.00	0.00	Fis = 0.000
Banten (209)	0.17	0.23	0.22	Fit = -2.345	0.01	0.02	0.02	Fit = -.612
Central Java (75)	0.24	0.43	0.38	Fst = -2.369	0.05	0.25	0.23	Fst = -.622
West Nusa Tenggara (103)	0.31	0.44	0.39		0.17	0.21	0.20	
South Sulawesi (107)	0.00	0.00	0.00		0.00	0.00	0.00	

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