HaeIII-RFLP Polymorphism of growth hormone gene in Savanna and Kalahari goats

Amie Marini, A.B.[1*], K. Aslinda[1], R. Mohd. Hifzan[2], A.B. Muhd. Faisal[2] and K. Musaddin[1]

[1]Strategic Livestock Research Centre, MARDI Headquarters, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia, [2]Strategic Livestock Research Centre, MARDI Station, Kluang, Johor.
*Corresponding author: amieza@mardi.gov.my

Abstract

Growth hormone (GH) plays an important role in several biological processes such as reproduction, lactation, metabolism and growth of livestock animals. The objectives of this study were to screen growth hormone (GH) gene variants and to identify the polymorphism of GH gene in Savanna and Kalahari goats using PCR-RFLP technique. Genomic DNA was obtained from goat blood samples and amplified by PCR-RFLP using five growth hormone genes. The present study showed two variations were present in growth hormone (GH1 and GH5) of goats. The analysis of 422 bp GH1 fragment revealed polymorphisms with three genotypes (AA, AB and BB). The AB genotype was identified as with the highest frequency in Savanna and Kalahari goats with values of 0.88 and 0.64, respectively. GH5 revealed three genotypes (GH, GG and HH) which differed in patterns from GH1. The highest genotype frequencies of GH5 in Savanna goats were 0.57 and 0.37 for GG and GH genotypes, respectively. Observed genotypes and frequencies in Kalahari goats differed from Savanna goats. The highest frequencies of GH and HH genotypes were found in Kalahari goats with value 0.5 and 0.36, respectively. Further sequence analysis showed that two single nucleotide polymorphisms (SNPs) were found in GH2 in exon 4 that caused a change in amino acid coding. One SNP located in exon 3 in GH6 was identified but did not change the amino acid coding.

Keywords: Goats, growth hormone gene, polymorphism, PCR-RFLP, SNP

Introduction

Growth hormone (GH) plays an important role in several biological processes such as reproduction, lactation, metabolism and growth of livestock animals (Burton et al., 1994; Ohlsson et al., 1998; Baldi, 1999; Jiang and Lucy, 2001; Supakorn et al., 2007). Besides the GH gene, there are growth hormone receptors (GHR) and insulin-like growth factor-1 (IGF-1) that influence the growth and body mass of an animal. The effects of GH on growth are observed on target tissue, including muscle, bone and adipose tissue. Expression of GH gene and their polymorphism at gene and protein level has been reported but only a few articles that discussed the nucleotide changes and position in GH sequence. The goat growth hormone gene of 2544 bp consists of five exons and four intervening introns (Accession: D00476).

Genetic polymorphism can be identified by several techniques, and there are many studies in this area. One of the most commonly used methods is polymerase chain reaction - restriction fragment length

polymorphisms (PCR-RFLP). It is a powerful method for identifying nucleotide sequence variation in amplified DNA and can detect single base substitutions in enzymatic restriction sites.

The Savanna and Kalahari are meat goat breeds known for their rapid growth, good mothering ability and reasonably high milk production to sustain growth of kids (North American Savannah Association, 2011, Stonehavenstud, 2012). Besides, these goats also can reproduce three times within two years and are well adapted to the tropical condition in Malaysia. A preliminary study indicated that Savanna and Kalahari goats are suitable alternative breeds for chevon production in a semi-intensive system in Malaysia. Therefore, the objectives of this study were to screen growth hormone gene (GH) variants and to identify the polymorphism of GH in Savanna and Kalahari goats using PCR-RFLP.

Materials and Methods

DNA extraction

A total of 106 head of Savanna and Kalahari goats were used in the study. The Savanna and Kalahari goats were kept at Mardi Research Station, Kluang, Johor. Genomic DNA was extracted using Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's procedure with minor modification. The DNA concentration was measured using NanoDrop machine (ND-1000, USA).

Primers and PCR-RFLP amplification

Caprine genomic DNA was amplified by PCR using five synthesis growth hormone genes shown in Table 1. The PCR reaction was performed in a total volume of 25 µl on PTC-200 DNA Engine Cycler (MJ Research, USA). Each PCR reactions mixture contained each primer (10 µM solution), 1X Buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTP, 1.5 U Taq DNA polymerase (Promega, USA) and 50 ng genomic DNA as a template. PCR amplification was conducted using two methods: GH1, GH5 and GH6 primers were amplified by PCR while GH2 and GH4 primers were amplified using touchdown PCR. The PCR programme consisted 94°C for 10 min, 35 cycles of 95°C for 30 sec, 62°C for 30 sec, 72°C for 45 sec and a final extension at 72°C for 7 min. Meanwhile touchdown PCR method was started with initial denaturation at 94°C for 5 min, 12 cycles of 95°C for 30 sec, 65°C for 30 sec (-1°C per cycle), 72°C for 45 sec, 22 cycles of 95°C for 30 sec, 62°C for 30 sec, 72°C for 45 sec and final extension at 72°C for 7 min. Electrophoresis of PCR products were conducted at 75V on a 1.5% agarose gel, stained with ethidium bromide and visualized using AlphaEaseFC Stand Alone Software of the gel documentation system (Alpha Innotech, California). The PCR products were selected and sequenced for further analysis. All the amplified bands were subjected to digest with HaeIII restriction enzyme (Promega, USA) according to the manufacturer's recommended protocol. The resulting products were separated in 4% metaphore agarose at 90V for 1 hour 45 min, and stained with ethidium bromide.

Results and Discussion

The five exons in caprine growth hormone gene have been characterized and successfully amplified by PCR-RFLP using five fragments growth hormone gene (GH1, GH2, GH4, GH5 and GH6). Only GH3 fragment was not amplified when tested on Savanna and Kalahari breeds in our local condition. However, the PCR products of five pairs of GH regions indicated, only GH1 and GH5 revealed polymorphisms. The GH2, GH4 and GH6 were detected as

monophormic when digested by *Hae*III restriction enzyme (Figure 1a, 1b, 1c). In GH2 and GH4 regions, both breeds were homozygous for CC and FF genotypes, respectively. Thus, the GH2 gene in Savanna and Kalahari goats differed from a study reported on Boer goats which showed polymorphism and correlation with GH1 (Hua et al., 2009). However, previous studies in Brazilian Canchim, Chorolais and Holstein cattle breed showed polymorphism in GH4 using *Alu I* and *Dde I* restriction enzymes (Yao et al., 1996; Kemenes et al., 1999; Silveira et al., 2008). Meanwhile, similar results were observed in GH6 with homozygous JJ genotype in Brahman, Charolais and Thai local native cattle (Supakorn et al., 2007).

The analysis of 422 bp GH1 fragment revealed polymorphisms with three genotypes (Amie Marini et al., 2010). Two genotypes AA (366 and 56 bp) and AB (422, 366 and 56 bp) were detected by HaeIII-RFLP, while genotype BB was absent (Figure 2). The genotype frequencies of AB in Savanna and Kalahari goats were 0.88 and 0.64, respectively (Table 2). The incidence of genotype AB was higher than AA in both breeds. However, five Savanna goats presented null GH1 gene. This result was similar to the report on polymorphism of GH1 on Boer goat (Hua et al., 2009). Most flock studies showed GH1 polymorphism and absence of BB genotype on Boer, Lubei white and Chengdu-Ma goats (Li et al., 2004; Bai et al., 2005).

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Table 1 Primers used for screening polymorphism in caprine growth hormone gene

Primer name	Primer sequence (5'-3')	Size (bp)	Reference
GH1	F: CTC TGC CTG CCC TGG ACT	422	Hua et al., 2009
	R: GGA GAA GCA GAA GGC		
GH2	AAC C	116	Hua et al., 2009
	F: TCA GCA GAG TCT TCA		
GH3	CCA AC	419	Supakorn et al.,
	R: CAA CAA CGC CAT CCT	(Not	2007
GH4	CAC	amplified)	
	F: CGA CGC CAT AGA CAG	404	
GH5	CAG		Silveira et al., 2008
	R: CAT TTA TGC AAG GAC	415	
GH6	CAC TGG		Supakorn et al.,
	F: TAG GGG AGG GTG GAA	417	2007
	AAT GGA		
	R: GAC ACC TAC TCA GAC		Supakorn et al.,
	AAT GCG		2007
	F: GCC AGT GGT CCT TGC ATA		
	AA		
	F: AGT CCA GGG CAG GCA		
	GAG		
	F: CCA TCC AGA ACA CCC		
	AGG T		
	R: CCA AGC TGT TGG TGA		
	AGA CTC		

Table 2. Genotypic frequency of the GH1 gene in Savanna and Kalahari goats

Breed	Observed genotype			Genotypic frequency		
	AA	AB	Null	AA	AB	Null
Savanna	6	81	5	0.07	0.88	0.05
Kalahari	5	9	0	0.36	0.64	0

Table 3. Genotype frequency of the GH5 gene in Savanna and Kalahari goats

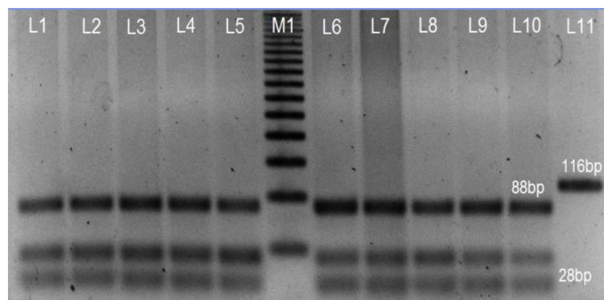
Breed	Observed genotype				Genotype frequency			
	GG	GH	HH	Null	GG	GH	HH	Null
Savanna	52	34	5	1	0.57	0.37	0.05	0.01
Kalahari	2	7	5	0	0.14	0.5	0.36	0

Three genotypes GH (228, 150, 78, 53 bp), GG (228, 78, 53 bp) and HH (150, 78, 53 bp) were detected by HaeIII-RFLP in GH5 gene but the pattern was not similar to GH1. The highest genotype frequencies in Savanna goats were 0.57 and 0.37 for GG and GH genotypes, respectively (Table 3). However, the genotype frequencies in Kalahari goats differed from Savanna goats. The frequencies of GH and HH genotypes in Kalahari goats were found to be 0.5 and 0.36, respectively. Only one Savanna goat was detected with null GH5 gene. In cattle, different result was reported on GH5 which showed absence of polymorphism in Brahman, Charolais and Thai local native beef cattle (Supakorn et al., 2007).

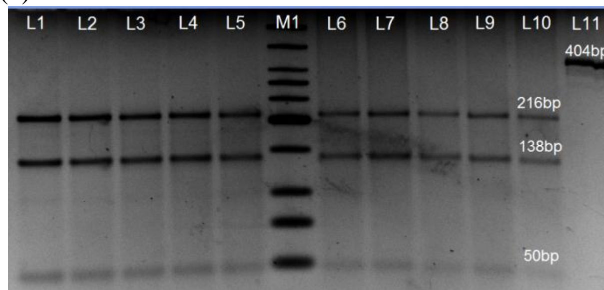
Analysis of the sequence GH gene in this study showed that the gene length was 2545 bp *in Savanna goats in comparison to the 2544 bp growth hormone complete codons in Capra hircus (Accession: D00476). A C nucleotide was inserted in intron 4 at site 1832.* There were two substitutions of SNP identified in GH2 in exon 4. The transition of GAC to GGC at position 1532 caused an amino acid change from Asp to Gly, while the substitution CGG to TGG at position 1585 caused an amino acid change from Arg to Trp. Meanwhile, a substitution of TCC to TCT at position 1148 located in exon 3 at GH6 was found but did not change amino acid coding. However, the GH gene sequence analysis could not be performed for Kalahari goats due to limited sample size. In the present study on Savanna and Kalahari, the locations of SNPs growth hormone gene were different from previous studies involving different goat breeds. These potential SNPs can be used for breed identification purposes.

Conclusion

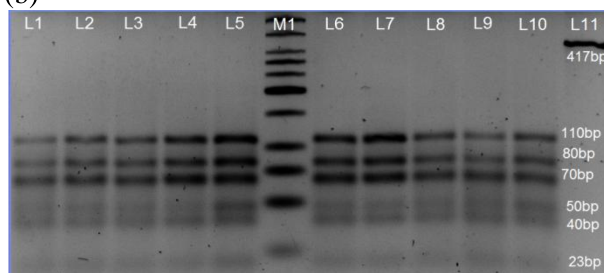
The present study to identify GH gene polymorphism at genetic level in Savanna and Kalahari goats showed that polymorphisms of GH1 and GH5 gene are present in both breeds. There were three substitutions of SNP identified in exon 4 and 3, in which the transition in exon 4 caused a change in protein coding. Further studies are being carried out to verify the incidence of polymorphism of GH gene in Savanna and Kalahari goats and that SNPs detection is associated with growth performance traits.



(a)



(b)



(c)

Figure 1. Different HaeIII-RFLP patterns of GH2 (a), GH4 (b) and GH6 (c)

For GH2 locus, lane 1-10 represented CC genotypes; for GH4 locus, lane 1-10 represented FF genotypes and for GH6 locus, lane 1-10 represented JJ genotypes. L11 represented amplified PCR product. M1 represented a 25 bp marker.

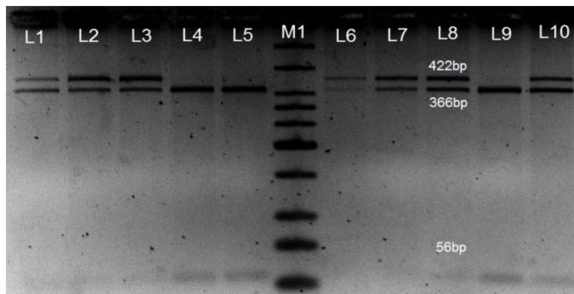


Figure 2. HaeIII-RFLP patterns of GH1
Lane 1, 2, 3, 6, 7 8 and 10 represented AB genotypes, lane 4, 5 and 9 represented AA. L11 represented amplified PCR product. M1 represented a 25 bp marker.

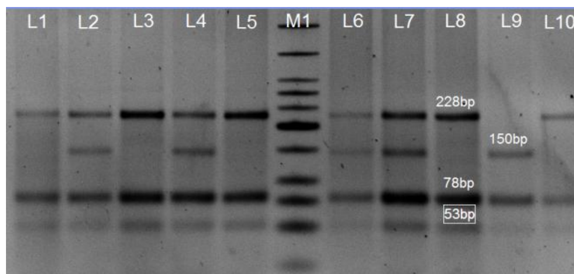


Figure 3. HaeIII-RFLP patterns of GH5
Lane 1, 3, 5, 8 and 10 represented GG genotypes, lane 2, 4, 6 and 7 represented GH genotypes, and lane 9 represented HH genotypes. L11 represented amplified PCR product. M1 represented a 25 bp marker.

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