Association of growth hormone gene polymorphisms with pre-weaning growth traits in Savanna goats

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Abstract

Expression of characteristics between generations depends on heritability and selection differential. The objective of this study was to determine inheritance of growth hormone gene (GH) and to evaluate the relationship of GH gene polymorphism and pre-weaning growth in Savanna goats. A total of 133 Savanna goats used in this study were obtained from MARDI Research Station, Kluang, Johor. From this group 63 does with GH1 and GH2 combinations were selected for breeding based on body condition score of 2.5 and above, history of kidding and reproductive soundness. The does were bred with three superior ABGG bucks in a natural breeding programme. F_1 progeny were evaluated for their genotypic patterns and growth performance. Two variants: GH1 and GH5 were identified. The GH1 gene revealed polymorphisms with two genotypes: AA and AB; and GH5 gene with three genotypes: GH, GG and HH. The combination of GH1 and GH5 genotypes of P_1 Savanna parents revealed four genotypic variants of ABGG, ABGH, AAGH and AAHH with frequencies of 0.47, 0.39, 0.06 and 0.08, respectively. F_1 progeny with genotypic variants of ABGG and ABGH at frequencies of 0.60 and 0.40 were observed, respectively. The four parental combinations of GH1 and GH5 genes produced F_1 genotypes which followed the Mendelian inheritance. The birth weight of ABGG F_1 Savanna kids was higher ($p<0.05$) than ABGH genotype. The genetic polymorphism of growth hormone genes can be a potential marker in genomic selection and breed improvement of the goats in line with good management and environmental condition.

Keywords: Gene variants, polymorphism, PCR-RFLP, goats

Introduction

Growth hormone (GH) plays a very important role in the growth of livestock as well as other biological processes such as metabolism, lactation and reproduction (Supakorn *et al*., 2007; Jiang and Lucy, 2001; Ge *et al*., 2003). Besides the GH gene, there are growth hormone receptors (GHR) and insulin-like growth factor-1 (IGF-1) that influence the growth and development of body mass of an animal. The effects of GH are observed on target tissues, including muscle, bone and adipose tissues. Expression of GH gene and its polymorphism at gene and protein levels has been reported but nucleotide changes and its position in the GH sequence has not been widely studied. The caprine growth hormone gene of 2544 bp consists of five exons and four introns, and is mapped on chromosome 19 (Dettori *et al*., 2013)

Genetic polymorphism can be identified by several techniques. The most common

method is polymerase chain reaction - restriction fragment length polymorphisms (PCR-RFLP). It is a powerful method that identifies nucleotide sequence variation in amplified DNA. Marker genotyping is expensive in the past thus only few major QTL were identified (Andrzej, 2002).

The Savanna is a meat goat breed known for its rapid growth, good mothering ability and reasonably high milk production to sustain growth of kids (Stonehavenstud, 2012; North American Savannah Association, 2011). The Savanna goats are also well adapted to the tropical climate and have great potential for goat meat production in Malaysia (Mohamad Hifzan and Musaddin, 2013). Therefore, the objectives of this study were to determine genotypic inheritance of growth hormone (GH) gene and its association with pre-weaning weight in Savanna goats.

Materials and Methods

Blood collection and DNA extraction

A total of 119 does and 14 bucks of the Savanna breed at MARDI, Kluang, Johor research station were selected for the study. The farm practiced semi-intensive production system with 5-h day grazing on *Bracharia humidicola* (Rendle) and *Panicum maximum* (Guinea) grasses. Caprine blood samples were collected via jugular vein puncture into EDTA vacutainer tubes. Then, genomic DNA was isolated using Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's procedure with minor modifications. The DNA concentration was estimated using NanoDrop ND-1000 Spectrophotmeter (NanoDrop Technologies, Inc., USA).

PCR-RFLP amplification

The genomic DNA was amplified by PCR using two synthesis GH gene fragments; GH1: F-5'CTC TGC CTG CCC TGG ACT3' and R-5'GGA GAA GCA GAA GGC AAC C3' and GH5: F-5'GCC AGT GGT CCT TGC ATA AA3' and R-5'AGT CCA GGG CAG GCA GAG3' (Hua *et al*., 2009; Supakorn *et al*., 2007). The PCR reaction was performed in a total volume of 25 µl on PTC-200 DNA Engine Cycler (MJ Research, USA). Each PCR reaction mixture contained 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. The PCR programme consisted of initial denaturation at 94°C for 10 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 45 s and a final extension at 72°C for 7 min. Electrophoresis of PCR products were conducted at 75V on a 1.5% agarose gel, and the PCR products visualized using AlphaEaseFC Stand Alone Software of the gel documentation system (Alpha Innotech, California). All amplified bands were subjected to digest with HaeIII restriction enzyme (Promega, USA) according to the manufacturer's protocol. The resulting products of digestion with restriction enzyme were separated into 4% metaphore agarose at 90V for 105 min.

Breeding and genetic analysis

The frequency of genotypes for GH1 and GH5 genes were assumed to be in Hardy-Weinberg Equilibrium (HWE). A group of 63 does representing four genotype variants: ABGG, ABGH, AAGH and AAHH were selected for breeding with 3 superior bucks with ABGG genotype in a restricted 60-d breeding period managed under semi-intensive system. The bucks were chosen based on physical assessment, age maturity,

body condition, health status, and reproductive organ normality (Musaddin, 2008). Does were fed supplemental concentrate with 16% CP at the rate of 400 g/head/d for 1 mo prior to breeding and throughout the breeding period. Salt lick and clean water were made available ad-libitum. All does were scanned for presence of active ovaries before the start of the experiment and pregnancy status was determined using ultrasound sonography 30 d after the end of the breeding period.

Evaluation of F_1 progeny

The does were allowed to kid naturally in individual pens. The F_1 kids were allowed to suckle freely up to 3 mo of age. At 1 mo, the kids were introduced to fresh cut fodder of *Bracharia humidicola* (Rendle) and *Panicum maximum* (Guinea) grasses. The kids were managed under intensive system. The F_1 progeny data for body weight, height at withers, body length and chest girth were collected monthly from birth to 3 mo of age. Blood samples were collected in EDTA via jugular vein puncture technique at weaning (90 d of age) and screened for the GH gene variants using PCR-RFLP technique and genotypic frequencies of F_1 progeny were estimated. The genotypic and phenotypic data of pre-weaning F_1 progeny were analysed using Generalized Linear Model Procedure of SAS v 9.3.

Results and Discussion

Genotypic variants

The present study showed the frequencies of GH1 and GH5 variants in Savanna goats deviated from Hardy Weinberg Equilibrium. Polymorphism of GH1 gene revealed two genotypes: AA (366, 56 bp) and AB (422, 366, 56 bp), while BB genotype was absent. GH5 gene revealed three genotypes: GH (228, 150, 78 and 53 bp) and GG (228, 78, 53 bp) and HH (150, 78 and 53 bp). These findings are similar to the previous reports on Savanna and Kalahari goats by Amie Marini *et al.* (2012) and Boer bucks by Hua *et al.* (2009). The polymorphic GH1 and GH2 genes were reported to be associated significantly with body weight in goats (Hua *et al*., 2009) and milk yield in dairy cattle (Zhou *et al*., 2005; Andrzej *et al*., 2002). The combination of GH1 and GH5 genotypes revealed four genotypic variants of ABGG, ABGH, AAGH and AAHH with frequencies of 0.47, 0.39, 0.06 and 0.08 for the P_1 Savanna goat parents.

The selective breeding of does with four combined genotypes: ABGG, ABGH, AAGH and AAHH resulted in F_1 genotypic progeny variants of ABGG and ABGH with frequencies of 0.60 and 0.40, respectively. Other variants were not observed. The mating of ABGG P_1 parents resulted in ABGG F_1 progeny. Meanwhile, the mating of the parents, P_2 (ABGH) x P_1 (ABGG) produced ABGH and ABGG genotypes of the F_1 progeny with frequencies of 0.60 and 0.40, respectively. P_3 parent (AAGH) mated to P_1 parent (ABGG) produced ABGH and ABGG genotypes of F_1 progeny with a frequency of 0.50 of each genotype while P_4 parent (AAHH) mated to P_1 parent (ABGG) produced F_1 progeny of ABGH genotype, in accordance to Mendelian inheritance.

The mating of ABGG parents of homozygous dominant genes produced F_1 progeny with homozygous dominant ABGG genotype while heterozygous ABGH and AAGH P_1 parents mated to homozygous dominant ABGG produced F_1 progeny with both homozygote ABGG and heterozygote ABGH genotypes. Meanwhile, mating between homozygous recessive AAHH genotype and homozygous dominant ABGG genotype produced F_1 progeny of ABGH heterozygote. Therefore, breeding the selected animals would express certain

characteristics and eliminate the genotypes of homozygous recessive when mated to homozygous dominant genotypes.

Pre-weaning performance

The body weight of F_1 Savanna kids for pre-weaning was observed to increase in both ABGG and ABGH genotypes (Figure 1). The ABGG genotype showed higher ($p<0.05$) birth weight than ABGH genotype (Table 1). Mohamad Hifzan *et al.* (2012) reported that birth weight was higher for the Savanna (4.00±0.53 kg) than Kalahari goats (3.68±0.79 kg) in the local environment. However, comparison of growth performance of livestock animals from different studies may not be accurate due to the influence of environment and nutritional differences which might have occured in different years. These factors are important for determining growth performance that indirectly affect the productivity of goats.

There was no significant difference in body height, body length and chest girth between ABGG and ABGH genotypes (Figures 2, 3 and 4). The pre-weaning average daily gain (ADG) of ABGG and ABGH genotypes were 76.33 g and 92.67 g, respectively. The pre-weaning ADG of ABGH genotype was higher ($p>0.05$) than ABGG genotype.

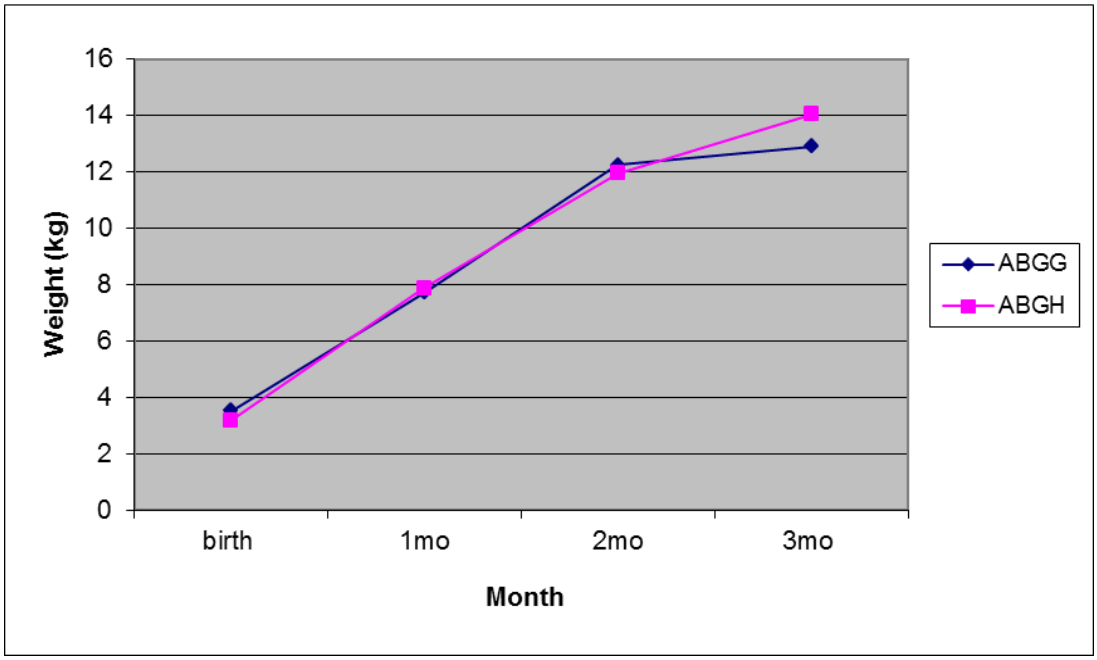

Figure 1. Pre-weaning body weight of ABGG and ABGH genotypes

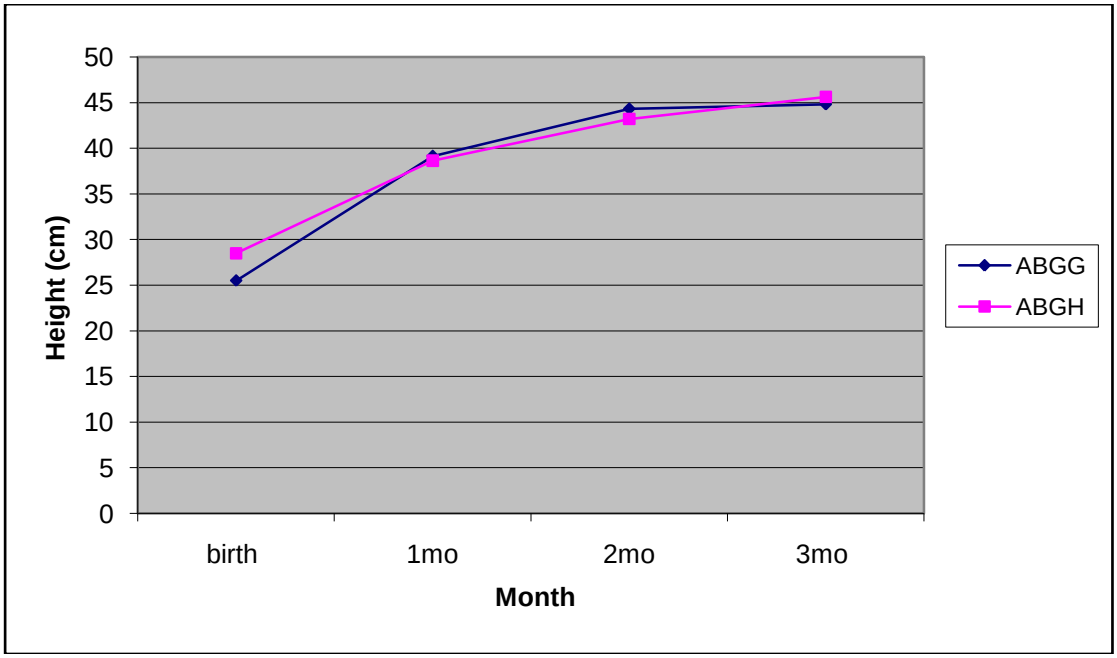

Figure 2. Pre-weaning height at withers of ABGG and ABGH genotypes

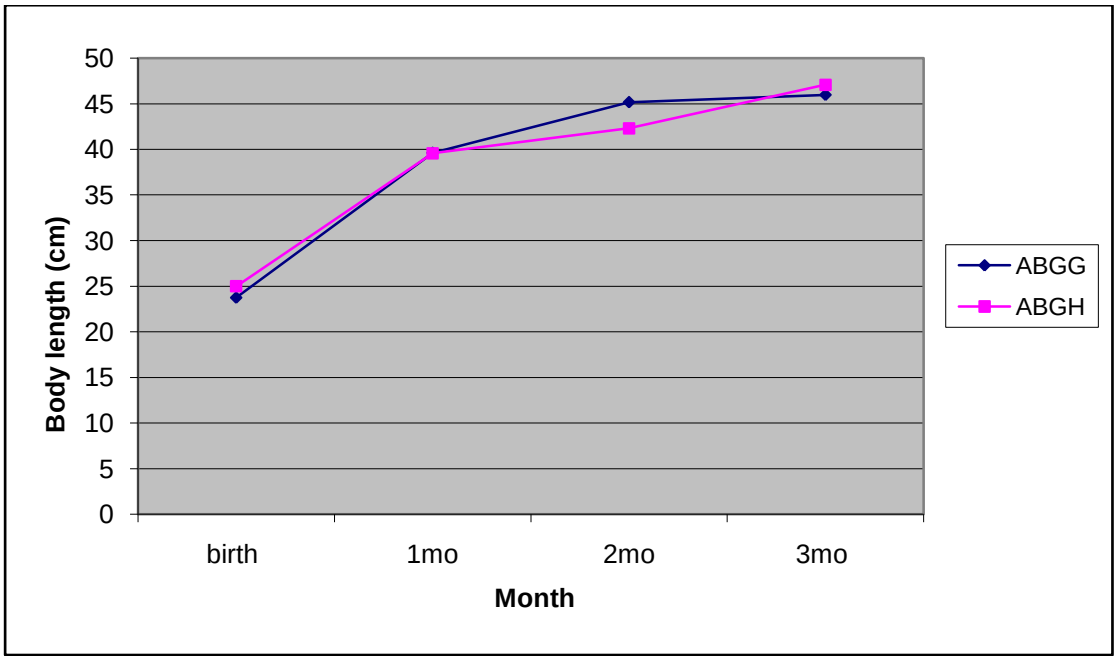

Figure 3. Pre-weaning body length of ABGG and ABGH genotypes

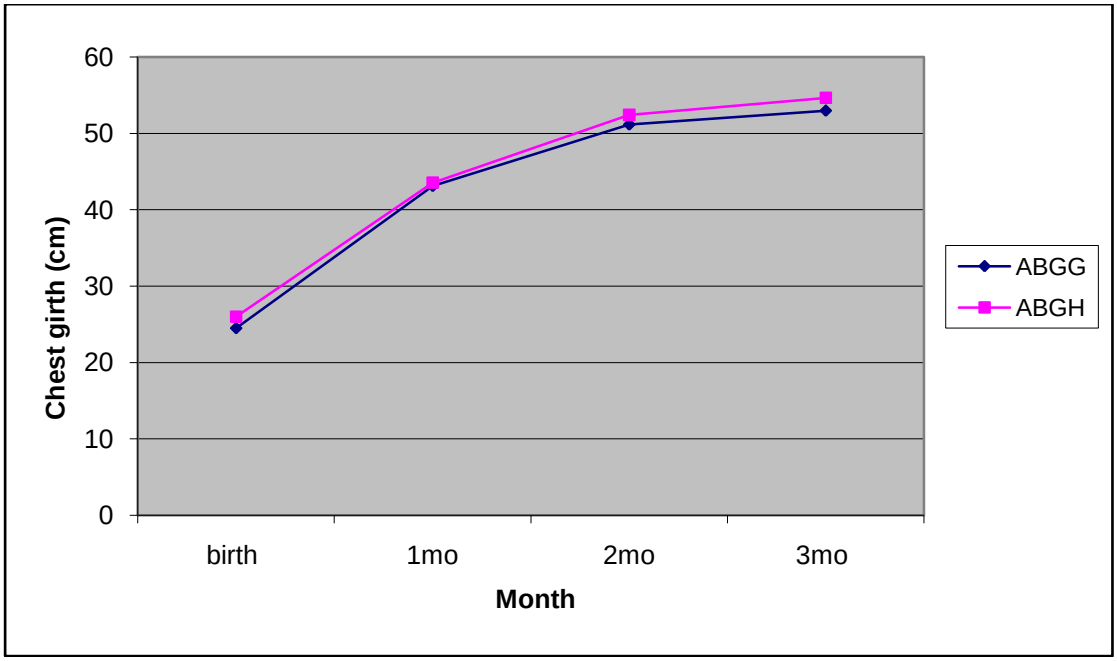

Figure 4. Pre-weaning chest girth of ABGG and ABGH genotypes

Table 1. Association between growth performance parameters and genotype effects of GH in F_1 Savanna goats

Parameter	ABGG Mean	SEM	ABGH Mean	SEM	*P*
Body weight (kg)					
Birth	3.50[a]	0.14	2.99[b]	0.08	0.04
3-mo	10.37	1.05	11.33	1.61	0.27
Body height (cm)					
Birth	25.50	1.50	29.00	1.15	0.16
3-mo	45.00	1.26	44.39	1.34	0.76
Body length (cm)					
Birth	23.75	0.75	25.33	0.33	0.11
3-mo	46.50	1.50	45.44	1.45	0.65
Chest girth (cm)					
Birth	24.50	3.00	25.33	0.33	0.73
3-mo	51.84	3.10	54.33	1.74	0.57
Pre-weaning ADG (g)	76.33	13.13	92.67	17.47	0.56

SEM = standard error of mean

[ab]Means with different superscripts within the same row were significantly different ($p<0.05$)

Conclusion

The combination of GH1 and GH5 genes revealed four genotypic variants of ABGG, ABGH, AAGH and AAHH. The mating of homozygous dominant genes would express certain characteristics and eliminate the homozygous recessive genotype. The results of this study can be applied in the selection of superior goats thus reducing the cost of replacement breeders. Genetic markers could be used for genomic selection in livestock improvement programme. The genetic polymorphism of growth hormone genes can be a potential marker for selection of high growth performance in goats. Further study is needed to validate the effects of genotypes before implementation of genomic selection associated with growth performance in goat populations.

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