THESIS

EFFECT OF GROWTH HORMONE AND GROWTH HORMONE RECEPTOR GENES FOR PREWEANING GROWTH TRAITS IN A MULTIBREED BEEF POPULATION

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THESIS

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China Supakorn
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LIST OF ABBREVIATIONS

ANOVA	= Analysis of Variance
BLUP	= Best Linear Unbiased Predictor
BR	= Brahman
BW	= Birth Weight
CH	= Charolais
CG	= Contemporary groups
EBV_a	= Direct estimated breeding value
EBV_m	= Maternal estimated breeding value
GH1 to GH6	= the first to the sixth PCR fragment of growth hormone gene
GHR1 to GHR3 gene	= the first to the third PCR fragment of growth hormone receptor
kg	= kilogram
mM	= millimolar
ml	= millilite
mg	= milligram
MSE	= mean square error
NA	= Thai local native
R^2	= coefficient of determination
SNPs	= Single Nucleotide Polymorphisms
SSCP	= Single Strand Conformation Polymorphism
µl	= microlite
WW	= Weaning Weight

EFFECT OF GROWTH HORMONE AND GROWTH HORMON RECEPTOR GENES FOR PREWEANING GROWTH TRAITS IN A MULTIBREED BEEF POPULATION

INTRODUCTION

Preweaning growth traits are economically important traits in beef production. These traits are controlled by polygenes and are affected by feeding and management under farm conditions. However, the breeding improvement for these traits of livestock in the past had been achieved by selection based on either phenotype or estimated direct genetic merit of superior animals.

In the past decade, molecular biology technique is popular among researchers. These techniques help to identify genetic variation at specific loci and the association between variation at QTL (quantitative trait loci) and interested traits. The aim of using QTL is for improvement of genetic gain by marker assisted selection (MAS) and the QTL technique is of high accuracy for the estimation of an animal's genetic value. The utilization of molecular biology techniques could save resources allocated progeny tests, reduce generation intervals and increase intensity of selection.

Current advances in molecular genetics are leading to the discovery of individual genes or candidate genes with substantial effect on traits of economic importance. Candidate gene strategy has been proposed by direct search for QTL (Tambasco *et al.*, 2003). In other words, the genetic variation of gene is affecting the physiological pathways and phenotype. The genetic variation of gene would be more likely to affect the phenotype variation than genes or chromosome regions that are chosen by chance. Several studies reported that the candidate genes have influenced for preweaning growth traits such as growth hormone and growth hormone receptor genes (e.g. Lucy *et al.*, 1998, Andrzej *et al.,* 1999 Ge *et al*., 2003; Kim *et al*., 2004). The growth hormone gene is located on 19^{th} chromosome and it is a major regulation gene for postnatal growth and metabolism in mammals. The growth hormone receptor gene is located on 20^{th} chromosome and it is a mediation gene of the biological actions of growth hormone on target cells.

The detection of polymorphism makes it possible to identify differences between individual in genotype at growth hormone and growth hormone receptor genes. The use of this information from two genes in breeding programs has potential to substantially enhance rates of genetic improvement. However, selection should not be based exclusively on candidate genes and would ignore all the other genes that affect performance. These unknown or unidentified genes would be referred to as polygenes. Therefore, to maximize genetic progress, selection should be on the animal's EBV for polygenes with adjusting information of gentic polymorphism for the major genes. In addition, this strategy is expected to accelerate genetic progress through increasing accuracy of selection and increasing selection differences (Johan *et al*., 1994).

The three important areas that need attention and focus for a multibreed beef population were: the detection genetic polymorphism of growth hormone and growth hormone receptor genes by using single strand conformation polymorphism (SSCP) technique, the analysis of effect of both genes for preweaning growth traits such as birth weight and weaning weight and prediction of estimated breeding values for preweaning growth traits with adjusting the best fit molecular information. This study was designed to undertaken at The Buffalo and Beef Production Research and Development Center (BPRDC), Kasetsart University. The feature of this herd is multibreed beef population. Not only purebreds but also crossbreds were selected as parent of the next generation. There was high variation of *Bos indicus* and *Bos taurus* breed fractions. It would be crated different assortment of gene controlling desirable traits. This characteristic of population could be found in many commercial beef population in Thailand. Consequently, farmers and breeders could utilize the result of this research to genetically improve preweaning growth traits and use it as basic information for studying about genetic marker or marker assisted selection in the future.

OBJECTIVES

The objectives of this study were:

1. To identify and characterize genetic polymorphism of growth hormone and growth hormone receptor genes in a multibreed beef population

2. To evaluate the effect of the genetic polymorphism of growth hormone and growth hormone receptor genes for preweaning growth traits in a multibreed beef cattle population

3. To compare the best fit models among model with molecular information (SNPs, regression of allelic and haplotypic effects) and without molecular information

4. To estimate genetic parameters and to predict of breeding values for preweaning growth traits with adjusted by molecular information of growth hormone and growth hormone receptor genes

5. To estimate rank correlation of breeding values for preweaning growth traits of individual multibreed beef cattle between model with and without molecular information in a multibreed beef population

LITERATURE REVIEW

1. Beef population in Thailand

In the past decade, Thai farmers raised cattle as a source of draft power for their agriculture. Nowadays, Thai farmers raised beef cattle for meat production. Therefore, the number of cattle in Thailand has increased since 1996 to 2005 (Table 1). In 2005, there were 1,202,306 farmers that raised beef cattle in Thailand. The 23,084 farmers that raised beef cattle for industry. The total number of beef cattle per household was 5 to 10 animals. The number of beef cattle of Northeastern part in Thailand was found higher than other parts. Thai native beef cattle was approximately 74 percent and 26 percent was other purebreds and crossbreds (Department of Livestock Department, 2005).

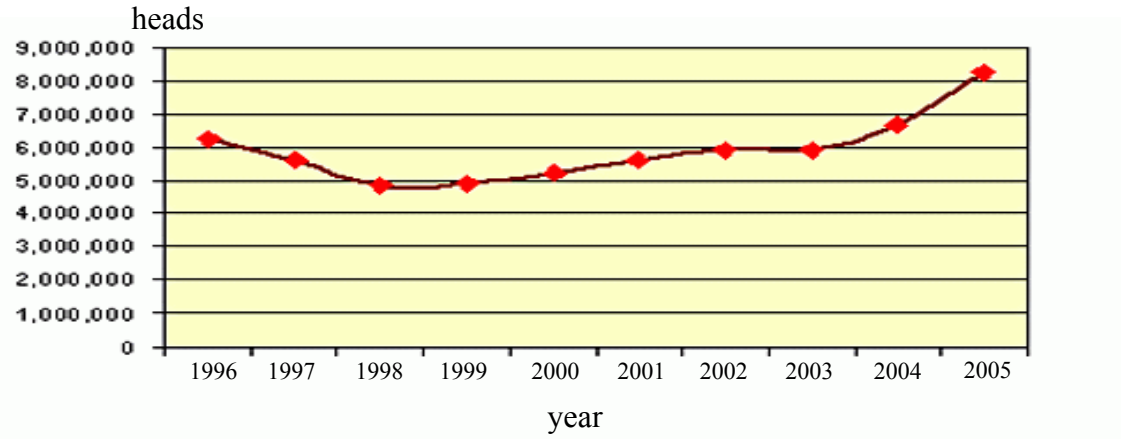


Figure 1 The number of beef cattle in Thailand since1996 to 2005

Source: Department of Livestock Department (2005)

The characteristic of beef populations in Thailand consists of unibreed and multibreed beef populations. Koonawootrittriron (2002) defined that unibreed populations are formed by animals of single breed. There are Charolais, Angus, Hereford, Brahman and Thai native. These foreign beef cattle from Europe, the United State and Australia could not tolerate insects, disease and Thai environmental conditions. On the other hand, Thai native or *Bos indicus* has a limitation of production performance. Therefore, Thai farmer have raising crossbred beef cattle that have some Thai native or *Bos indicus* fraction and *Bos taurus* fraction.

The multibreed populations are those composed of purebred and crossbred animals that interbred. Crossbred animals in multibreed populations are not just the ends of product of some purebred x purebred, purebred x crossbred and crossbred x crossbred mating. They also act as parents of the next generation if they chosen by selection process (Koonawootrittriron, 2002). Selection within a breed exploit additive genetic variation for selected traits. On the other hand, selection among population and crossbreeding system exploits both additive and non-additive effect

(heterosis) and complementarity among breeds (Koch *et al.*, 1985). The utilization of differences among breeds would obtain optimizing average genetic merit of performance traits under various conditions (Gregory and Cundiff, 1980). As a result, animal genetic improvement programs involve two main methodologies for increasing the productivity of farm animals such as selection of the best animals within a breed or population or using the best breeds or breed combination through crossbreeding system.

2. Economically importance of preweaning growth traits

Performance testing has been concentrating on measuring live weights at regular intervals such as birth weight, weaning weight, yearling weight, eighteen months weight or final weight (Payne and Hodge, 1997). However, two preweaning growth traits (birth weight and weaning weight) were emphasized for the preferable requirement of beef breeding services in Thailand. Moreover, preweaning growth traits are relatively easy traits to be recorded.

Weaning weight is an important trait in the efficiency of beef production. The efficiency depends on two basic elements such as maternal performance, growth of the young after weaning (Waldron *et al.*, 1993). Even though high weaning weight contributes to the efficiency, selection should not base on only weaning weight. The high weaning weight was associated with an increased birth weight (Robinson, 1996). Nevertheless, high birth weight is often associated with dystocia. This can cause calf losses, reduce calf performance and reduce cow fertility (Kinghorn and Simm, 1999). Robinson (1996) stated that extreme birth weight could in turn cause production problems and economic losses for beef producers. For that reason, birth weight and weaning weight are considered to be two primary traits in breeding strategy for growth traits.

3. Preweaning growth performance of beef cattle

The publications are available on descriptive statistic and genetic parameters for preweaning growth traits of beef cattle in aboard and Thailand. These publications could be separated into two categories, which are least square means of the performance and genetic parameters for preweaning growth traits. These reviewed publications represent mainly in relative breed with this study such as Charolais, Brahman and Thai native.

Means of birth weight and weaning weight of Brahman in the United Stated were found to be 39.60 and 211.80 kilograms (Jenkins and Ferrell, 2004). However, means of BW and WW of Charolais ranged from 36.00 to 47.00 and 196.00 to 279.00 kilograms, respectively (Aziz *et al*., 2003; Phocas and Laloe, 2004; Ozluturk *et al*., 2006). Splan *et al*. (2002) reported that means of weaning weight of Charolais and Brahman crossbreds in the United Stated was found to be 183.10 kilogram.

In Thailand, Tumwasorn *et al.* (1993) reported that least square means of birth weight in Charolais, Brahman and Thai native purebreds at Buffalo and Beef Production Research and Development Center (BPRDC), Kasetsart University were found to be 16.86, 21.95 and 26.83 kilograms, respectively. Likewise, weaning weight in Thai native, Brahman and Charolais purebreds were found to be 87.05, 111.67 and 147.13 kilograms, respectively. In addition, they carried the studies out in crossbreds, which were the offspring from mating between three breeds. The least square means of birth weight and weaning weight of these crossbreds were found to be 26.38 to 29.73 and 122.22 to 146.35 kilograms, respectively. Likewise, Markvichitr *et al*. (1996) studied in crossbred population (1/2 of Charolais, 1/4 of Brahman and 1/4 of Thai native) and found that the least square means of birth weight ranged from 19.65 to 28.19 kilograms and weaning weight from 88.96 to 143.96 kilograms. Certainly, the ranges of least square means for birth weight and weaning weight in crossbred of Charolais, Brahman and Thai native were higher than purebred.

Many publications were reported genetic parameters for both two traits (BW and WW). The additive, maternal and maternal permanent environmental effects for growth should be considered when beef producers formulate the breeding plans (Meyer, 1992). The direct and maternal heritabilities in Charolais ranged from 0.13 to 0.42 and 0.12 to 0.17 for birth weight and 0.14 to 0.33 and 0.11 to 0.13 for weaning weight, respectively (Trus and Wilton, 1988; Van Vleck *et al.*, 1996; Phocas and Laloe 2004). Hetzel *et al.* (1990) reported that the direct and maternal heritabilities in Brahman crosses from Australia were found to be 0.23 and 0.03 for birth weight and 0.12 and 0.08 for weaning weight, respectively. Splan *et al.* (2002) reported that direct and maternal heritabilities in Charolais and Brahman crossbred for weaning weight were found to be 0.40 and 0.19.

In Thailand, Commungkhun *et al.* (1998) reported that the estimated direct heritabilities in Brahman purebred beef population in field data set were found to be 0.11 for birth weight and 0.29 for weaning weight. However, Chitprasan *et al.* (1999) carried out in a commercial beef population at Supanburi province, Thailand in the same breed. The estimated direct heritability for birth weight was higher than studied by Commungkhun *et al.* (1998). In their's study, the estimated direct heritability of birth weight was found to be 0.44. In crossbred population at BPRDC, Racksasri (1996) studied estimated heritabilities of birth weight and weaning weight and found out to be 0.25 and 0.17, respectively.

4. Candidate genes for economic traits in beef cattle

Candidate genes or direct markers are expected genes to influence on several economic traits. Candidate genes strategy has been proposed to direct searching for QTL, which assumed that the genetic variation at genes affecting the physiological pathways related to a phenotype (Tambasco *et al.*, 2003). The studies on candidate genes in beef cattle from other countries are carried out on growth (e.g. Moody *et al.,* 1996, Reis *et al*., 2001, Tambasco *et al*., 2003; Kim *et al.*, 2004), carcass composition and quality (Schlee *et al*., 1994) and disease resistance (Weigel *et al*., 1990).

5. Expression of growth hormone and growth hormone receptor genes

In cattle, the genome is composed of 29 pairs of autosomes and two sex chromosomes. The growth hormone and growth hormone receptor genes are encoded by a single gene. The growth hormone gene is located on the 19th chromosome and the growth hormone receptor gene is located on the 20th chromosome. These two genes are consisting of unlike number of exons and introns. The growth hormone gene consisted of five extrons and four introns. The growth hormone receptor gene consisted of ten exons and nine introns (Hediger *et al*., 1990).

Expression of genes is integrated hormonal mechanism such as growth hormone, growth hormone receptor and insulin like growth factor I (IGF-I). The growth hormone plays a central role in the regulation of growth and metabolism in animals (Jiang and Lucy, 2001). In this case, growth hormone is released from the anterior pituitary into the circulation in secretory burst in response to signal peptides from the hypothalamus such as growth hormone releasing hormone and somatostatin. The actions of growth hormone are mediated through the growth hormone receptor. Moreover, growth hormone binds growth hormone receptor with high affinity and specificity. Expression of receptor is a requirement for cellular responsiveness to growth hormone. Furthermore, the growth hormone released to circulation has a direct effect on the liver where it is a signal to synthesis and release of *IGF*-I. In other words, the effect of growth depends on the interactions with growth hormone receptor and other hormones (Fariborz, 1997; Switenski, 2002).

6. Genetic polymorphism of growth loci

6.1 Growth hormone gene

Growth hormone (*GH*) gene is a key factor to produce growth hormone, which is necessary for postnatal growth and metabolism in mammals. The growth hormone is synthesized in the pituitary gland (Ge *et al*., 2003). The mutation of this gene (Figure 2) is likely to occur on the promoter region and the 5th exon (e.g. Moody *et al.,* 1996; Reis *et al*., 2001; Kim *et al.,* 2004).

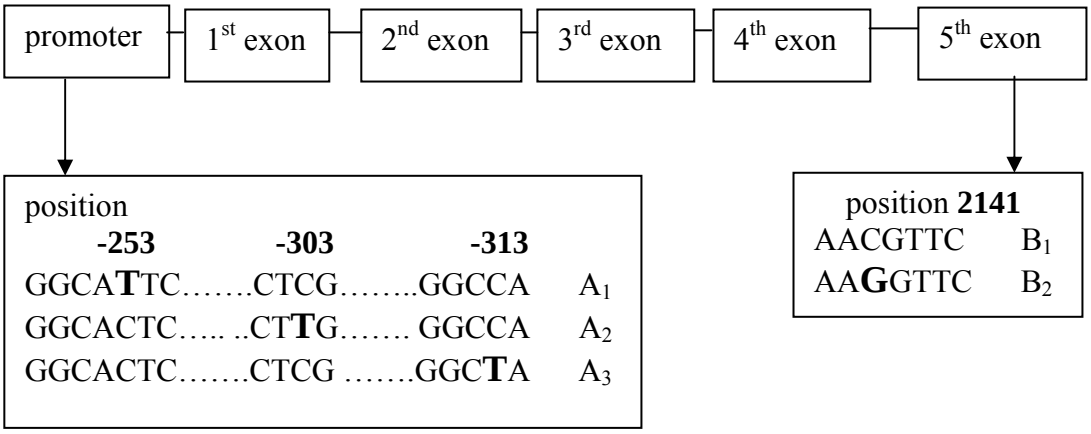


Figure 2 The bovine growth hormone gene, and the polymorphism on promoter and 5th exon

Note the A_1, A_2 and A_3 = the 1th, 2nd and 3rd allele of growth hormone gene present from mutation on promoter region and the B_1 and B_2 = the 1thand 2nd allele of growth hormone gene present from mutation on the 5th exon.

Source: modified from Moody *et al.* (1996), Reis *et al*. (2001), Ge *et al*. (2003) and Kim *et al.* (2004)

On the promoter region, mutation has been by detected at nucleotide position -253, -303 and -313 where cytocine were substituted thymine at all position. On 5th exon, an amino acid was changed at position 127 in the peptide, where a leucine was exchanged for valine, due to the base substitutions between cytocine to guanine at position 2141 (Schlee *et al*., 1994; Kim *et al.,* 2004). The research reviews of different authors are as follows:

In Europe, on the 5th exon, allelic frequencies of B_1 in cattle were found to be ranged from 0.59 to 0.90 and allelic frequencies of B_2 ranged from 0.10 to 0.41 (eg. Schlee *et al.,* 1994; Pilla *et al*., 1996; Lechniak *et al.,* 1999; Stasio *et al.*, 2002; Table 1). In all breeds from Table 1, the allelic frequency of B_1 was higher than allelic frequency of B_2, except Hereford and some beef cattle in Poland. By contrast, Lechniak *et al*. (1999) insisted that allelic frequency of B_1 was lower than allelic frequency of B_2 in Limousine, Charolais, Piedmontese, Angus, Hereford and

Maronesa. They found that the estimated allelic frequencies of B_1 and B_2 ranged from 0.38 to 0.39 and 0.61 to 0.62 respectively.

In North America, Ge *et al.* (2003) reported that allelic frequencies of A_1, A_2 and A_3 on the promoter region of high IGF-I Angus line were found to be 0.27, 0.30 and 0.43 respectively and allelic frequencies of A_1, A_2 and A_3 in low IGF-I line were found to be 0.32, 0.28 and 0.40 respectively. Allelic frequencies of B_1 on the 5th exon ranged from 0.60 to 0.64 and allelic frequencies of B_2 ranged from 0.36 to 0.40. On the other hand, Moody *et al.* (1996) reported that the frequency of allele B_1 in Hereford cattle (Table 1) was lower than that reported by Ge *et al.* (2003).

In Asia, Kim *et al.* (2004) and Pal *et al.* (2004) reported that allelic frequencies of A_1 in a Korean Hanwoo cattle ranged from 0.82 to 0.94, allelic frequencies of A_2 ranged from 0.06 to 0.18 and the A_3 allele was not detected (Table 1).

From these reviews, the mutation of growth hormone gene could occur on promoter region (3 alleles) and on the 5th exon (2 alleles) because of different selection in each particular population and distinct of cattle breeds (Schlee *et al.*, 1994). Allelic frequencies of A_1 on promoter region were mostly higher than A_2 and A_3 (Ge *et al.,* 2003; Kim *et al.,* 2004; Pal *et al.,* 2004). Allelic frequencies of B_1 on the 5th exon were higher than B_2 (eg. Schlee *et al*., 1994; Pilla *et al*., 1996; Regitano *et al.,* 1999; Grochowska *et al.,* 2001).

Table 1 Allelic frequencies of growth hormone gene on promoter region and on the 5th exon

Breed	n (head)	country	Alleles					Reference
			Promoter			5th exon		
			A_1	A_2	A_3	B_1	B_2	
Simmental	41	Germany	-	-	-	0.71	0.29	Schlee *et al*. (1994)
Hereford	35	USA	-	-	-	0.33	0.67	Moody *et al*. (1996)
Piedmontese x Chianina	53	Italy	-	-	-	0.67	0.33	Pilla *et al*. (1996)
Holstein crossbreds	100	Poland	-	-	-	0.86	0.14	Lechniak *et al*. (1999)
Beef cattle[1]	13	Poland	-	-	-	0.38	0.62	
Canchim	154	Brazil	-	-	-	0.79	0.21	Regitano *et al.* (1999)
Charolais	36	Brazil	-	-	-	0.74	0.26	
Polish Friensian	214	Poland	-	-	-	0.69	0.31	Grochowska *et al*. (2001)
Piedmontese	54	Italy	-	-	-	0.72	0.28	Stasio *et al*. (2002)
Angus								
high IGF-I	40	USA	0.27	0.30	0.43	0.60	0.40	Ge *et al*. (2003)
low IGF-I	40		0.32	0.28	0.40	0.64	0.36	
Aberdeen Angus	52	Brazil	-	-	-	0.77	0.23	Luciana *et al*. (2003)
Hanwoo cattle	108	Korea	0.82	0.18	-	-	-	Kim *et al.* (2004)
Karan Fries cattle	26	India	-	-	-	0.94	0.06	Pal *et al*. (2004)

Note [1] beef cattle = Limousin, Chalorais, Piedmontese, Angus and Hereford

6.2 Growth hormone receptor gene

Growth hormone receptor is a transmembrane protein that binds growth hormone with high affinity and specificity (Ge *et al*., 2003). Several studies have reported the genetic polymorphism of growth hormone receptor gene in various points region (e.g. Lucy *et al.*, 1998; Andrzej *et al.*, 1999; Ge *et al.*, 2003). The review works could be summarized as an abnormal sequence DNA or mutation on promoter, 5'–flanking region, the 10^{th} exon and 3'–flanking region (Figure 3). The reasons of mutation were deletion, transition and transversion on the DNA strand. Different authors carried out the research works for specific mutable region as follows:

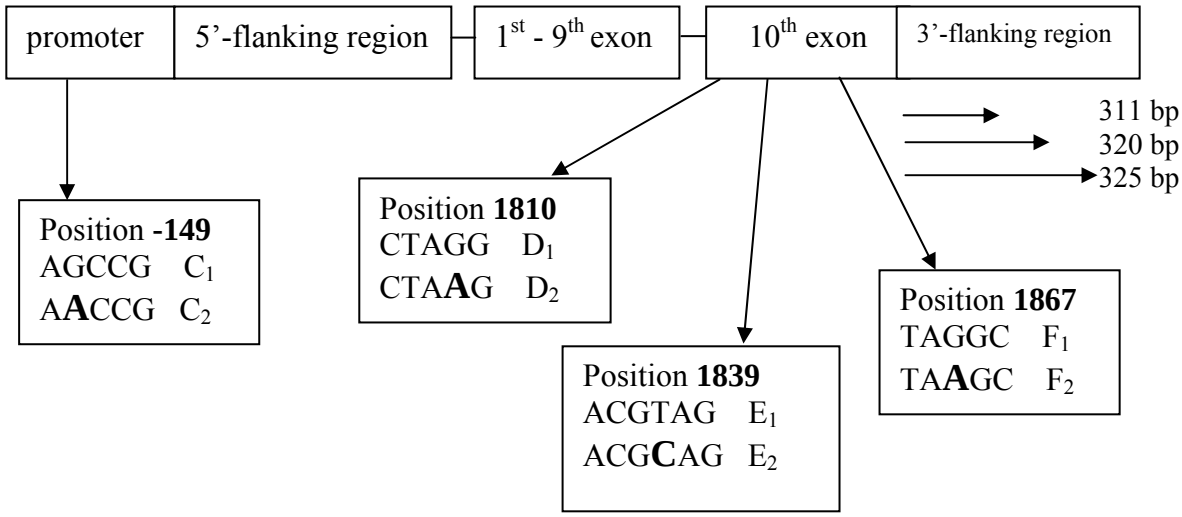


Figure 3 The bovine growth hormone receptor gene and the polymorphism on promoter, the 10^{th} exon and 3' –flanking region

Note The $C_1$and C_2 = the $1^{th}$and 2^{nd} allele of growth hormone receptor gene present from mutation on promoter region. The D_1 and D_2 = the $1^{th}$and 2^{nd} allele of growth hormone receptor gene present from mutation at position 1810 on the 10^{th} exon. The E_1 and E_2 = the $1^{th}$and 2^{nd} allele of growth hormone receptor gene presented from mutation at position 1839 on the 10^{th} exon and the F_1 and F_2 = the 1^{th} and 2^{nd} allele of growth hormone receptor gene present from mutation at position 1867 on 10^{th} exon.

Source: modified from Lucy *et al.* (1998), Andrzej *et al.* (1999) and Ge *et al.* (2003)

Lucy *et al*. (1998) reported genetic polymorphisms of $(GT)_n$ microsatellite (GT short tandem repeats) on the promoter region of growth hormone receptor gene in 111 cattle. The result identified five alleles that were 10, 15, 16, 17 and 19 GT repeats, respectively. Allelic frequencies of 10, 15, 16, 17 and 19 GT repeats were found to be 0.12, 0.03, 0.48, 0.35, and 0.03, respectively.

Andrzej *et al.* (1999) reported polymorphic sites on the 5'-flanking region of growth hormone receptor gene by using PCR-RFLP methods. This study found out that three genotypes, which were digested by *Fnu4 HI* enzyme, were identified as homozygous (-/- and +/+) and heterozygous (-/+). Allelic frequencies of non digested (-) and digested (+) ranged from 0.17 to 0.55 and 0.45 to 0.83, respectively. Their findings were concluded that the allelic frequencies between breeds were different, except to Polish Friesian and Aberdeen Angus. Allelic frequency of non-digested was higher than allelic frequency of digested, excepting to Simmental breed.

Ge *et al*. (2003) confirmed polymorphisms on the promoter and the 10^{th} exon of growth hormone receptor gene in Angus cattle. On the promoter region, genotypic frequencies of C_1C_1, C_1C_2 and C_2C_2 were found to be 0.35, 0.50 and 0.15 and allelic frequencies of C_1 and C_2 were found to be 0.60 and 0.40, respectively. On 10^{th} exon, genotypic frequencies of D_1D_1, D_1D_2 and D_2D_2 at position 200 were found to be 0.35, 0.52 and 0.13 and allelic frequencies of D_1 and D_2 were found to be 0.61 and 0.39, respectively. Genotypic frequencies of E_1E_1, E_1E_2 and E_2E_2 at position 229 were found to be 0.39, 0.50 and 0.11 and allelic frequencies of E_1 and E_2 were found to be 0.59, 0.41, respectively. Genotypic frequencies of F_1F_1, F_1F_2 and F_2F_2 at position 257 were found to be 0.04, 0.35 and 0.61 and allelic frequencies of F_1 and F_2 were found to be 0.22 and 0.78 respectively.

Moisio *et al*. (1998) detected DNA length variants on the 3'-flanking region of growth hormone receptor gene (*GHR*) in 3 forms: 311 bp (GHR_{311}), 320 bp (GHR_{320}) and 325 bp (GHR_{325}). The allelic frequencies of GHR_{311}, GHR_{320} and GHR_{325} ranged from 0.13 to 0.66, 0.32 to 0.70 and 0.00 to 0.17, respectively. Their result also recommended that the allelic frequencies had become more diverse. In this case, GHR_{320} and GHR_{325} alleles were more frequent in the selected dairy breeds such as Finnish Ayrshire and Finnish Friesian whereas the shortest allele GHR_{311} predominates in Northern Finncattle, Western Finncattle and Eastern Finncattle.

7. Effect of growth hormone and growth hormone receptor genes for preweaning growth traits

Several studies proved that the genetic polymorphism of growth hormone and growth hormone receptor genes correlated with preweaning growth traits (eg. Schlee *et al*., 1994; Tambasco *et al*., 2003; Stasio *et al*., 2002; Hale *et al*., 2000). In other words, the genetic polymorphism for both genes was found responsible to improve preweaning growth traits.

7.1 Growth hormone gene

The mutation of growth hormone gene on promoter or on the 5^{th} exon depicted one of changing phenotype since polypeptide chains that are translated from converting DNA. For example, the transversion of cytocine to guanine on the 5^{th} exon at nucleotide position 2141 was the cause for amino acid substition leucine to valine at position 127 of growth hormone polypeptide. This substitution illustrated

low body weight and average daily gain (Lucy *et al*., 1998). The detail review works by different authors are as follows:

Schlee *et al*. (1994) demonstrated the relationship between genetic polymorphisms of growth hormone gene on the 5th exon and growth hormone in blood samples by radioimmuno-assays. Moreover, animals with B_1B_1 genotype generally showed higher concentrations of growth hormone than B_1B_2 in German Black and White, Bavarian and Tyrolean Brown and Simmental. Likewise, a significant concentration of growth hormone was observed in the Black and White bulls. Their work also concluded that genotype B_1B_1 had influenced with higher levels of growth hormone than B_1B_2 genotype animals but it did not occur in B_2B_2 genotype animals.

In the similar way, Tambasco *et al*. (2003) explained the effect of growth hormone gene polymorphism on growth traits by restriction fragment length polymorphisms (RFLPs) method. The authors demonstrated that the effect of growth hormone gene was influenced for average daily gain from weaning to yearling weight. Consequently, average daily gain from birth to weaning weight and average daily gain from weaning to yearling weight of animals with B_1B_1 genotype of growth hormone gene on the 5th exon were higher than B_1B_2 genotype animals ($P<0.05$). Pal *et al*. (2004) reported that the genotype of growth hormone gene had a significant effect for birth weight, three month body weight and average daily body weight gain in Karan Fries cattle. The B_1B_1 genotype animals had birth weight, three months body weight and average birth weight higher than B_1B_2 genotype ($P<0.05$; Table 2).

However, Reis *et al*. (2001) showed the effect of genetic polymorphism of growth hormone gene for mature weight (WTM) in eight major indigenous Portuguese cattle breeds by *AluI* enzyme. Significant effect of the growth hormone genotype was observed in the Alentejana breeds (Table 2). Their results suggested that B_1B_2 genotype animals tended to be in high mature body weight.

Kim *et al.* (2004) found out the least square means of estimated breeding value (EBV) in each genotypes of growth hormone gene for production traits in Korean Hanwoo cattle. The A_2A_2 genotype had a high estimated breeding value animals for weight at 3 months ($P<0.05$) when compared with genotypes A_1A_1 and A_1A_2. As a result, the authors suggested that A_2A_2 animals should be selected for improving weight at 3 month. Moreover, it is advised that polymorphisms may be useful for selection at DNA level.

In contrast, Stasio *et al*. (2002) confirmed the association of polymorphisms in the growth hormone gene on the 5th exon for growth traits in Piedmontese male calves. Concerning the growth hormone locus the substitution effect was found to be negligible. However, the effect of growth hormone gene tended to be high for body weight at 5, 7 and 11 months. Ge *et al*. (2003) reported that growth hormone gene on promoter region and on 5th exon did not influence for growth traits in Angus cattle.

Adequate attention and various research works were done on the 5th exon because this exon could be more influential compared to others (Lucy *et al*., 1998; Reis *et al*., 2001). Research works supported that B_1B_1 genotype of growth hormone gene on 5th exon were higher than the others (Tambasco *et al*., 2003; Pal *et al*., 2004). In some cases, the study reported that genotypes of growth hormone gene on 5th exon did not influence on improvement of growth traits (Ge *et al*., 2003).

Table 2 The least square means and standard errors of mature weight (WTM), average daily gain from birth to weaning (GBW), average daily gain from weaning to yearling (GWY) birth weight (BW), three months body weight (WT 3 M) and average daily body weight gain (ADG)

Traits	n (head)	Breed	Genotype on 5th exon			Reference
			B_1B_1	B_1B_2	B_2B_2	
WTM[1]	22	Alentejana	594.60 ± 8.41[a]	648.30 ± 22.20[]	-	Reis *et al.*
	24	Arouquesa	465.80 ± 20.23	484.80 ± 15.72	-	(2001)
	23	Barrosa	438.60 ± 22.15	410.10 ± 15.60	490.40 ± 31.32	
	32	Marinhoa	537.10 ± 22.83	-	-	
	24	Maronesa	433.70 ± 21.36	492.00 ± 15.11	482.80 ± 12.33	
	22	Mertolenga	488.60 ± 6.63	-	-	
	21	Mirandesa	574.10 ± 8.83	-	-	
	27	Preta	577.90 ± 21.78	533.90 ± 24.60	-	
GBW[2]	72	Angus,	0.84 ± 0.01[a]	0.78 ± 0.02[b]	-	Tambasco
GWY[2]	74	Canchism,	0.65 ± 0.02[a]	0.74 ± 0.02[b]	-	*et al*.
	65	Simmental				(2003)
BW[1]	26	Karan	28.80 ± 31.13	24.00 ± 2.52[b]	-	Pal *et al.*
WT 3 M[1]		Fries Cattle	59.38 ± 2.67[a]	56.67 ± 5.46[b]	-	(2004)
ADG[2]			0.59 ± 0.22[a]	0.56 ± 0.52[b]	-	

Note [1] = the unit in kilograms, [2] = the unit in kilograms per day and [a, b] superscript letter differ between the column ($P<0.05$)

7.2 Growth hormone receptor gene

Few studies reported the correlation between growth hormone receptor gene and preweaning growth traits since this gene is recently discovered. Hale *et al*. (2000) reported a microsatellite (TG short tandem repeats) polymorphism of the growth hormone receptor gene on promoter region in Angus breed. So-called short allele (eleven consecutive TG sequences) is common in *Bos indicus* while a longer one (sixteen to twenty repeats) is common in *Bos taurus* cattle. The comparison of Angus steers of the homozygous (long/long) genotype was higher ($P<0.01$) for weaning and carcass weight than heterozygous (long/short). However, the homozygous genotype (short/short) was found absent. Curi *et al*. (2005) studied the effect of polymorphism microsatellites in the regulatory region of this gene in Nellore, Canchism and their's crossbreds by PCR technique. They observed that the effect of substitution from one allele to another allele had significantly effect on decreasing in daily weight gain and body weight ($P<0.05$). On the other hand, Ge *et*

al. (2003) reported that the growth hormone receptor gene was independent with growth traits.

8. Use of marker information in genetic evaluation

Molecular information is integrated with production and pedigree information for evaluation genetic effect. Indeed, the estimation of marker effect would be incorporated in animal model genetic evaluations, providing the best linear unbiased predictors (BLUP) of individual animals in a breeding population (Dekkers, 2004). Kennedy *et al.* (1992) and Hayes and Goddard (2001) suggested that the use of mixed model procedures under individual animal model with inclusion of identifiable single genes as a fixed effect could separate effect of single gene from those of polygenes and could provide unbiased prediction of breeding values.

The benefit of adding molecular information in breeding program is expected to accelerate genetic progress through accuracy of selection, reduction of generation interval and increasing selection differentials (Meuwissen and van Arendonk, 1992). The studies of molecular genetic information on quantitative traits in selection showed highest responses to selection in early generations, followed by a decline in later generations. Gibson (1994) examined the long-term consequences of markers using computer simulation of selection on known major genes. The result confirmed that although marker resulted in greater cumulative response to selection in the short term, the phenotypic selection achieved greater response in the longer term.

MATERIALS AND METHODS

Trial I

Genetic polymorphism of growth hormone and growth hormone receptor genes in a multibreed beef population

Animals and Equipments

1. The multibreed beef cattle population and dataset

The animals in this study were raised at Buffalo and Beef Production Research and Development Center (BPRDC), Kamphaeng Sean campus, Kasetsart University, Nakhon Prathom. All animals had complete pedigree and preweaning growth traits informations. In total, 130 animals were born during the year 1987 to 2004. Crossbred animals were several types of breed fractions, which consisted of CH, BR and NA breed fractions. These different breed fractions were separate into six breed groups. The breed combination for 1/2 of CH, 1/4 of BR and 1/4 of NA was found to be highest in frequency in the population (Appendix Table 1.). The number of birth weight (BW) and weaning weight (WW) data of 1/2CH1/4BR1/4NA, 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA, other breed groups (CH<1/2, CH=1/2 and CH>1/2) were found to be 34, 25, 15, 13, 33 and 10 records, respectively. The general description of dataset of studied animals is presented in Table 3.

Table 3 General descriptive of the dataset in this study

Group	n	Frequency (%)	Means± SD[1] BW (kg)	Means± SD[1] WW (kg)
Sex				
Male	35	26.93	30.79±5.31	178.46±47.54
Female	95	73.07	27.41±5.58	161.88±34.11
Breed group				
1/2CH1/4BR1/4NA[2]	34	26.15	27.20±6.00	163.04±43.42
1/2CH5/16BR3/16NA	25	19.24	31.33±4.30	183.77±34.73
1/2CH3/8BR1/8NA	15	11.54	29.76±4.71	162.50±41.13
CH<1/2	13	10.00	28.86±7.47	159.36±39.98
CH=1/2	33	25.38	27.64±5.97	162.44±29.09
CH>1/2	10	7.69	25.82±4.46	156.03±35.99
Total	130	100.00	28.32±5.69	166.00±38.70

Note [1]SD = standard deviation, [2]CH=Charolais, BR=Brahman, NA=Thai native and kg=kilogram

2. Animal management system

The management system in the herd was such that all the calves those were born all year round and were raised along with their parent until weaning. The age at weaning weight data of beef cattle that ranged from 180 to 240 days. Calving seasons were classified as winter (November to February), summer (March to June) and rainy (July to October). These seasons were cited from Thai meteorological department (2002).

The cattle had an access to grazing pasture during the day and were confined in large pens during the night. Animals of all breed groups were raised under the same management conditions. The animals were maintained on improved pasture and were supplemented with concentrate containing 14 percent of crude protein. Animals were fed two to three kg of concentrate meal. The concentrate composed of local grains, modified-rice straw, molasses, vitamins and minerals. The paragrass (*Brachilia mutica*), guinea (*Panicum maximum*) and caribbean stylo (*Stylosanthes hamata*) were cultivated for feeding. The farm followed rotational grazing method. Dry lick mineral blocks and drinking water were fed *ad libitum*.

All animals in the herd were treated against internal and external parasites (IVOMEC®) every year. Breeding age and young bulls and heifers were vaccinated against viral (Foot and Mounth Disease) annually beginning at eight months of age. Moreover, only heifers were vaccinated against bacterial (*Blucella abortus*) once time at weaning age (seven to eight month of age).

Artificial insemination was used for mating in this herd. Cows were mated throughout the year. Live weight and body measurements of all animals were taken at birth and at weaning using weighing scale. All collected informations were inputted into the computers.

3. Laboratory equipment

3.1 Equipment and chemical substances used for collect blood sample and DNA extract were:

a. Syringes with needles
b. 10 ml tube with Ethylenediaminetetraacetic acid (EDTA)
c. Microcentrifuge tube
d. Lysis buffer (250 mM NaCl, 100mM EDTA, 0.5 percent of SDS (sodium dodecyl sulfate))
e. Proteinase K
f. Phenol: Choroform: Isoamyl (25: 24: 1)
g. 95% Ethanol
h. 70% Ethanol
i. Tris-EDTA buffer (TE) buffer (10mM Tris HCl, 1mM EDTA)
j. Centrifuges
k. Vortex mixer

l. Incubator
m. Refrigerator (-20 ^{o}C)

3.2 Equipment used for checking qualitative and quantitative of DNA by agarose gel electrophoresis or spectrophotometry were

a. Molecular weight marker

b. Tracking dye (70% of glycerol, 2% of Tris-acetate/EDTA solution (TAE), 4% of o.5M EDTA, 0.01% of 20% SDS, 0.09% of bromophenol blue, 0.09% of xylene cyanol and 0.23% of de-ionized H_2O)

c. Agarose gel

d. 1X TBE buffer (10.8g of Tris-Base, 5.5g of Boric acid, 4 ml of 0.5M EDTA, 100 ml of de-ionized H_2O)

e. Ethidium bromide (10mg/ml in water)
f. Horizontal gel electrophoresis
g. Gel document
h. Spectrophotometry

3.3 Equipments and chemical substances used for amplification of growth hormone and growth hormone receptor genes by Polymerase Chain Reaction (PCR) were

a. 0.9 µl of DNA (20 ng/µl)
b. Primers

Primers were designed from Primer 3 (http://www.genome.wi.mit.edu/cgi.-bin/primer/primer3.cg1/). They were considered from database of Genbank (http://www.ncbi.nlm.nih.gov) of growth hormone (GeneID280804) and growth hormone receptor (GeneID280805) nucleotide sequences in *Bos taurus* and *Bos indicus*. Two genes from upstream of promoter region to downstream of the last exon were analyzed for each beef cattle.

The growth hormone gene consisted of five exons and four introns. The growth hormone receptor gene consisted of ten exons and nine introns. However, growth hormone receptor nucleotide sequences from Genbank had many gap regions. Therefore, growth hormone receptor was selected some regions to study. The primer sequences, melting temperature and size of amplified fragments for growth hormone and growth hormone receptor genes are represented in Table 4 and Table 5.

Table 4 Primer sequences used for screening polymorphisms of the growth hormone gene (*GH*)

Fragment	Primer Forwards (5'⟶ 3')	Primer Reverse (5' ⟶3')	Tm (^{o}C)	Size (bp)
GH1	CGACGCCATAGACAGCAG	CATTTATGCAAGGACCACTGG	60	419
GH2	GCCAGTGGTCCTTGCATAAA	AGTCCAGGGCAGGCAGAG	60	415
GH3	CTCTGCCTGCCCTGGACT	CTGGGTGTTCTGGATGGAG	60	404
GH4	CCATCCAGAACACCCAGGT	CCAAGCTGTTGGTGAAGACTC	62	417
GH5	CCTGCAGTTCCTCAGCAGA	GGGTGCCATCTTCCAGCT	59	417
GH6	CTTCGGCCTCTCTGTCTCTC	GCACTTCATGACCCTCAGGT	63	254

Note Tm = melting temperature, ^{o}C = Celsius degree and bp = base pair

Table 5 Primer sequences used for screening polymorphisms of the growth receptor hormone gene (*GHR*)

Fragment	Primer Forwards (5'⟶ 3')	Primer Reverse (5'⟶ 3')	Tm (^{o}C)	Size (bp)
GHR1	CTGACATTCTGGAGGCTGAT	GGACAGCTTGATGAGTTGAC	60	416
GHR2	CTGACATTCTGGAGGCTGAT	GGACAGCTTGATGAGTGGAC	55	404
GHR3	GCTAACTTCATCGTGGAC	ATTCATGCCCCAGCCAAC	55	414

Note Tm = melting temperature, ^{o}C = Celsius degree and bp = base pair

c. Master mix was prepared to be 14.1 µl per 1 reaction. Its composition were as follow
   - 4.50 µl of 10x Thermo Buffer
   - 1.80 µl of Primer Forwards (5 µM)
   - 1.80 µl of Primer Rewards (5 µM)
   - 1.20 µl of $MgCl_2$ (25 mM)
   - 0.60 µl of dNTPs (5 mM)
   - 7.08 µl of De-ionized H_2O
   - 0.12 µl of Taq DNA Polymerase (1 unit)

d. Thermal cyclers

3.4 Equipments and chemical substances used for detection by Single Strand Conformation Polymorphism (SSCP) were

a. Dye of SSCP (48.5% of formalmide, 0.005% of 1M NaoH, 0.025% of bromophenol blue, 0.025% of xylene cyanol and 51% of de-ionized H_2O)

b. Binding solution (5 µl bisilane and 5 µl glacial acetic acid to 1 ml of 95% ethanol)

c. 10% non-denaturing polyacrylamide gel (40% of acrylamide : bisacrylamide, 49:1 (%T = 40, %C = 2; T = Total monomer concentration, C = The weight percentage of crosslinker: 20ml, 10X TBE buffer 8 ml, de-ionized H_2O 52 ml)

d. N′, N′, N′, N′ - Tetra methyl ethylene diamine (TEMED) 80 µl

e. 10% ammonium persulfate 800 µl (ammonium persulfate 0.08 g and de-ionized H_2O 800 µl)

f. 1X TBE buffer

g. Sequi-gen ® GT nucleic acid vertical gel electrophoresis

h. Electrophoresis power supply

i. Straining solution

(a.) 10% acetic acid 2 liter (glacial acetic acid 200 ml and de-ionized H_2O 1800 ml)

(b.) De-ionized H_2O 2 liter

(c.) Silver solution (silver nitrate 4 g, de-ionized H_2O 2 liter and 37% formaldehyde 3 ml)

(d.) Developing solution (2.5% of sodium carbonate anhydrous, 0.02% of 37% formaldehyde and 0.0002% of sodium thiosulfate)

Methodology

1.1 Blood sampling

The whole blood samples were collected from 130 beef cattle for DNA extraction. From individual animal, 8 ml blood was collected from jugular vein using syringe with 18 gauze needle. The blood was then collected in 10 ml tubes containing EDTA used as anticoagulant. The blood sample after collection were kept on ice and DNA was extracted from sample as soon as possible.

1.2 DNA extraction

Genomic DNA was extracted from whole blood sample. The chilled blood was poured into a microcentrifuge tube. It was centrifuged at 3,000 rpm for 10 minutes and the supernatant was discarded. To lyse leukocytes, 600µl of lysis buffers was added to it and the mixture was incubated at 55-60 ^oC for 10 minutes. Proteinase K was added to the mixture and incubated for 2 hours at 55-60 ^oC. DNA was extracted by the combination of phenol : chloroform : isoamyl alcohol method (Sambrook *et al*., 1989). The solution was centrifuged at 3,000 rpm for 10 minutes and was separated. From the upper layer, the phenol was extracted; the centrifuged upper layer was then kept in a clean microcentrifuge tube. The DNA was precipitated by 95 percent of ethanol. The DNA pellet was washed with 70 percent of ethanol twice and was discarded 70 percent of ethanol. Pellets were air-dried for 20 minutes. The DNA sample was dissolved in 100 µl of TE buffer and was kept in water bath at 65 ^oC for 10 minutes. DNA samples were kept at -20^oC.

1.3 The following methods were used for checking qualitative and quantitative of DNA by agarose gel electrophoresis or spectrophotometry.

1.3.1 Concentration of DNA were determined by using agarose gel electrophoresis as follows

The gel apparatus was prepared end wall, comb and plastic gel tray. Microwave was used for melting 0.8 g of agarose with 100 ml of 1X TBE buffer. The solution was then checked for its complete melting and for homogenity. The warm gel was poured in the gel tray and allowed it to cool at room temp. After that 2 ml of DNA sample and 8 µl of tracking dye were loaded along with of molecular weight or lamda marker. The gel was allowed to run 1XTBE buffer at 100 volts for 1 hour until the bromophenol blue was found half the way down the gel. The agarose gel was then stained in ethidium bromide for 30 minutes followed by 10 minutes destaining in normal water. The gel was placed on UV transilluminator or gel document and DNA was determined using molecular weight marker.

1.3.2 Concentration of DNA were determined by using a spectrophotometer as follows

The spectrophotometer was turned on and kept for 5 minutes for getting warm up. Then 2 µl of DNA and 98 µl of H_2O were added in the quartz cuvette. The determination of A_{260} and A_{280} of DNA sample was carried out. The absorption of A_{260} and A_{280} were averaged. The DNA with A_{260}/A_{280} ratio between 1.8 and 1.9 were considered as good quality and can be used for further study. If the ration was found greater than 1.9, the sample was considered as RNA contamination and protein contamination when the ratio was found less than 1.8. The following equation was used for calculation of DNA concentration: DNA (µg/ml) = A260 x 50µg/ml x 50(dilution factor).

1.4 Amplification of DNA fragments

The PCR tubes or plate were kept in programmed thermocycler for amplification. The amplification was performed in a systematic manner. The first cycler was denatured at 94^{o}C for 3 minutes, followed by second cycle denaturation at 94 ^{o}C for 50 second. In third cycle, annealing in Tm-5 ^{o}C for 1 minutes was done depend on each primer. Then extension at 72 ^{o}C for 1 minute was carried out in fourth cycle. After that the cycle from second to fourth was repeated for 40 cycles and finally extension at 72 ^{o}C for 7 minutes was done. After that PCR products were kept at -20 ^{o}C.

The 3 µl of each PCR products were mixed 7 µl of tracking dye and electrophoresed on 2 percent of agarose gel. Gel was run in 1X TBE buffer at 100 volts about 30 second. Then, the agarose gel was stained in ethidium bromide about 15 minutes and destrained in water for about 10 minutes. The size of PCR product was visualized by UV fluorescence and compared with DNA marker (50 bp DNA ladder).

1.5 Detection of genetic polymorphisms

1.5.1 Pre-treatment of fragment of SSCP

The SSCP fragment was pre-treated by mixing of 2 µl of PCR product and 2µl of loading dye of SSCP. The mixture was denatured at 94 ^{o}C for 7 minutes. The samples were then snapping cooled on ice water for preventing heteroduplex formation and renaturation.

1.5.2 Preparing electrophoresis tray and gel

The glass plates (one large and one small) were cleaned in soap solution. The glass plates were then cleaned with 95 percent of ethanol to remove any lipid from the surface. The plates were cleaned with dust free tissue. The small glass plate was wiped with 1 ml binding solution for fixing gel into the glass plates. Two plates of different size were put together separated with plastic spacers. Then the electrophoresis tray was checked the alignment of bottom of edge of glass plates and fixed them with two clamps. Both the sandwich glass plates were then gel casting.

1.5.3 Preparing polyacrylamide gel solution

The 10 percent of non-denaturing polyacrylamide gel, TEMED and 10 percent of ammonium persulfate were mixed in a clean fat free beaker and stir well carefully to avoid air-bubbles. The gel solution was poured between two glass plates. The gel was allowed to polymerize for one and half hour at room temperature. Then, the complete unit was placed into the electrophoresis tray partly filled with 1XTBE buffer. The gel was allowed to run in 1XTBE buffer that was precooled to 4^{o}C. The power supply was adjusted at 10 watts and the gel was allowed to run for about 8 hours.

1.5.4 Staining SSCP gel

After electrophoresis was completed, the glass plates were removed with the gel from the tank. The gel with 10 percent acetic acid was fixed for 30 minutes at room temperature (approximately 24 to 26 ^{o}C) by gentle shaking. The gel was then incubated with distilled water at room temperature for 10 minutes each time for three times with gentle shaking. The gel was developed with developing solution at room temperature for about 25 minute by gentle shaking. Then, the developing reaction was stopped using 10 percent of acetic acid for at least 30 minutes at room temperature by gentle shaking. It was then rinsed with distilled water for at least 30 minutes at room temperature by gentle shaking. After that the small glass plate was set up with gel and kept at room temperature for dry it.

1.6 Classification genetic polymorphisms

The similar pattern of each region of both genes was combined in the same group. The difference between groups was identified by the genotypes. Every genotype was confirmed by sequencing. Genetic polymorphism considered two kinds such as single nucleotide polymorphisms (SNPs) and haplotypes. The SNPs is defined as that polymorphism (variation in sequence between individuals) caused by a change in a single nucleotide. This is responsible for most of the genetic variation between individual. Haplotype is defined as the particular combination of genotypes in a defined region of the gene (Zaid *et al*., 1999). The following alphabetal symbols of genotype of SNPs were used in this study. The following numerical symbols were used for the *GH* and *GHR* haplotypes. The numbers were represented by polymorphism SNPs in each fragment.

1.7 Data Analysis

Measuring allelic and genotypic frequencies were calculated as describe by Falconer and Mackay (1996). Allele and genotypic frequencies were found by adding up the number of copies of each allele or each genotype in the population and expressing it as a frequency. These frequencies were compared by chi-square test between genotypes and alleles (SAS, 1996).

$$\text{Allelic frequency} = \frac{\text{The number of copies of an interested allele in the population}}{\text{The total number of copies of all alleles at a locus in the population}}$$

$$\text{Genotypic frequency} = \frac{\text{The number of copies of interested genotype in the population}}{\text{The total number of copies of all genotypes at a locus in the population}}$$

Trial II

Effect of genetic polymorphisms of growth hormone and growth hormone receptor genes on preweaning growth traits in a multibreed beef population

Data Structure

Data for this trial were received from the two imformations. The initial information was preweaning growth traits such as birth weight (BW) and weaning weight (WW) These traits were collected from existing animals at BPRDC. Data consisted of 130 records collected from 1987 to 2004 for BW and WW. The animals were withdrawn blood sample for detection genetic polymorphism of growth hormone and growth hormone receptor genes by PCR-SSCP technique in trial I. Also, these molecular informations such as SNPs and haplotypes were used in this trial. The growth performance and molecular informations were merged for data analysis in trial II.

Methodology

1. Factor influencing for the traits

The possible effects found in available in the dataset and preliminary literature review. Data were classified into breed groups, sex, and contemporary groups (year-season of birth). Breed groups consisted of six breed groups such as 1/2CH1/4BR1/4NA, 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA and other breed groups (CH<1/2, CH=1/2 and CH>1/2). Sex was identified male and female. The performance of BW and WW in each sex and breed group are represented in Table 3 (page 14). Season of birth was recorded as winter (November to February), summer (March to June) and rainy (July to October). The interested traits were BW and WW. The possible contemporary groups were found to be 33 levels. Furthermore, effect of SNPs and haplotypes were included in model.

2. Model testing

The effects in the model were assumed to be fixed effects, except for the residual term that was assumed to be normally, identically distributed with mean zero and a common variance. Least square analyses of variance were conducted for each traits using generalized linear model (GLM) procedure of Statistic Analysis System (SAS, 1996).

The statistical models for determination of BW and WW composed of two models. The first fitted model included fixed effects of BW and WW were breed group, sex, and contemporary group (year-season of birth). The genetic fixed effect was SNPs effect. The second model consisted of the similar fixed effects but the genetic fixed effect was haplotypic effect.

2.1 The first model: The effect of SNPs on both the traits

$$y_{ijklmn} = \mu + GH_i + GHR_j + CG_k + Sex_l + BG_m + e_{ijklmn}$$

The terms in the models were:

y_{ijklmn} = individual performance for each trait
μ = the overall means
GH_i = the fixed effect of genotypes of growth hormone gene polymorphic sites
GHR_j = the fixed effect of genotypes of growth hormone receptor gene polymorphic sites
CG_k = the fixed effect of contemporary groups (year-season of birth) (k = 1, 2, …, 33)
Sex_l = the fixed effect of sex (l = male and female)
BG_m = the fixed effect of breed groups (m =1, 2, …, 6)
e_{ijklmn} = random residual effect ($e_{ijklmn} \sim NID (0, \sigma^2_e)$)

2.2 The second model: The effect of haplotypes on both the traits

$$y_{ijklm} = \mu + H_i + CG_j + Sex_k + BG_l + e_{ijklm}$$

The terms in the models were:

y_{ijklm} = individual performance for each trait
μ = the overall means
H_i = the fixed effect of haplotypes (i = pattern combinations of polymorphism growth hormone and growth hormone receptor genes)
CG_j = the fixed effect of contemporary groups (year-season of birth) (j = 1, 2, …, 33)
Sex_k = the fixed effect of sex (k = male and female)
BG_l = the fixed effect of breed groups (l =1, 2, …, 6)
e_{ijklm} = random residual effect ($e_{ijklm} \sim NID (0, \sigma^2_e)$)

3. Least square means comparison

Least square means of BW and WW per different SNPs or haplotypes and comparison among SNPs or haplotypes were obtained using LSMEANS statement and PDIFF option of PROC GLM (SAS, 1996). Significant effects were considered at 95 and 99 percents of confidence interval.

Trial III

Comparison for the best fit model among models with SNPs, regression of allelic, haplotypic effects and without molecular information

Data Structure

The mean square error (MSE) coefficient of determination (R^2) and log likelihood (log L) and accuracy of prediction (ACC) were considered for the best fit model between model with and without molecular information. This trial composed of three informations. The first information was growth performance such as birth weight (BW) and weaning weight (WW) of individual animal. The second information was the fixed and random effects affecting for both traits. The fixed effects were breed groups, sexes and contemporary groups (year-season of birth). The random effects were direct genetic effect, maternal genetic effect and random residual error effects. The last information was the genetic fixed effect such as molecular information of growth hormone and growth hormone receptor genes. This information was that getting from the analyses results from trial I. Molecular information was divided into three types: SNPs, regression of allelic and haplotypic effects. Details of data structure form a multibreed population at BPRDC for this trial are summarized in Table 6.

Table 6 Data structure of birth weight (BW) and weaning weight (WW)

Traits	BW	WW
No. of animal with records	130	130
No. of animals	238	238
No. of sire	35	35
No. of dam	73	73
No. of cg[a]	33	33
No. of SNPs	14	14
No. of haplotypes	23	23
Mean±SD (kg)	28.32±5.69	166.00±38.70
Range (kg)	16.00 to 45.00	85.00 to 285.00

Note [a] cg = contemporary groups (year-month of birth)

Methodology

1.1 Testing among models with and without molecular information (SNPs, regression of allelic and haplotypic effects) by considering MSE, R^2 and log L from SAS (1996)

Comparison among four models was determined for MSE, R^2 and log L. The assumption in this study was ignoring relationship among animals. Moreover, the assumption of random residual effect was normally independently distributed. The first and second moments were zero and σ^2_e. In addition, if MSE of one model was found lower than another model, it indicates that the model was the best for genetic evaluation. The R^2 can be used to explain the variability of predicted value to total variation. The R^2 ranged from 0 to 1. If it closes to one, it will determine the good model (Carlson and Thorne, 1997). Therefore, The MSE or residual variance and R^2 were that received from PROC GLM. The log L was received from PROC MIXED.

1.2 Model testing among models with and without molecular information (SNPs, regression of allelic and haplotypic effects) by considering the log L and ACC from ASREML software (Gilmour *et al*., 2001)

Univariate and bivariate animal model were fitted to the data including all pedigree information. The assumption for fixed effect testing of this methodology was accounting relationship among animals. Also, the pedigree information of the 130 animals was included for estimation of log L and ACC. Moreover, the assumption of random residual effect was identically independently distributed. The first and second moments were zero and σ^2_e. The log L was received from an animal model throughout using ASREML software (Gilmour *et al*., 2001).

The ACC of direct genetic effect for BW and WW was calculated from standard error (SE) from direct genetic effect of individual animal and direct additive genetic variances (σ^2_a) and the ACC of maternal genetic effect was calculated from standard error (SE) from maternal genetic effect of individual animal and maternal genetic variances (σ^2_m)

Accuracy of prediction was calculated by the following equations (Henderson, 1975).

Accuracy (ACC) of direct genetic effect of individual animal $= \sqrt{1 - \frac{PEV}{\sigma_a^2}} = \sqrt{1 - \frac{(SE)^2}{\sigma_a^2}}$

Accuracy (ACC) of maternal genetic effect of individual animal $= \sqrt{1 - \frac{PEV}{\sigma_m^2}} = \sqrt{1 - \frac{(SE)^2}{\sigma_m^2}}$

The SE of individual animals was received from univariate and bivariate analyses with mixed model equation by BLUP from ASREML (Gilmour *et al*., 2001). The means accuracy of prediction among three kinds of model with molecular information and model without molecular imformations of individual animals was compared by using option PDIFF from PROC GLM (SAS, 1996).

The four statistical model for analysis by SAS and for univariate analysis are as follow:

a. The first model did not accounted for molecular information.

$$y_{ijklmn} = \mu + CG_i + Sex_j + BG_k + A_l + D_m + e_{ijklmn}$$

The terms in the models were:

y_{ijklmn} = individual performance for each trait
μ = the overall means
CG_i = the fixed effect of contemporary groups (year-season of birth) (i = 1, 2, …, 33)
Sex_j = the fixed effect of sex (j = male and female)
BG_k = the fixed effect of breed groups (k = 1, 2, …, 6)
A_l = the random effect of direct additive genetic effect of animal (l = 1, 2, …, 130)
D_m = the random effect of maternal genetic effect of dam (m = 1, 2, …, 73)
e_{ijklmn} = random residual effect ($e_{ijklmn} \sim NID(0, \sigma^2_e)$)

b. The second model accounted for SNPs effect.

$$y_{ijklmnop} = \mu + GH_i + GHR_j + CG_k + Sex_l + BG_m + A_n + D_o + e_{ijklmnop}$$

The terms in the models were:

$y_{ijklmnop}$ = individual performance for each trait
μ = the overall means
GH_i = the fixed effect of genotypes of growth hormone gene polymorphic sites
GHR_j = the fixed effect of genotypes of growth hormone receptor gene polymorphic sites
CG_k = the fixed effect of contemporary groups (year-season of birth) (k = 1, 2, …, 33)
Sex_l = the fixed effect of sex (l = male and female)
BG_m = the fixed effect of breed groups (m = 1, 2, …, 6)
A_n = the random effect of direct additive genetic effect of animal (n = 1, 2, …, 130)
D_o = the random effect of maternal genetic effect of dam (o = 1, 2, …, 73)
$e_{ijklmnop}$ = random residual effect ($e_{ijklmnop} \sim NID\ (0, \sigma^2_e)$)

c. The third model accounted for regression of allelic effect.

$$y_{ijklmn} = \mu + CG_i + Sex_j + BG_k + b_1 GH_{ijklmn} + b_2 GHR_{ijklmn} + A_l + D_m + e_{ijklmn}$$

The terms in the models were:

y_{ijklmn} = individual performance for each trait
μ = the overall means
CG_i = the fixed effect of contemporary groups (year-season of birth) (i = 1, 2, …, 33)
Sex_j = the fixed effect of sex (j = male and female)
BG_k = the fixed effect of breed groups (k = 1, 2, …, 6)
GH_{ijklmn} and GHR_{ijklmn} = a number of copies of each allele of growth hormone and growth hormone receptor genes present in an individual
b_1 and b_2 = the regression coefficients which correspond to allelic effect
A_l = the random effect of direct additive genetic effect of animal (l = 1, 2, …, 130)
D_m = the random effect of maternal genetic effect of dam (m = 1, 2, …, 73)
e_{ijklmn} = random residual effect ($e_{ijklmn} \sim NID\ (0, \sigma^2_e)$)

d. The fourth model accounted for haplotypic effect

$$y_{ijklmno} = \mu + H_i + CG_j + Sex_k + BG_l + A_m + D_n + e_{ijklmno}$$

The terms in the models were:

$y_{ijklmno}$ = individual performance for each trait
μ = the overall means
H_i = the fixed effect of haplotypes (i = pattern combinations of polymorphism growth hormone and growth hormone receptor genes)
CG_j = the fixed effect of contemporary groups (year-season of birth) (j = 1, 2, …, 33)
Sex_k = the fixed effect of sex (k = male, female)
BG_l = the fixed effect of breed groups (l = 1, 2, …, 6)
A_m = the random effect of direct additive genetic effect of animal (m = 1, 2, …, 130)
D_n = the random effect of maternal genetic effect of dam (n = 1, 2, …, 73)
$e_{ijklmno}$ = random residual effect ($e_{ijklmno} \sim NID (0, \sigma^2_e)$)

Trial IV

Estimation of genetic parameters for preweaning growth traits with adjusted by molecular information of growth hormone and growth hormone receptor genes and comparison prediction of breeding values for these traits between model with and without molecular information in a multibreed beef population

Data structure

Data of this trial consisted of 130 records belonging to the defined six breed groups (1/2CH1/4BR1/4NA, 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA, CH<1/2, CH=1/2 and CH>1/2. The data structure composed of three informations. The first information was pedigree information. The number of sire and dam for estimation were found to be 15 and 31 percents from total evaluated animals. The data of those animals for this analysis must birth after theirs offspring and having proper sex records. The second information was preweaning growth performance such as birth weight (BW) and weaning weight (WW). The age at weaning weight data of beef cattle that ranged from 180 to 240 days. The last information was the best-fit molecular information of growth hormone and growth hormone receptor genes. The molecular information was received from trial I. The best-fit molecular information among three types: SNPs, regression of allelic and haplotypic effects for accounted in the model was decided from trial III. These informations were applied for evaluation in this trial.

1.1 Model for genetic evaluation

Bivariate analysis was incorporated in all pedigree information available. The effects were divided into 3 types: fixed, random genetic (direct and maternal genetic effect) and random residual error effects. Genetic evaluation model require the construction of fixed effect such as contemporary groups (year-month of birth), sex, breed groups and the best fit molecular information of growth hormone and growth hormone receptor genes. Unfortunately, preliminary analyses showed that sex was unfeasible in both BW and WW. Effect of contemporary groups was significant for only BW and effect of breed groups was significant for only WW.

The random effects for BW and WW were direct genetic, maternal genetic and residual effect. The maternal genetic effect was included in bivariate animal model because this effect represented mainly the dam's milk production and mothering ability, through effects of the uterine environment and extra-chromosomal inheritance may contribute (Meyer, 1992). However, the cows that produced offspring more than one were a few records (5 percent of evaluated animals). In addition, the maternal permanent environmental effect was close to zero from the preliminary univariate analysis. Therefore, this effect was not included in the bivariate animal model.

1.2 Estimation of covariance components

Covariance components were estimated by restriction maximum likelihood (REML) using the average information (AI) and fitting an animal model throughout using ASREML software (Gilmour *et al*., 2001). The information or prior values for estimation of (co)variance in bivariate analysis values were those getting from univariate analysis in the same data set.

The used models were two-trait (BW and WW) animal models. Each trait was assumed to have direct and maternal genetic effects. Fixed genetic effect was the best fit molecular information of growth hormone and growth hormone receptor genes and fixed environmental effects were breed groups, sex and contemporary groups. The random effects in these models were additive genetic, maternal genetic and residual effects.

The bivariate animal model used is as follows:

$$\begin{bmatrix} y_{BW} \\ y_{WW} \end{bmatrix} = \begin{bmatrix} X_{BW} & 0 \\ 0 & X_{WW} \end{bmatrix} \begin{bmatrix} \beta_{BW} \\ \beta_{WW} \end{bmatrix} + \begin{bmatrix} Z_{BW} & 0 \\ 0 & Z_{WW} \end{bmatrix} \begin{bmatrix} a_{BW} \\ a_{WW} \end{bmatrix} + \begin{bmatrix} M_{BW} & 0 \\ 0 & M_{WW} \end{bmatrix} \begin{bmatrix} m_{BW} \\ m_{WW} \end{bmatrix} + \begin{bmatrix} e_{BW} \\ e_{WW} \end{bmatrix}$$

The terms in the models were: y is an N x 1 vector of observation; X is an incidence matrix relating the fixed effects (β) to the vector of observation, Z is an incidence matrix relating the vector of direct genetic effect (a) to observation, M is an incidence matrix relating the vector of maternal genetic effect (m) to observation and e is the vector of residual error effects associated with y.

The first and second moments for above model was assumed as follows:

$$E\begin{bmatrix} y_{BW} \\ y_{WW} \end{bmatrix} = \begin{bmatrix} X\beta_{BW} \\ X\beta_{WW} \end{bmatrix}$$

$$V\begin{bmatrix} a_{BW} \\ a_{WW} \\ m_{BW} \\ m_{WW} \\ e_{BW} \\ e_{WW} \end{bmatrix} = \begin{bmatrix} A\sigma^2_{a_{BW}} & A\sigma_{a_{BW}a_{WW}} & A\sigma_{a_{BW}m_{WW}} & A\sigma_{a_{BW}m_{WW}} & 0 & 0 \\ A\sigma_{a_{BW}a_{WW}} & A\sigma^2_{a_{WW}} & A\sigma_{a_{WW}m_{BW}} & A\sigma_{a_{WW}m_{WW}} & 0 & 0 \\ A\sigma_{a_{BW}m_{WW}} & A\sigma_{a_{WW}m_{BW}} & A\sigma^2_{m_{BW}} & A\sigma_{m_{BW}m_{WW}} & 0 & 0 \\ A\sigma_{a_{BW}m_{WW}} & A\sigma_{a_{WW}m_{WW}} & A\sigma_{m_{BW}m_{WW}} & A\sigma^2_{m_{WW}} & 0 & 0 \\ 0 & 0 & 0 & 0 & I\sigma^2_{e_{BW}} & I\sigma_{e_{BW}e_{WW}} \\ 0 & 0 & 0 & 0 & I\sigma_{e_{BW}e_{WW}} & I\sigma^2_{e_{WW}} \end{bmatrix}$$

In the (co)variance structures, A was the numerator relationship matrix among animals in the pedigree file, I was identity matrix, σ^2_a, σ^2_m, and σ^2_e were variances due to direct genetic, maternal genetic and residual effects, respectively; σ_{am} was a direct-maternal covariance genetic effects; σ_{aa} was a covariance of direct genetic effect between traits; σ_{mm} was a covariance of maternal genetic effect between traits and σ_{ee} was a covariance of random residual effect between traits.

1.3 Estimation of direct and maternal heritabilities

Estimated variance components such as direct genetic variance, the maternal genetic variance and the phenotypic variance were calculated for heritability. The direct heritability (h^2_a) and maternal heritability (h^2_m) were calculated from formula given by Falconer and Mackay (1996).

$$h^2_a = \frac{\sigma^2_a}{\sigma^2_p} \quad , \qquad h^2_m = \frac{\sigma^2_m}{\sigma^2_p}$$

1.4 Estimation of direct, maternal and phenotypic correlations

Estimated variance components and covariances between traits were calculated as direct genetic correlation (r_{aBWaWW}), maternal genetic correlation (r_{mBWmWW}), direct-maternal genetic correlation within the traits (r_{aBWmBW} and r_{aWWmWW}), direct-maternal correlation between traits (r_{aBWmWW} and r_{aWWmBW}) and phenotypic correlation (r_{pBWpWW}) from the equation (Falconer and Mackay, 1996)

$$r_{a_{BW}a_{WW}} = \frac{\mathrm{cov}\, a_{BW}a_{WW}}{\sqrt{\sigma^2_{a_{BW}}\sigma^2_{a_{WW}}}} \qquad r_{m_{BW}m_{WW}} = \frac{\mathrm{cov}\, m_{BW}m_{WW}}{\sqrt{\sigma^2_{m_{BW}}\sigma^2_{m_{WW}}}}$$

$$r_{a_{BW}m_{BW}} = \frac{\mathrm{cov}\, a_{BW}m_{BW}}{\sqrt{\sigma^2_{a_{BW}}\sigma^2_{m_{BW}}}} \qquad r_{a_{WW}m_{WW}} = \frac{\mathrm{cov}\, a_{WW}m_{WW}}{\sqrt{\sigma^2_{a_{WW}}\sigma^2_{m_{WW}}}}$$

$$r_{a_{BW}m_{WW}} = \frac{\mathrm{cov}\, a_{BW}m_{WW}}{\sqrt{\sigma^2_{a_{BW}}\sigma^2_{m_{WW}}}} \qquad r_{a_{WW}m_{BW}} = \frac{\mathrm{cov}\, a_{WW}m_{BW}}{\sqrt{\sigma^2_{a_{WW}}\sigma^2_{m_{BW}}}}$$

$$r_{p_{BW}p_{WW}} = \frac{\mathrm{cov}\, p_{BW}p_{WW}}{\sqrt{\sigma^2_{p_{BW}}\sigma^2_{p_{WW}}}}$$

The terms in the equation are:

r_{aBWaWW} = direct genetic correlation between BW and WW
r_{mBWmWW} = maternal genetic correlation between BW and WW
r_{aBWmBW} = direct-maternal genetic correlation of BW
r_{aWWmWW} = direct-maternal genetic correlation of WW
r_{aBWmWW} = correlation between direct genetic of BW and maternal genetic of WW
r_{aWWmBW} = correlation between direct genetic of WW and maternal genetic of BW
r_{pBWpWW} = phenotypic correlation between BW and WW
cov_{aBWaWW} = covariance of direct genetic between BW and WW
cov_{mBWmWW} = covariance of maternal genetic between BW and WW
cov_{aBWmBW}
cov_{aWWmWW}

cov_{aBWmWW} = covariance of direct-maternal genetic of BW
cov_{aWWmBW} = covariance of direct-maternal genetic of WW
cov_{pBWpWW} = covariance of direct genetic of BW and maternal genetic of WW
$\sigma^2_{a_{BW}}, \sigma^2_{a_{WW}}$ = covariance of direct genetic of WW and maternal genetic of BW
$\sigma^2_{m_{BW}}, \sigma^2_{m_{WW}}$ = covariance of phenotype between BW and WW
$\sigma^2_{p_{BW}}, \sigma^2_{p_{WW}}$ = direct genetic variances of BW and WW
= maternal genetic variances of BW and WW
= phenotypic variances of BW and WW

1.5 The direct estimated breeding values (EBV_a) and maternal breeding values (EBV_m)

The EBV_a and EBV_m were obtained by solving the mixed model equations using the estimated variance components obtained at convergence. The both EBVs were calculated means, standard deviation, maximum and minimum values by PROC MEANS (SAS, 1996).

1.6 Comparison of ranking on estimated breeding values between the models with and without the best fit molecular information.

Comparison of ranking on EBVs was analyzed by Spearman rank correlations. The direct estimated breeding values (EBV_a) and maternal breeding values (EBV_m) were compared between model with the best fit molecular information and without molecular information by using PROC CORR and option SPEARMAN (SAS, 1996). The rank correlations of EBV_a and EBV_m between models with and without molecular information were divided into two groups:

1.6.1 All animals in each breed group had the records.
1.6.2 top 50 percent of top animals in each breed group. They would be candidates to be selected in order to produce replacement animals.

1.7 Means comparison for EBV_a and EBV_m of model with the best fit molecular information between sort through the model with and without the best fit molecular information.

The objective to study means comparison for EBV_a and EBV_m of model with the best fit molecular information between sort through the model with and without the best fit molecular information was to evaluate genetic progress when accounted in the best fit molecular information in the model. Both EBVs (EBV_a and EBV_m) of model with the best fit molecular information from bivariate analysis were sorted highest to lowest and the means were calculated from 50 percent of the top data in each breed group. These EBVs from model with the best fit molecular information were sort through ranking of the model without molecular information and means were calculated for 50 percent of the highest EBVs of data in each breed group. Means EBVs of model with the best fit molecular information from 2 methods of sorting were compared by using PROC TTEST (SAS, 1996).

RESULTS

Trial I

Genetic polymorphism of growth hormone and growth hormone receptor genes in a multibreed beef population

1. Single Nucleotide Polymorphisms (SNPs) of growth hormone gene

Amplification in six growth hormone PCR product fragments (*GH1* to *GH6*) of individual beef cattle in this population was covering almost the entire length of growth hormone gene. The result of PCR technique indicated that the six fragments of growth hormone gene had similar patterns (Appendix Figure 1). Therefore, the single strand conformation polymorphism (SSCP) method was used for detection a DNA polymorphism when the electrophoresis mobility of DNA bands was reproducibly different. A PCR product was subjected to SSCP analysis to discover the DNA sequence variation. The position and length of the fragments are illustrated in Figure 4.

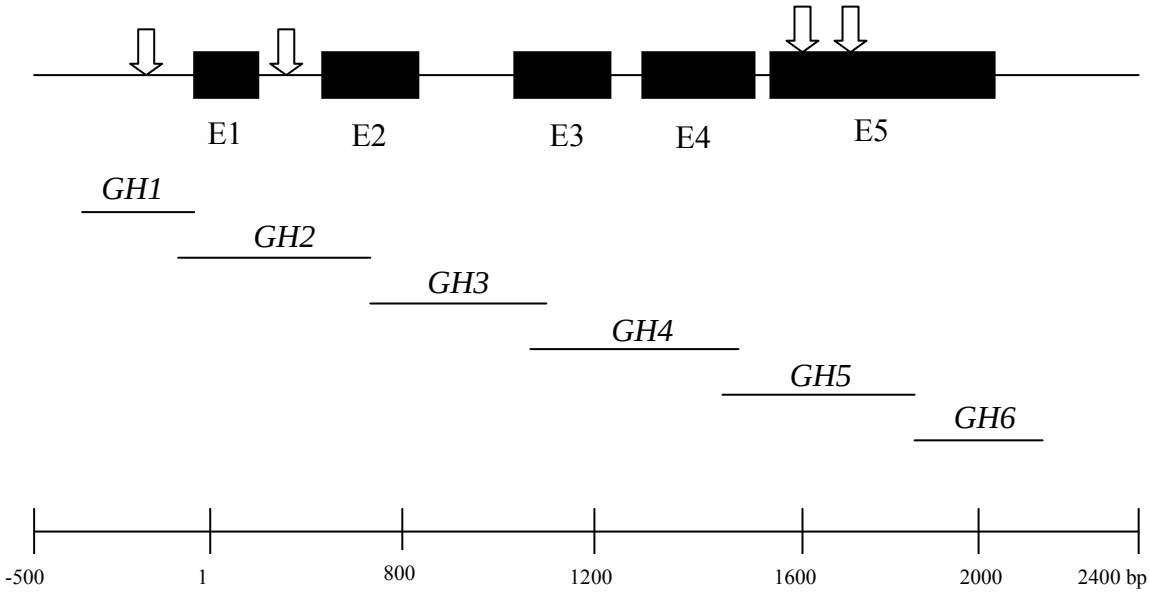


Figure 4 Map of the growth hormone gene with the position and length of the growth hormone fragments. The arrowheads indicate the location of the polymorphisms in this study.

Note The E1 to E5 represent the first to fifth exons and *GH1* to *GH6* represent PCR product of the first to sixth fragments, respectively.

The *GH1*, *GH2* and *GH5* fragments were detected polymorphic within the cattle population that was analyzed. However, the *GH3*, *GH4* and *GH6* fragments were found monomorphic.

The GH1 fragment was identified as three different SSCP patterns (Figure 5).

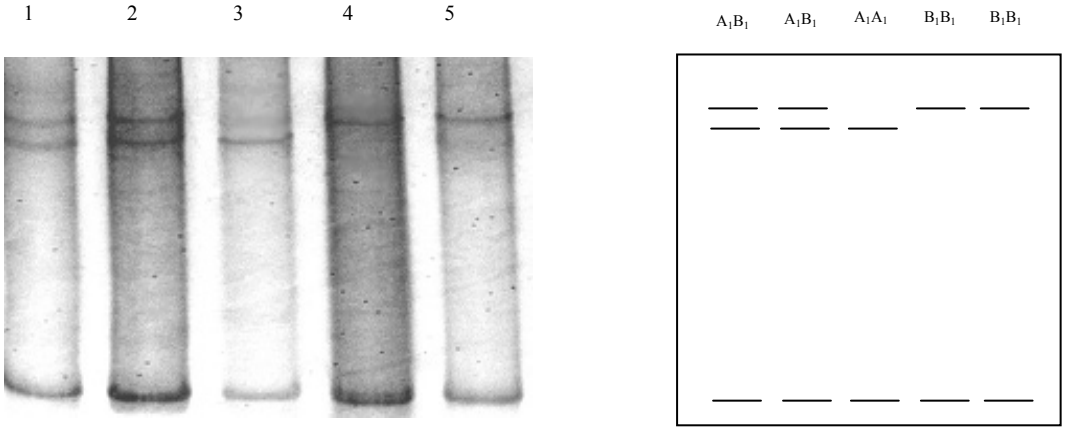


Figure 5 The different SSCP patterns of *GH1*. Lanes 3 showed the pattern 1 (A_1A_1). Lane 4 and 5 showed pattern 2 (B_1B_1) and lane 1 and 2 showed pattern 3 (A_1B_1).

The *GH1* fragment revealed three genotypes. The heterozygous (A_1B_1) presented three distinct bands. It indicated that only one strand of an allele had a different conformation with an unlike electrophoretic mobility. The sequence analysis proved the difference in mobility, which had A to C transversion in the promoter region at nucleotide position -303. The sequence at this position of genotype A_1A_1 was found to be base A. The genotype B_1B_1 was mutated by substitution A to C. The heterozygous of A_1B_1 consisted of base A from A_1A_1 and base C from B_1B_1 at nucleotide position -303. These could be designated as A_1 and B_1 alleles, respectively.

The *GH1* fragment was identified in 7, 111 and 12 heads with A_1A_1, A_1B_1 and B_1B_1 genotypes, respectively. Genotypic frequencies of A_1A_1, A_1B_1 and B_1B_1 were found to be 5.40, 85.40 and 9.20 percents, respectively. It indicated that genotypic frequency of A_1B_1 animals was found to be highest ($P<0.01$) on an average proportion in the population. The appearance of different GH1 fragments genotypes in the both sexes is presented in Table 7. Genotype A_1B_1 appeared to be the most frequent in both sexes. However, A_1A_1 genotype was not detected in bulls. The allelic frequency of A_1 and B_1 in a population was almost close to 48.08 and 51.92 percent. The B_1 allele was found slightly lower than allele A_1 in male but B_1 was slightly higher than allele A_1 in female.

Table 7 Genotypic and allelic frequencies of *GH1* in the multibreed beef population

Animals		Genotypes			Alleles	
		A_1A_1	A_1B_1	B_1B_1	A_1	B_1
Total	n	7^a	111^b	12^a		
(n=130)	%	5.40	85.40	9.20	48.08^a	51.92^a
Male	n	-	29^a	6^b		
(n=35)	%	-	82.90	17.10	41.43^a	58.57^a
Female	n	7^a	82^b	6^a		
(n=95)	%	7.40	86.30	6.30	50.53^a	49.47^a

Note [a] and [b] within the same row values marked with the different letter are significantly different at $P < 0.01$

The *GH2* fragment was detected three as different SSCP patterns (Figure 6).

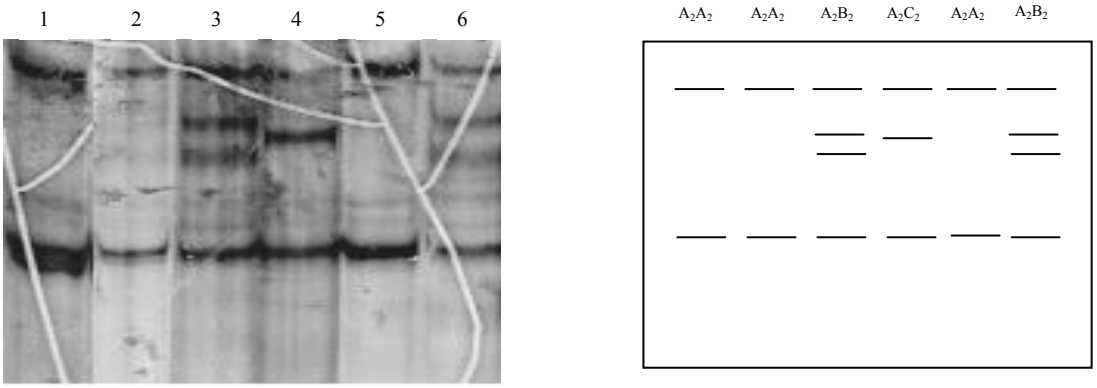


Figure 6 The different SSCP patterns of *GH2*. Lanes 1, 2 and 5 showed SSCP pattern 1 (A_2A_2). Lanes 3 and 6 showed SSCP pattern 2 (A_2B_2) and lane 4 showed pattern 3 (A_2C_2).

Figure 6 shows the SSCP pattern of *GH2* fragment of the gene. Three alleles were observed and designated as A_2, B_2 and C_2, respectively. The mutation at nucleotide position 670 was T to G transversion and T to C transition. The A_2A_2 genotype had base T at nucleotide position 670. The A_2B_2 heterozygous genotype occurred that one strand changed base T to G but the A_2C_2 heterozygous genotype was found substituted as one strand from base T to C at the same position. Furthermore, three alleles that were observed from genotypes were designated as A_2, B_2 and C_2, respectively.

Genotypic and allelic frequencies of *GH2* are reflected in Table 8. The A_2A_2, A_2B_2 and A_2C_2 genotypes were found in 117, 11 and 2 heads, respectively. It indicated that genotypic frequency of A_2C_2 was lowest when compared with A_2A_2 and A_2B_2. Genotypes A_2A_2 of both sexes were the most frequent. Genotype A_2C_2 was detected in a low frequency in bulls but it was not detected in cows. Allelic frequency of A_2 was found to be 0.95. It was higher than B_2 alleles in both sexes but C_2 genotype was absent.

Table 8 Genotypic and allelic frequencies of *GH2* in the multibreed beef population

Animals		Genotypes			Alleles		
		A_2A_2	A_2B_2	A_2C_2	A_2	B_2	C_2
Total	N	117[a]	11[b]	2[c]			
(n=130)	%	90.00	8.50	1.50	95.00[a]	4.23[b]	0.77[b]
Male	N	30[a]	3[b]	2[b]			
(n=35)	%	85.70	8.60	5.70	92.86[a]	4.29[b]	2.85[b]
Female	N	87[a]	8[b]	-			
(n=95)	%	91.60	8.40	-	95.79[a]	4.21[b]	-

Note [a] and [b] within the same row values marked with the different letter are significantly different at $P < 0.01$

The pattern of *GH3* and *GH4* PCR fragments are represented in Figure 7. These fragments were found monomorphic because every band was similar locations. It indicated that DNA sequence of *GH3* and *GH4* in this multibreed population were similar. Therefore, these two regions were not used to identify the different animal.

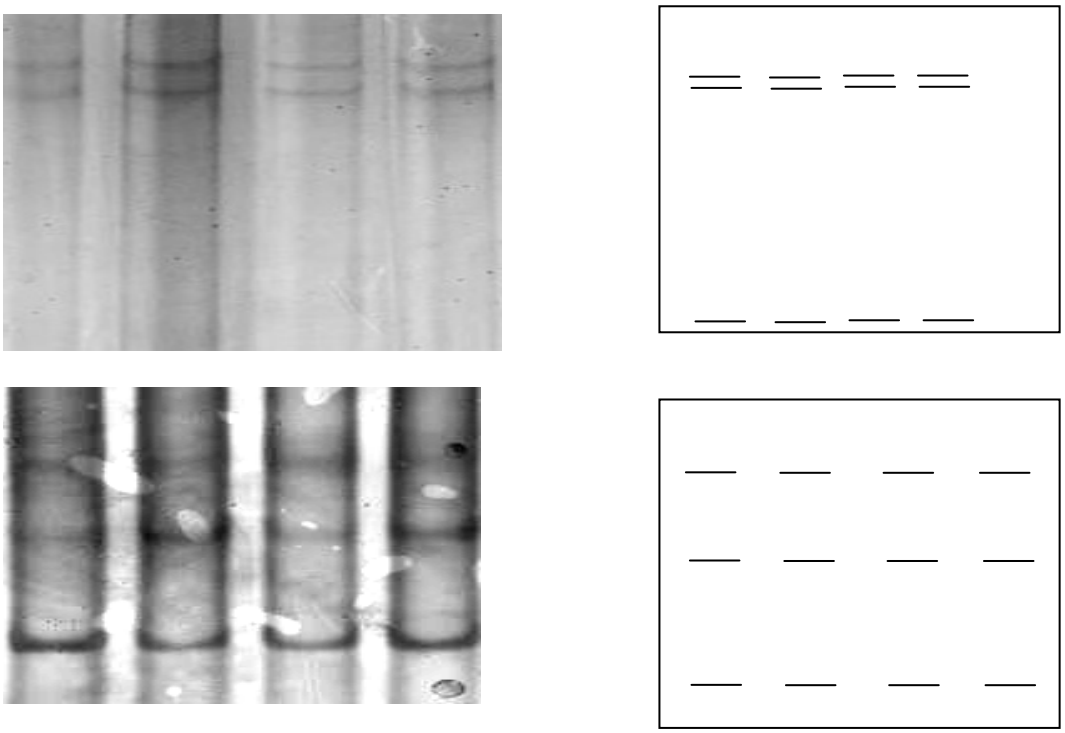


Figure 7 The similar SSCP patterns for *GH3* (above) and *GH4* (below).

The *GH5* fragment was identified as five different SSCP patterns (Figure 8).

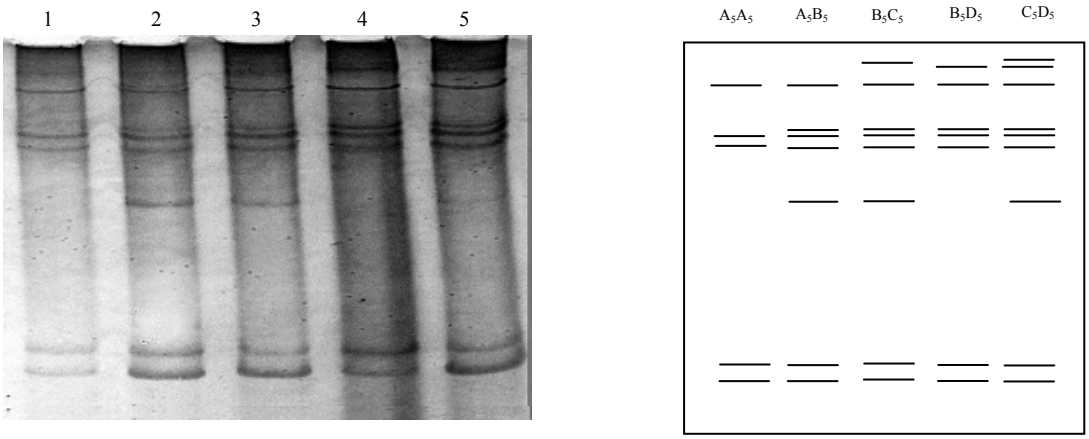


Figure 8 The different SSCP patterns of *GH5*. Lanes 1, 2, 3, 4 and 5 represent SSCP pattern 1, 2, 3, 4 and 5. They are represented with genotypes A_5A_5, A_5B_5, B_5C_5, B_5D_5 and C_5D_5, respectively.

The *GH5* region harboured two mutation points at fifth exon of growth hormone gene. The first position of mutation had G to C transversion at nucleotide position 2141. Moreover, the amino acid was changed in the peptide from valine to leucine. The second position of mutation had G to C and G to T transversions at nucleotide position 2354. Therefore, an amino acid at the second position was mutated from tryptophane to cystein for G to C transversion and was mutated from tryptophane to proline for G to T transversion.

The genotype A_5A_5 had base G and C at nucleotide position 2141 and 2354, respectively. The A_5B_5 heterozygous genotype was found mutated as one strand by substitution G to C at nucleotide position 2141. Likewise, the B_5C_5 heterozygous genotype was found mutated as one strand by substitution G to C and C to G at nucleotide position 2141 and 2354, respectively. Beside, the B_5D_5 heterozygous genotype was found mutated as one strand by substitution G to C and C to T at nucleotide position 2141 and 2354, respectively. Additionally, the C_5D_5 heterozygous genotype was found mutated as one strand by substitution G to T at nucleotide position 2354. Further, four alleles that were observed from genotypes were designated as A_5, B_5, C_5 and D_5, respectively.

The *GH5* fragment was identified in 3, 40, 23, 18 and 46 heads with A_5A_5, A_5B_5, B_5C_5, B_5D_5 and C_5D_5 genotypes, respectively. In multibreed beef population, the genotypic frequency of C_5D_5 was found to be higher than the others. The appearance of different *GH5* fragment genotypes in both sexes is presented in Table 9. The genotypic frequency of C_5D_5 in male was noted with the highest frequency. Genotypic frequency of A_5B_5 of female was higher than the other genotypes. The A_5A_5 genotype was the lowest. This genotype was not detected in bulls. Allelic frequency of B_5 appeared as the most frequent one in the population. It indicated that this multibreed beef population had B_5 allele animals more than the other alleles.

Table 9 Genotypic and allelic frequencies of *GH5* in the multibreed beef population

Animals		Genotypes					Alleles			
		A_5A_5	A_5B_5	B_5C_5	B_5D_5	C_5D_5	A_5	B_5	C_5	D_5
Total	N	3^a	40^b	23^c	18^c	46^b				
(n=130)	%	2.30	30.80	17.70	13.80	35.40	17.69^a	31.15^a	26.54^a	24.62^a
Male	N	-	6^a	6^a	6^a	17^b				
(n=35)	%	-	17.10	17.10	17.10	48.60	8.57^a	25.71^b	32.86^b	32.86^b
Female	N	3^a	34^b	17^{cd}	12^d	29^d				
(n=95)	%	3.20	35.80	17.90	12.90	30.50	21.05^a	33.16^a	24.21^a	21.58^a

Note $^{a, b, c}$ and ^{d} within the same row values marked with the different letter are significantly different at $P < 0.01$

The *GH6* PCR fragment showed monomorphic because all lanes had similar patterns. This region is illustrated in Figure 9. It indicated that the DNA sequences of animals in this region were similar. It only means that the primers could not detect polymorphism in this region. Also, this region would not used to identify animals in this population.

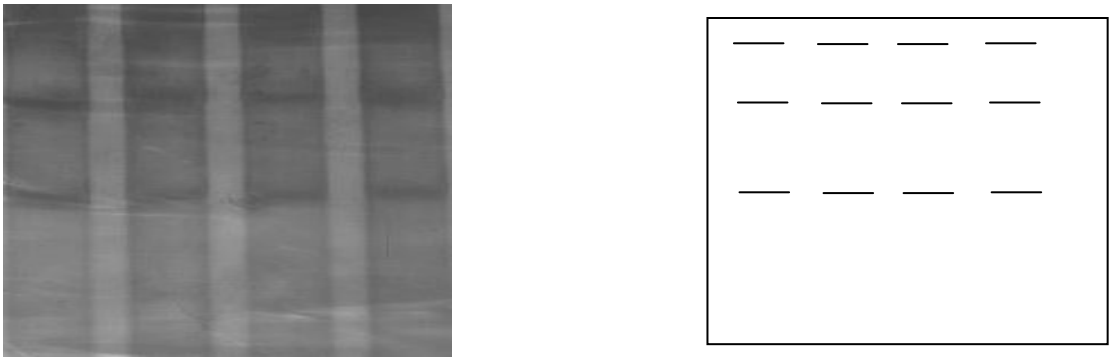


Figure 9 The similar SSCP patterns of *GH6*

2. SNPs of growth hormone receptor gene

Amplification in three growth hormone receptor PCR product fragments (*GHR1* to *GHR3*) of individual beef cattle in this population. The result of PCR technique indicated that the six fragments of growth hormone gene had similar patterns (Appendix Figure 2). Therefore, the SNPs in growth hormone receptor gene were analyzed in three regions from 4th exon to 10th exon by SSCP technique (Figure 10). There is because the characteristic of this gene was to fill the gap region from promoter region to 3th intron, so the complete sequences of this region were not reported.

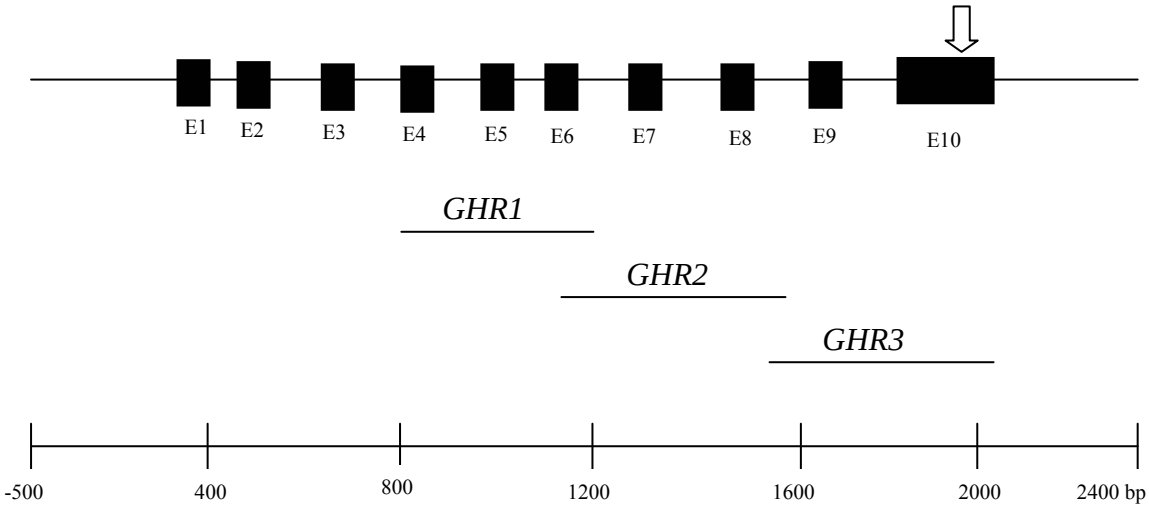


Figure 10 Map of the growth hormone receptor gene with the position and length of the growth hormone receptor fragments. The arrowheads indicate the location of the polymorphism.

Note The E1 to E10 represent first to tenth exons and *GHR1*, *GHR2* and *GHR3* represent PCR product of the first, second and third fragments, respectively.

The *GHR1* and *GHR2* fragments were monomorphic within this population (Figure 11). It indicated that animals in the population had similar pattern on *GHR1* and *GHR2*. Therefore, not only *GHR1* but also *GHR2* were not used to identify cattle in the population.

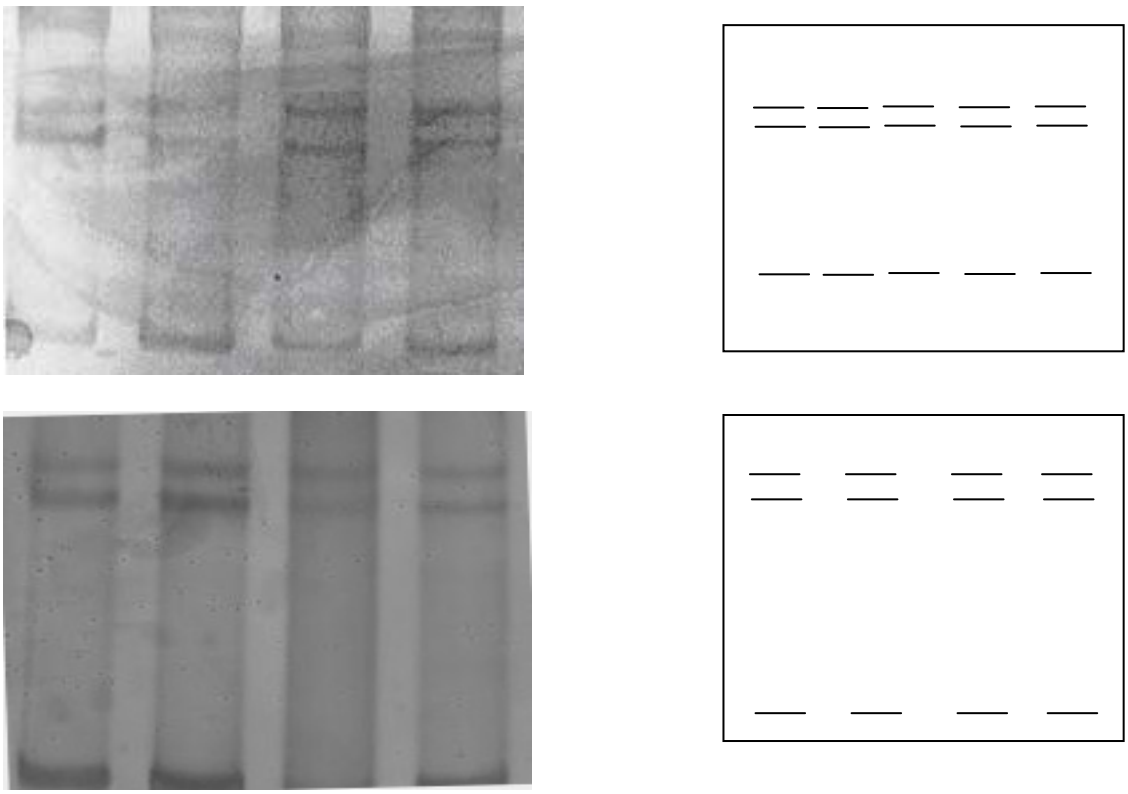


Figure 11 The similar SSCP patterns of *GHR1* (above) and *GHR2* (below).

Only *GHR3* fragment was found to be polymorphic. The *GHR3* were detected as three SSCP patterns (Figure 12).

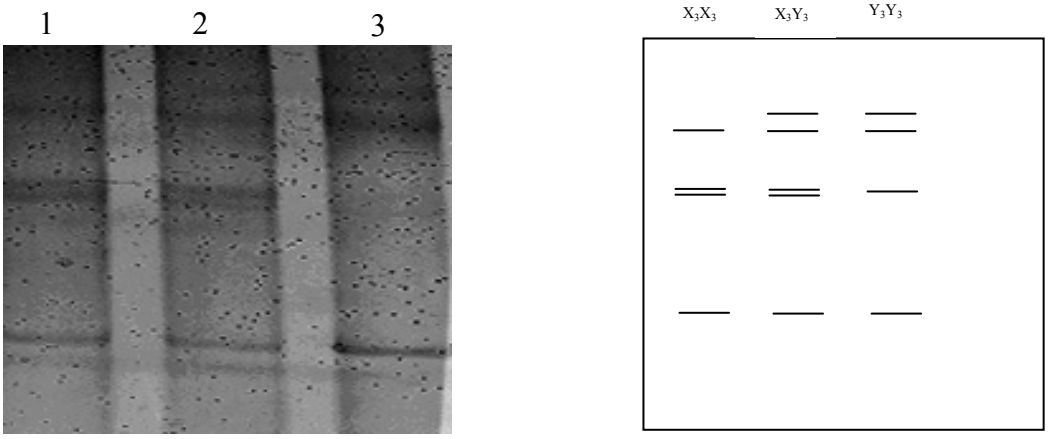


Figure 12 The different SSCP pattern of *GHR3*. Lanes 1, 2 and 3 represented SSCP pattern 1, 2 and 3 of *GHR3*. They represented with genotypes X_3X_3, X_3Y_3 and Y_3Y_3, respectively.

Figure 12 illustrated the SSCP results for *GHR3* fragment. This region was identified and was observed for all possible genotypes. Sequence determination of the entire length of the fragment revealed G to A transition at nucleotide position 1867 in the *GHR3* fragment. The sequence nucleotide at position 1867 of genotype

X_3X_3 was found to be base G. The genotype Y_3Y_3 was mutated by substitution G to A. The heterozygous of X_3Y_3 consisted of base G from X_3X_3 and base A from Y_3Y_3 at nucleotide position 1867. They could be designated as X_3 and Y_3 alleles, respectively. An amino acid was changed in the peptide, where a methionine was exchanged for isoleucine.

The *GHR3* was observed in 3, 67 and 60 heads with X_3X_3, X_3Y_3 and Y_3Y_3 genotypes, respectively (Table 10). Genotype X_3Y_3 and Y_3Y_3 were mostly detected in this population but the genotypic frequency of X_3X_3 was the lowest. The X_3X_3 genotype was lost in male and it was detected only in 3 cows. Moreover, the frequency of Y_3Y_3 in male was higher than the frequency of X_3Y_3. In contrast, the frequency of Y_3Y_3 in female was lower than the X_3Y_3. Allelic frequencies of Y_3 of both sexes were higher than X_3.

Table 10 Genotypic and allelic frequencies of *GHR3* in the multibreed beef population

Animals		Genotypes			Alleles	
		X_3X_3	X_3Y_3	Y_3Y_3	X_3	Y_3
Total	n	3^a	67^b	60^b		
(n=130)	%	2.31	51.54	46.15	28.08^a	71.92^b
Male	n	-	15^a	20^a		
(n=35)	%	-	42.86	57.14	21.43^a	78.57^b
Female	n	3^a	52^b	40^b		
(n=95)	%	3.16	54.74	42.11	30.53^a	69.47^b

Note [a] and [b] within the same row values marked with the different letter are significantly different at $P < 0.01$

3. Haplotypes

From total of 135 (3^3x5) all possible haplotypes, 112 haplotypes having theoretically possible combinations of 4 individual genotypes (*GH1*, *GH2*, *GH5* and *GHR3*) were not detected from the samples. Therefore, only 23 haplotypes were identified in this multibreed beef cattle population. (Table11). Nearly 3/4 percent of the possible genotype combinations were missing.

The 2153 and 2122 haplotypes were found in highest frequency in the population. It indicated that the animals with 2153 and 2122 haplotypes could be favorably haplotypes to discover in the population. Bulls with 2153 haplotype were found to be highest in exclusive sires in the population (Table 12). Similar with dam, the 2122 and 2153 haplotypes were highest genotypic frequency (Table 13).

Table 11 Haplotypic frequency of *GH* and *GHR* genes in the multibreed beef population

Haplotypes	Number of animals (heads)	Genotypic frequencies (%)
1111	2[a]	1.50
1121	1[a]	0.80
1122	4[a]	3.10
2112	1[a]	0.80
2122	30[b]	23.10
2123	2[a]	1.50
2132	15[c]	11.50
2133	3[a]	2.30
2142	10[c]	7.70
2143	6[c]	4.60
2152	1[a]	0.80
2153	31[b]	23.80
2222	1[a]	0.80
2233	2[a]	1.50
2243	1[a]	0.80
2253	7[c]	5.40
2353	1[a]	0.80
3122	2[a]	1.50
3132	2[a]	1.50
3133	1[a]	0.80
3142	1[a]	0.80
3153	5[ab]	3.80
3253	1[a]	0.80
Total	130	100.00

Note [a, b] and [c] within the same column values marked with the different letter are significantly different at $P < 0.01$

Table 12 Haplotypic frequency of *GH* and *GHR* genes in bulls

Haplotypes	Number of animals (heads)	Genotypic frequencies (%)
2122	5^{ab}	14.29
2132	4^{ab}	11.43
2133	1^{a}	2.86
2142	4^{ab}	11.43
2153	10^{b}	28.55
2253	3^{a}	8.57
2243	1^{a}	2.86
2353	1^{a}	2.86
3122	1^{a}	2.86
3133	1^{a}	2.86
3142	1^{a}	2.86
3153	3^{a}	8.57
Total	35	100.00

Note ^{a} and ^{b} within the same column values marked with the different letter are significantly different at $P < 0.01$

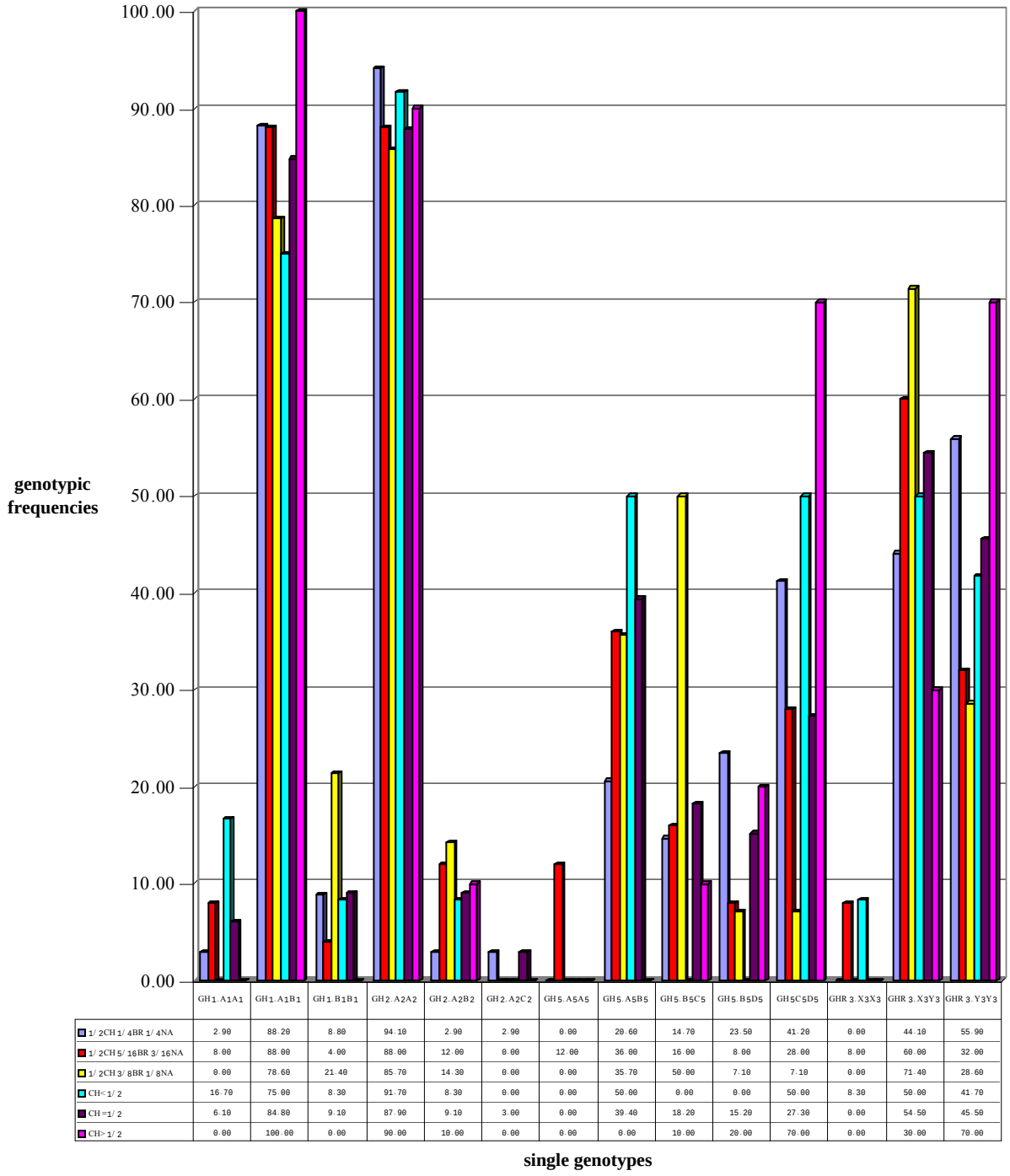
Table 13 Haplotypic frequency of *GH* and *GHR* genes in cows

Haplotypes	Number of animals (heads)	Genotypic frequencies (%)
1111	2[a]	2.11
1121	1[a]	1.05
1122	4[a]	4.21
2112	1[a]	1.05
2122	25[b]	26.31
2123	2[a]	2.11
2132	11[c]	11.58
2133	2[a]	2.11
2142	6[a]	6.32
2143	6[a]	6.32
2152	1[a]	1.05
2153	21[b]	22.10
2222	1[a]	1.05
2233	2[a]	2.11
2253	4[a]	4.21
3122	1[a]	1.05
3132	2[a]	2.11
3153	2[a]	2.11
3253	1[a]	1.05
Total	95	100.00

Note [a, b] and [c] within the same column values marked with the different letter are significantly different at $P < 0.01$

4. Genotypic frequencies of SNPs in each breed group

The characteristic of this population is a multibreed beef population. It consisted of 25 breed groups. The frequency of these breed groups is represented in Appendix Table 1. From the frequency of breed groups, these groups were divided into 6 groups to study genotypic frequency such as 1/2CH1/4BR1/4NA, 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA and other breed (CH<1/2, CH=1/2 and CH>1/2). Frequencies of SNPs varied among breed groups studied. The appearance of SNPs of growth hormone and growth hormone receptor genes is shown in Figure13.



	GH1.A1A1	GH1.A1B1	GH1.B1B1	GH2.A2A2	GH2.A2B2	GH2.A2C2	GH5.A5A5	GH5.A5B5	GH5.B5C5	GH5.B5D5	GH5C5D5	GHR3.X3X3	GHR3.X3Y3	GHR3.Y3Y3
1/2CH1/4BR1/4NA	2.90	88.20	8.80	94.10	2.90	2.90	0.00	20.60	14.70	23.50	41.20	0.00	44.10	55.90
1/2CH5/16BR3/16NA	8.00	88.00	4.00	88.00	12.00	0.00	12.00	36.00	16.00	8.00	28.00	8.00	60.00	32.00
1/2CH3/8BR1/8NA	0.00	78.60	21.40	85.70	14.30	0.00	0.00	35.70	50.00	7.10	7.10	0.00	71.40	28.60
CH<1/2	16.70	75.00	8.30	91.70	8.30	0.00	0.00	50.00	0.00	0.00	50.00	8.30	50.00	41.70
CH=1/2	6.10	84.80	9.10	87.90	9.10	3.00	0.00	39.40	18.20	15.20	27.30	0.00	54.50	45.50
CH>1/2	0.00	100.00	0.00	90.00	10.00	0.00	0.00	0.00	10.00	20.00	70.00	0.00	30.00	70.00

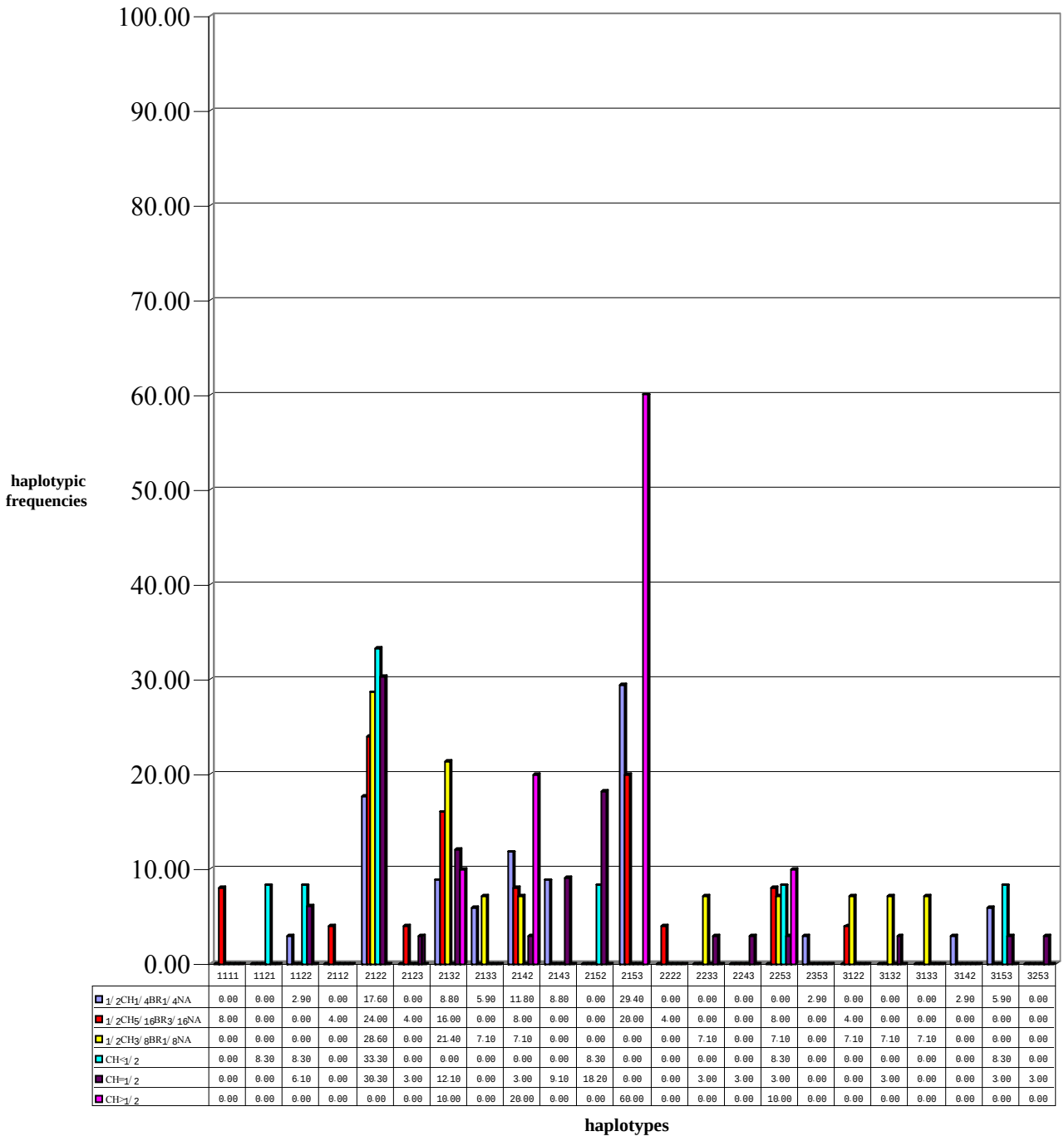
Figure 13 Genotypic frequencies of SNPs of growth hormone and growth hormone receptor genes

Note CH = Charolais, BR = Brahman and NA = Thai native

The A_1B_1, A_2A_2, A_2B_2, C_5D_5, X_3Y_3 and Y_3Y_3 genotypes were detected in six breed groups. The genotypic frequency of A_1B_1 in six breed groups was higher than other genotypes. Moreover, the genotypic frequency of A_2A_2 was higher than other genotypes in every breed groups. Animals with A_1A_1 of GH1 were not detected in 1/2CH3/8BR1/8NA and other breed group (CH>1/2). Moreover, the other breed group (CH>1/2) animals were not detected B_1B_1 genotype of GH1. Genotype A_2C_2 of GH2 was found in two breed groups such as 1/2CH1/4BR1/4NA and other breed group (CH=1/2). Animals with A_5A_5 of GH5 were found only 1/2CH5/16BR3/16NA breed group. The other breed group (CH>1/2) was found to be with higher frequency in C_5D_5 genotype of GH5 than other breed groups. Animals with X_3X_3 of GHR3 were found in two breed groups such as 1/2CH5/16BR3/16NA and other breeds (CH<1/2). The genotypic frequencies of X_3Y_3 and Y_3Y_3 were higher than X_3X_3 genotypes in every breed groups.

5. Haplotypic frequency of *GH* and *GHR* genes in each breed group

The appearance of haplotypes of growth hormone and growth hormone receptor genes is shown in Figure14. The 2153 haplotypes were detected with a high frequency in 1/2CH1/4BR1/4NA and other breed group (CH>1/2). However, the 2122 haplotypes were detected with a high frequency in 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA and the other breed groups (CH>1/2 and CH=1/2). Animals with 2353 and 3142 haplotypes were detected only in 1/2CH1/4BR1/4NA breed group. Furthermore, the animals with 3253 were detected only in other breed (CH=1/2). Animals with 1111, 2112 and 2222 haplotypes were detected only in 1/2CH5/16BR3/16NA. Moreover, the animals with 3133 were detected only in 1/2CH3/8BR1/8NA breed group. Only other breed group (CH<1/2) was detected in 1121 haplotype. In addition, animals with 2243 haplotype were detected only in other breed group (CH=1/2).



	1111	1121	1122	2112	2122	2123	2132	2133	2142	2143	2152	2153	2222	2233	2243	2253	2353	3122	3132	3133	3142	3153	3253
1/2CH1/4BR1/4NA	0.00	0.00	2.90	0.00	17.60	0.00	8.80	5.90	11.80	8.80	0.00	29.40	0.00	0.00	0.00	0.00	2.90	0.00	0.00	0.00	2.90	5.90	0.00
1/2CH5/16BR3/16NA	8.00	0.00	0.00	4.00	24.00	4.00	16.00	0.00	8.00	0.00	0.00	20.00	4.00	0.00	0.00	8.00	0.00	4.00	0.00	0.00	0.00	0.00	0.00
1/2CH3/8BR1/8NA	0.00	0.00	0.00	0.00	28.60	0.00	21.40	7.10	7.10	0.00	0.00	0.00	0.00	7.10	0.00	7.10	0.00	7.10	7.10	7.10	0.00	0.00	0.00
CH<1/2	0.00	8.30	8.30	0.00	33.30	0.00	0.00	0.00	0.00	0.00	8.30	0.00	0.00	0.00	0.00	8.30	0.00	0.00	0.00	0.00	0.00	8.30	0.00
CH=1/2	0.00	0.00	6.10	0.00	30.30	3.00	12.10	0.00	3.00	9.10	18.20	0.00	0.00	3.00	3.00	3.00	0.00	0.00	3.00	0.00	0.00	3.00	3.00
CH>1/2	0.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00	20.00	0.00	0.00	60.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 14 Genotypic frequencies of haplotypes of growth hormone and growth hormone receptor genes

Note CH = Charolais, BR = Brahman and NA = Thai native

Trial II

Effect of genetic polymorphisms of growth hormone and growth hormone receptor genes on preweaning growth traits in a multibreed beef population

1. Fixed effects testing

The analysis of variance (ANOVA) tables for fixed effect testing are represented in Appendix Table 3 and Appendix Table 4 for BW and Appendix Table 5 and Appendix Table 6 for WW. The fixed effects for BW and WW composed of contemporary group, sex and breed group. Moreover, the molecular information, with separation SNPs and haplotypic effects were accounted in the model. They are concluded in Table 14. The result reported that contemporary groups had a significant effect (P<0.01) only on BW for SNPs and haplotypes models. On the other hand, they did not influence on WW for the same model. Effect of sex was non-significance in models with SNPs and haplotypic effect in both traits. Nonetheless, BW and WW of bulls tended to be higher than cows in SNPs and haplotypes model (Appendix Table 2). Effect of breed group was significant in only WW in SNPs and haplotypic model. The high BW and WW in both models tended to be 1/2CH5/16BR3/16NA breed group. The low BW and WW in both models tended to be other breed (CH>1/2) (Appendix Table 2). The SNPs of *GH1* and *GH5* was highly significant for BW but only *GH1* was significant for WW. Haplotypic effect influenced for both traits.

Table 14 Level of significant for factors included in statistical models (model with adjusted SNPs and haplotypes) for birth weight (BW) and weaning weight (WW)

Factors	SNPs model		Factors	Haplotypes model	
	BW	WW		BW	WW
Contemporary groups (year-season of birth)	**	ns	Contemporary groups (year-season of birth)	**	ns
Sex	ns	ns	Sex	ns	ns
Breed groups	ns	*	Breed groups	ns	*
SNPs			Haplotypes	**	*
GH1	**	*			
GH2	ns	ns			
GH5	**	ns			
GHR3	ns	ns			

Note * = P<0.05, ** = P<0.01 and ns = non significant (P>0.05)

2. Effect of SNPs of growth hormone gene

The effect of SNPs of growth hormone gene for BW and WW is presented in Table 15. The SNPs of *GH1* fragment influenced for BW ($P<0.01$) and WW ($P<0.05$). In addition, the SNPs of *GH5* fragment influenced for WW. Animals with A_1A_1 of *GH1* and A_5A_5 genotype of *GH5* performed the lowest on BW ($P<0.05$). Moreover, animals with B_1B_1 of *GH1* performed the highest on WW ($P<0.05$). Animals with A_1A_1 of *GH1* tended to be lowest WW. No statistically significant difference of *GH2* region was observed in this population ($P>0.05$).

Table 15 Least square means and standard error (SE) of SNPs of growth hormone gene for birth weight (BW) and weaning weight (WW) in the multibreed beef population

SNPs	n (heads)	BW (kg)	WW (kg)
GH1			
A_1A_1	7	21.78±1.36[a]	142.45±19.74[a]
A_1B_1	111	25.50±1.09[b]	153.50±16.01[a]
B_1B_1	12	29.40±1.43[c]	186.16±20.88[b]
GH2			
A_2A_2	117	25.59±0.74	155.35±10.79
A_2B_2	11	25.12±1.22	179.55±17.88
A_2C_2	2	25.96±2.06	147.21±30.15
GH5			
A_5A_5	3	19.14±1.91[a]	148.92±27.97
A_5B_5	40	22.79±1.28[a]	147.87±18.76
B_5C_5	23	25.85±1.27[b]	166.10±18.58
B_5D_5	18	27.62±1.25[b]	161.47±18.33
C_5D_5	46	32.41±1.41[c]	179.14±20.56

Note *GH1*, *GH2* and *GH5* = First, second and fifth of PCR product fragments of growth hormone gene, [a, b, c, d] and [e] within the same column values marked with the different letter are significantly different at $P < 0.01$

3. Effect of SNPs of growth hormone receptor gene

The least square means and standard errors for BW and WW in *GHR3* are indicated in Table 16. The effect of SNPs of growth hormone receptor gene in the population was not significant on BW and WW. The Y_3Y_3 genotype was tended to be highest BW but the X_3Y_3 was tended to be higher WW. The X_3X_3 was tended to be lowest on BW and WW.

Table 16 Least square means and standard error (SE) of SNPs of growth hormone receptor gene (*GHR3*) for birth weight (BW) and weaning weight (WW) in the multibreed population

SNPs	n (heads)	BW (kg)	WW (kg)
X_3X_3	3	25.29±1.45	133.45±35.91
X_3Y_3	67	24.99±1.09	178.49±15.88
Y_3Y_3	66	26.40±1.15	170.17±16.88

4. Haplotypes

The least square means and standard errors of haplotypes for BW and WW are presented in Table 17. The haplotypes appeared to have statistically significant effects on both the traits ($P<0.01$). The result indicated that animals with the 3153 and 3133 haplotypes tended to be highest BW and WW, respectively. On the other hand, animals with 1111 and 2243 haplotypes tended to be lowest BW and WW ($P<0.05$).

Table 17 Least square means and standard error (SE) of haplotypes for birth weight (BW) and weaning weight (WW) in the multibreed population

Haplotypes	n (heads)	BW (kg)	WW (kg)
1111	2	16.12 ± 1.83^{a}	100.53 ± 25.44^{abcd}
1121	1	20.59 ± 2.67^{ab}	88.37 ± 37.14^{ab}
1122	4	19.94 ± 1.32^{abc}	138.08 ± 18.32^{abcdef}
2112	1	18.12 ± 2.51^{ab}	136.03 ± 34.94^{abcdef}
2122	30	23.45 ± 0.62^{bcde}	149.78 ± 8.60^{abcdef}
2123	2	21.96 ± 1.82^{bcd}	135.07 ± 25.34^{abcde}
2132	15	26.63 ± 0.75^{def}	151.99 ± 10.45^{abcdef}
2133	3	26.03 ± 1.49^{def}	187.84 ± 20.68^{def}
2142	10	28.06 ± 0.84^{efg}	154.33 ± 11.63^{abcdef}
2143	6	30.09 ± 1.14^{fgh}	150.16 ± 15.91^{abcdef}
2152	1	24.59 ± 2.67^{bcd}	178.37 ± 37.14^{cdef}
2153	31	33.65 ± 0.51^{hij}	164.65 ± 7.08^{bcdef}
2222	1	22.12 ± 2.51^{bcd}	186.03 ± 34.94^{def}
2233	2	23.78 ± 1.83^{bcd}	140.71 ± 25.47^{abcd}
2243	1	30.26 ± 2.52^{fgh}	85.30 ± 35.15^{a}
2253	7	33.00 ± 0.98^{hi}	192.34 ± 13.65^{def}
2353	1	34.39 ± 2.50^{ijk}	185.08 ± 34.81^{def}
3122	2	23.42 ± 1.79^{cde}	176.75 ± 24.95^{bcdef}
3132	2	26.69 ± 1.81^{def}	180.66 ± 25.18^{bcdef}
3133	1	31.72 ± 2.56^{ghi}	225.47 ± 35.60^{f}
3142	1	26.39 ± 2.50^{def}	175.08 ± 34.81^{def}
3153	5	38.89 ± 1.16^{k}	208.00 ± 16.15^{def}
3253	1	37.12 ± 2.51^{jk}	211.03 ± 34.94^{ef}

Note $^{a, b, c, d, e, f, g, h, i, j}$ and ^{k} within the same column values marked with the different letter are significantly different at $P < 0.05$

Trial III

Comparison for the best fit model among models with SNPs, regression of allelic, haplotypic effects and without molecular information

1. Model testing

Comparison of the best fit model for BW and WW between model with and without molecular information by considering mean square error (MSE) and coefficient of determination (R^2) from PROC GLM and log likelihood (log L) from PROC MIXED is represented in Table 18. The results of model with three kinds of molecular informations were lower of MSE and higher of R^2 and log L than model without molecular information. A difference for R^2 between models with and without molecular information was approximately 50 percent for BW and 40 percent for WW. Moreover, the result indicated that MSE of model with haplotypic effect for both traits was the lowest MSE. This corresponds with the results was received from PROC MIXED analysis. Moreover, this model was found to be the highest of log L for both traits. Furthermore, the result indicated that the coefficient of determination (R^2) of model with haplotypic effect for BW and WW was the highest.

Table 18 Model testing among model with SNPs, regression of allelic, haplotypic effects and without molecular information

Model	MSE		R^2		log L	
	BW	WW	BW	WW	BW	WW
with SNPs effect	37.56	2,542.03	0.53	0.25	-287.50	-466.19
with regression of allelic effect	39.26	2,575.02	0.42	0.21	-284.21	-465.99
with haplotypic effect	30.22	2,332.09	0.62	0.38	-249.33	-400.54
without molecular information	52.09	2,665.75	0.26	0.16	-328.21	-508.26

Note MSE = means square error, R^2 = coefficient of determination, log L = log likelihood, SNPs= single nucleotide polymorphisms, BW= birth weight and WW = weaning weight

2. The log likelihood (log L) and accuracy of prediction (ACC) among models with SNPs, regression of allelic effect, haplotypic effect and without molecular information from univariate analysis

The comparison of log L for BW and WW among model with molecular imformations (SNPs, regression of allelic and haplotypic effects) from univariate analysis is represented in Table 19. It indicated that the log L of model with haplotypic effect for BW and WW was higher than model with regression of allelic effect and model with SNPs effect. Moreover, the log L of model with haplotypic effect for both traits was higher than model without molecular information. It indicated that model with haplotypic effect from univariate analysis was better fit than the other models (SNPs, regression of allelic and without molecular information effects).

Table 19 The log L among models with SNPs, regression of allelic effect, haplotypic effect and without molecular information from univariate analysis

Model	log L	
	BW	WW
with SNPs effect	-4,039.01	-2,703.42
with regression of allelic effect	-4,073.12	-2,708.18
with haplotypic effect	-4,011.60	-2,654.97
without molecular information	-4,088.56	-2,746.76

Note log L = log likelihood, SNPs= single nucleotide polymorphisms, BW= birth weight and WW = weaning weight

The study of comparison means of ACC considered among model with SNPs, regression of allelic effect, haplotypic effect and without molecular information. The means of ACC of both estimated breeding values (EBV_a and EBV_m) were compared between with and without molecular information from univariate analysis (Table 20). The result indicated that mean ACC of EBV_a and EBV_m for BW and WW between models with and without molecular information in this population was found different ($P<0.05$). The means of ACC of model with three types of molecular information of both EBVs for BW and WW were higher than model without molecular information ($P<0.05$). The difference means ACC of EBV_a and EBV_m for BW between models with three types molecular information and model without molecular information were approximately found to be 15 and 20 percents. The difference means ACC of EBV_a and EBV_m for WW between models with three types molecular information and model without molecular information were approximately found to be 12 and 20 percents. The means of ACC of EBV_a and EBV_m among three types of molecular information for both traits were not significant ($P>0.05$). The result reported that model with haplotypic effect tended to be the highest means ACC of EBV_a and EBV_m for BW and WW.

When considered detail of each breed group, the results of means ACC in each breed group was most likely from total population. The result reported that the ACC means of EBV_a and EBV_m for BW and WW of model with molecular information were higher than model without molecular information in each breed group ($P<0.05$). The model with haplotypic effect tended to be the highest ACC means of EBV_a and EBV_m for BW and WW in each breed groups.

Table 20 Statistical description of accuracy of prediction (ACC) for birth weight (BW) and weaning weight (WW) among models with SNPs, regression of allelic effect, haplotypic effect and without molecular information from univariate analysis

	n (records)	EBV_a for BW	EBV_m for BW	EBV_a for WW	EBV_m for WW
1/2CH1/4BR1/4NA [1]	35				
- With SNPs		0.63±0.01[a]	0.40±0.02[a]	0.56±0.01[a]	0.34±0.02[a]
- With regression of allelic effect		0.62±0.01[a]	0.40±0.02[a]	0.55±0.01[a]	0.35±0.02[a]
- With haplotypic effect		0.65±0.01[a]	0.42±0.02[a]	0.58±0.01[a]	0.40±0.02[a]
- Without molecular information		0.59±0.01[b]	0.36±0.02[b]	0.49±0.01[b]	0.31±0.02[b]
1/2CH5/16BR3/16NA	25				
- With SNPs		0.65±0.01[a]	0.39±0.01[a]	0.59±0.01[a]	0.44±0.01[a]
- With regression of allelic effect		0.64±0.01[a]	0.38±0.01[a]	0.57±0.01[a]	0.44±0.01[a]
- With haplotypic effect		0.68±0.01[a]	0.41±0.02[a]	0.61±0.01[a]	0.46±0.01[a]
- Without molecular information		0.50±0.01[b]	0.32±0.01[b]	0.51±0.01[b]	0.38±0.01[b]
1/2CH3/8BR1/8NA	14				
- With SNPs		0.70±0.02[a]	0.42±0.02[a]	0.56±0.02[a]	0.41±0.01[a]
- With regression of allelic effect		0.69±0.02[a]	0.41±0.02[a]	0.55±0.02[a]	0.43±0.01[a]
- With haplotypic effect		0.71±0.02[a]	0.46±0.02[b]	0.58±0.02[a]	0.44±0.01[a]
- Without molecular information		0.67±0.02[b]	0.40±0.02[a]	0.48±0.02[b]	0.36±0.01[b]

Note EBV_a and EBV_m = direct and maternal estimated breeding values, [1]CH=Charolais, BR=Brahman and NA=Thai native, n = number of observation, [a] and [b] within the same column values marked with the different letter are significantly different at $P < 0.05$

Table 20 (Continued)

	n (records)	EBV_a for BW	EBV_m for BW	EBV_a for WW	EBV_m for WW
CH<1/2	14				
- With SNPs		0.65±0.01[a]	0.35±0.02[a]	0.57±0.02[a]	0.39±0.02[a]
- With regression of allelic effect		0.63±0.01[a]	0.34±0.02[a]	0.55±0.02[a]	0.36±0.02[a]
- With haplotypic effect		0.66±0.01[a]	0.39±0.02[a]	0.58±0.02[a]	0.44±0.02[a]
- Without molecular information		0.59±0.01[b]	0.33±0.02[b]	0.49±0.02[b]	0.36±0.02[b]
CH=1/2	33				
- With SNPs		0.64±0.01[a]	0.36±0.01[a]	0.58±0.01[a]	0.37±0.02[a]
- With regression of allelic effect		0.62±0.01[a]	0.31±0.01[b]	0.55±0.01[a]	0.37±0.01[a]
- With haplotypic effect		0.65±0.01[a]	0.37±0.01[a]	0.59±0.01[a]	0.42±0.01[a]
- Without molecular information		0.57±0.01[b]	0.28±0.01[b]	0.49±0.01[b]	0.30±0.01[b]
CH>1/2	9				
- With SNPs		0.63±0.01[a]	0.33±0.01[a]	0.57±0.01[a]	0.38±0.02[a]
- With regression of allelic effect		0.62±0.01[a]	0.31±0.01[a]	0.56±0.01[a]	0.38±0.02[a]
- With haplotypic effect		0.66±0.01[a]	0.37±0.01[b]	0.59±0.01[a]	0.43±0.02[a]
- Without molecular information		0.59±0.01[b]	0.31±0.01[a]	0.50±0.01[b]	0.35±0.02[b]
Total	130				
- With SNPs		0.64±0.01[a]	0.34±0.01[a]	0.57±0.01[a]	0.39±0.01[a]
- With regression of allelic effect		0.62±0.01[a]	0.35±0.01[a]	0.54±0.01[a]	0.38±0.01[a]
- With haplotypic effect		0.66±0.01[a]	0.37±0.01[a]	0.59±0.01[a]	0.44±0.01[a]
- Without molecular information		0.58±0.01[b]	0.30±0.01[b]	0.50±0.01[b]	0.32±0.01[b]

Note EBV_a and EBV_m = direct and maternal estimated breeding values, [1]CH=Charolais, BR=Brahman and NA=Thai native, n = number of observation, [a] and [b] within the same column values marked with the different letter are significantly different at $P < 0.05$

3. The log L and ACC among models with SNPs, regression of allelic effect, haplotypic effect and without molecular information from bivariate analysis

The comparison of log L among model with molecular imformation (SNPs, regression of allelic and haplotypic effects) indicated that the log L of model with haplotypic effect (-6,612.19) was higher than model with SNPs effect (-6,690.75) and model with regression of allelic effect (-6,727.07). Moreover, the log L of model with haplotypic effect was lower than model without molecular information (-6,775.78). It indicated that model with haplotypic effect from bivariate analysis was better fit than the other models (SNPs, regression of allelic and without molecular information effects).

Therefore, the study of comparison means of ACC considered between model with SNPs, regression of allelic effect, haplotypic effect and without molecular information. The means of ACC of both estimated breeding values (EBV_a and EBV_m) were compared between with and without molecular information (Table 21). The result indicated that mean ACC of EBV_a and EBV_m for BW and WW between models with and without molecular information in the total population was found different ($P<0.05$). The means of ACC of model with three types of molecular information of both EBVs for BW and WW were higher than model without molecular information ($P<0.05$). The ACC means of model with three types molecular informations of EBV_a and EBV_m for BW were approximately found to be 20 percent when compared with model without molecular information. The means ACC of model with three types molecular informations of EBV_a and EBV_m for WW were approximately found to be 20 and 10 percents when compared with model without molecular information. The means ACC among three types of molecular information were not significant for only the ACC of EBV_a for BW ($P>0.05$). The means ACC of EBV_m for BW and WW between models with SNPs and regression of allelic effect were not significant ($P>0.05$) but they were lower than model with haplotypic effect. The means ACC of EBV_a for WW between models with regression of allelic effect and haplotypic effect were not significant ($P>0.05$) but they were higher than model with SNPs. The result reported that model with regression of allelic effect tended to be the highest means ACC of EBV_a for BW and WW. The means ACC of EBV_m for both traits were the highest in model with haplotypic effect.

When considered detail of each breed group, the results of means ACC in each breed group was most likely from total population. The result reported that the ACC means of EBV_a and EBV_m for BW and WW of model with molecular information were higher than model without molecular information in each breed group ($P<0.05$). The ACC means of model with regression of allelic effect of EBV_a for BW and WW in each breed groups tended to be the highest, except only the ACC means of EBV_a for both traits in 1/2CH5/16BR3/16NA breed group. The ACC means of model with haplotypic effect of EBV_m for BW and WW tended to be the highest in each breed groups.

Table 21 Statistical description of accuracy of prediction (ACC) for birth weight (BW) and weaning weight (WW) among models with SNPs, regression of allelic effect, haplotypic effect and without molecular information from bivariate analysis

	n (records)	EBV_a for BW	EBV_m for BW	EBV_a for WW	EBV_m for WW
1/2CH1/4BR1/4NA [1]	35				
- With SNPs		0.64±0.01[a]	0.46±0.01[a]	0.56±0.01[a]	0.34±0.01[a]
- With regression of allelic effect		0.66±0.01[a]	0.45±0.01[a]	0.59±0.01[a]	0.35±0.01[b]
- With haplotypic effect		0.64±0.01[a]	0.49±0.01[b]	0.57±0.01[a]	0.37±0.01[b]
- Without molecular information		0.51±0.01[b]	0.41±0.01[c]	0.50±0.01[b]	0.32±0.01[a]
1/2CH5/16BR3/16NA	25				
- With SNPs		0.68±0.01[a]	0.54±0.01[a]	0.57±0.01[a]	0.44±0.01[a]
- With regression of allelic effect		0.69±0.01[a]	0.56±0.01[a]	0.59±0.01[a]	0.44±0.01[a]
- With haplotypic effect		0.70±0.01[a]	0.57±0.01[a]	0.60±0.01[a]	0.45±0.01[a]
- Without molecular information		0.50±0.01[b]	0.40±0.01[b]	0.52±0.01[b]	0.38±0.01[b]
1/2CH3/8BR1/8NA	14				
- With SNPs		0.69±0.02[a]	0.52±0.02[a]	0.52±0.02[a]	0.40±0.01[a]
- With regression of allelic effect		0.72±0.02[a]	0.54±0.02[a]	0.51±0.02[a]	0.43±0.01[b]
- With haplotypic effect		0.70±0.02[a]	0.55±0.02[a]	0.54±0.02[a]	0.44±0.01[b]
- Without molecular information		0.58±0.02[b]	0.45±0.02[b]	0.48±0.02[b]	0.39±0.01[a]

Note EBV_a and EBV_m = direct and maternal estimated breeding values, [1]CH=Charolais, BR=Brahman and NA=Thai native, n = number of observation, [a, b] and [c] within the same column values marked with the different letter are significantly different at $P < 0.05$

Table 21 (Continued)

	n (records)	EBV_a for BW	EBV_m for BW	EBV_a for WW	EBV_m for WW
CH<1/2	14				
- With SNPs		0.63±0.01[a]	0.45±0.01[a]	0.55±0.02[a]	0.40±0.02[a]
- With regression of allelic effect		0.67±0.01[b]	0.48±0.01[b]	0.56±0.02[a]	0.41±0.02[a]
- With haplotypic effect		0.66±0.01[b]	0.50±0.01[b]	0.54±0.02[a]	0.42±0.02[a]
- Without molecular information		0.59±0.01[a]	0.37±0.01[c]	0.46±0.02[b]	0.38±0.02[b]
CH=1/2	33				
- With SNPs		0.61±0.01[a]	0.40±0.01[a]	0.53±0.01[a]	0.35±0.02[a]
- With regression of allelic effect		0.64±0.01[a]	0.41±0.01[a]	0.57±0.01[b]	0.38±0.01[b]
- With haplotypic effect		0.62±0.01[a]	0.44±0.01[b]	0.55±0.01[b]	0.38±0.01[b]
- Without molecular information		0.57±0.01[b]	0.38±0.01[a]	0.49±0.01[c]	0.30±0.01[c]
CH>1/2	9				
- With SNPs		0.64±0.01[a]	0.43±0.01[a]	0.56±0.01[a]	0.40±0.01[a]
- With regression of allelic effect		0.67±0.01[b]	0.44±0.01[a]	0.56±0.01[a]	0.41±0.01[a]
- With haplotypic effect		0.67±0.01[b]	0.48±0.01[b]	0.54±0.01[a]	0.41±0.01[a]
- Without molecular information		0.59±0.01[c]	0.39±0.01[c]	0.48±0.01[b]	0.36±0.01[b]
Total	130				
- With SNPs		0.66±0.01[a]	0.44±0.01[a]	0.53±0.01[a]	0.42±0.01[a]
- With regression of allelic effect		0.68±0.01[a]	0.45±0.01[a]	0.57±0.01[b]	0.43±0.01[a]
- With haplotypic effect		0.66±0.01[a]	0.49±0.01[b]	0.55±0.01[b]	0.46±0.01[b]
- Without molecular information		0.55±0.01[b]	0.40±0.01[c]	0.49±0.01[c]	0.36±0.01[c]

Note EBV_a and EBV_m = direct and maternal estimated breeding values, [1]CH=Charolais, BR=Brahman and NA=Thai native, n = number of observation, [a, b] and [c] within the same column values marked with the different letter are significantly different at $P < 0.05$

Trial IV

Estimation of genetic parameters for preweaning growth traits with adjusted by molecular information of growth hormone and growth hormone receptor genes and comparison prediction of breeding values for these traits between model with and without molecular information in a multibreed beef population

1. Estimates of (co)variance components and heritabilities

The effect of haplotypic effect was accounted in the model for estimates of (co)variance components and heritabilities because this model was the best fit. It confirmed from the result of trial III. Variances, direct heritabilities and maternal heritabilities for BW and WW are presented in Table 22. The random residual variances of both traits for BW and WW were found to be 61 and 53 percents when compared with phenotypic variances. The maternal genetic variances of BW and WW were found to be 16 and 42 percents when compared with total genetic variances. The direct genetic effects were approximately more than six times for BW and two times for WW when compared with maternal genetic effects.

Estimated direct heritabilities of BW and WW were of moderate values (0.33 and 0.27). The maternal heritability of WW was approximately three times than the maternal heritability of BW. Estimated maternal heritabilities of BW and WW were low value (0.06 and 0.19). Also, this population had low variation of milking ability.

Table 22 Estimates of (co)variance components and genetic parameters for birth weight (BW) and weaning weight (WW)

Traits/Parameters[a]	BW	WW
σ_a^2 (kg^2)	10.30	314.80
σ_m^2 (kg^2)	1.89	226.70
σ_e^2 (kg^2)	19.50	619.40
h_a^2	0.33±0.08[1]	0.27±0.10
h_m^2	0.06±0.03	0.19±0.07

Note [a] σ^2_a = direct genetic variance; σ^2_m = maternal genetic variance σ^2_e = random residual variance; h^2_a = direct heritability, h^2_m = maternal heritability,

[1] = heritability ± standard error and kg = kilogram

2. The genetic and phenotypic correlations

The direct-maternal, direct genetic, maternal genetic and phenotypic correlations between BW and WW are presented in Table 23. In this population, the direct-maternal correlation of BW was moderate and negative and the direct-maternal correlation of WW was high and negative. The direct and maternal genetic correlations between BW and WW were high and positive. The environmental and phenotypic correlations were moderate and positive values.

The genetic correlations between direct genetic effect for BW and maternal genetic effect for WW was negative and of moderate value. The genetic correlations between direct genetic effect for WW and maternal genetic effect for BW was negative and low value.

Table 23 The correlations of birth weight (BW) and weaning weight (WW)

parameter[a]	
$r_{a(BW),\ m(BW)}$	-0.28±0.13
$r_{a(WW),\ m(WW)}$	-0.69±0.18
$r_{a(BW),\ m(WW)}$	-0.21±0.10
$r_{m(BW),\ a(WW)}$	-0.07±0.04
$r_{a(BW),\ a(WW)}$	0.88±0.14
$r_{m(BW),\ m(WW)}$	0.81±0.28
$r_{e(BW),\ e(WW)}$	0.22±0.09
$r_{p(BW),\ p(WW)}$	0.47±0.05

Note [a]$r_{a(BW)m(BW)}$ = direct-maternal genetic correlation of birth weight; $r_{a(WW)m(WW)}$ = direct-maternal genetic correlation of weaning weight; $r_{a(BW),\ m(WW)}$ = correlation between direct genetic effect for BW and maternal genetic effect for WW; $r_{m(BW),\ a(WW)}$ = correlation between maternal genetic effect for BW and direct genetic effect for WW; $r_{a(BW),\ a(WW)}$ = direct genetic correlation between BW and WW; $r_{m(BW),\ m(WW)}$ = maternal genetic correlation between BW and WW; $r_{e(BW),\ e(WW)}$ = environmental correlation between BW and WW and $r_{p(BW),\ p(WW)}$ = phenotypic correlation between BW and WW

3. Coefficient of breed group and haplotypic effects

The coefficient of breed group effect is presented in Table 24. The estimate of coefficient of breed group effect for BW in the model with haplotypic effect was lower than model without molecular information. However, the estimate of coefficient of breed group effect for WW in the model with haplotypic effect was higher than in the model without molecular information. The superiority for BW and WW of 1/2CH5/16BR3/16NA breed group in the models with haplotypic effect and without molecular information represented in this multibreed population. The inferiority for BW and WW of other breed (CH>1/2) in the both models represented in this population.

The coefficients of haplotypic effects are presented in Table 25. The superiority for BW of 3153 haplotype in the model with haplotypic effect but the inferiority for BW of 1111 haplotype represented in this population. The superiority for WW of 3133 haplotype in the model but the inferiority for WW of 2243 haplotype represented in this multibreed beef population.

Table 24 Coefficient of breed groups on six breed groups for birth weight (BW) and weaning weight (WW) of models with haplotypic effect and without molecular information

Breeds	Model with haplotypic effect		Model without Molecular information	
	BW (kg)	WW (kg)	BW (kg)	WW (kg)
1/2CH1/4BR1/4NA[1]	0.62 (0.57)[2]	17.98(4.70)	1.22(0.61)	0.00(0.00)
1/2CH5/16BR3/16NA	2.01(0.65)	21.18(5.36)	2.47(0.68)	1.62(5.01)
1/2CH3/8BR1/8NA	0.78(0.64)	15.20(5.38)	1.14(0.69)	-2.43(5.14)
CH<1/2	-0.30(0.65)	3.61(5.38)	0.37(0.66)	-16.72(5.13)
CH=1/2	0.00(0.00)	7.65(5.57)	2.15(0.64)	-12.47(5.33)
CH>1/2	-1.86(0.61)	0.00(0.00)	0.00(0.00)	-19.47(4.75)

Note [1]CH=Charolais, BR=Brahman and NA=Thai native, [2] = the number in the brackets is standard error and kg = kilogram

Table 25 Coefficient of haplotypes for birth weight (BW) and weaning weight (WW) of models with haplotypic effect

Haplotypes	BW	WW
1111	-10.86(3.66)	-46.47(21.94)
1121	-7.36(5.30)	-45.44(32.08)
1122	-6.60(2.74)	-1.82(17.44)
2112	-7.05(5.04)	-2.46(31.17)
2122	-3.35(1.07)	6.18(7.92)
2123	-6.52(3.63)	4.35(21.78)
2132	-0.62(1.44)	12.33(9.87)
2133	-0.77(3.63)	54.73(22.94)
2142	0.00(0.00)	16.49(11.91)
2143	3.84(2.12)	15.41(13.81)
2152	-3.88(5.02)	0.00(0.00)
2153	5.38(1.13)	14.62(8.14)
2222	-4.89(5.20)	54.00(31.56)
2233	-7.20(5.22)	-18.99(21.54)
2243	2.81(2.02)	35.19(13.88)
2253	3.17(5.19)	-47.63(31.15)
2353	8.48(5.17)	68.41(30.92)
3122	-5.72(3.64)	42.03(21.59)
3132	-1.89(4.20)	31.87(27.50)
3133	-1.01(4.93)	104.00(39.54)
3142	-1.65(5.35)	21.63(31.96)
3153	10.97(2.43)	57.00(15.53)
3253	10.33(5.84)	82.95(34.73)

Note the number in the parentheses represent standard errors

4. Estimated breeding values (EBVs)

The number and breed groups of sires and dams in this multibreed were substantially different. Sires represented only 15 percent of evaluated animals. Most of sires were 1/2CH1/4BR1/4NA (60 percent). Most of dams were 1/2CH3/8BR1/8NA (12 percent) and 1/2CH1/4BR1/4NA (11 percent). Also, ranges of EBVs were separated for both sires and dams and each breed group. The means and standard deviation of EBVs for all animals, sires, dams and evaluated animals in each breed group are represented in Table 26. The range of direct estimated breeding values (EBV_a) and maternal estimated breeding values (EBV_m) of all evaluated animals for BW and WW in this population were found wide. Furthermore, the range of both EBVs of dams was wider than the range of both EBVs of sires. When considered in detail of each breed group, the range of EBV_a and EBV_m for BW and WW of 1/2CH5/16BR3/16NA were wider than the other breed groups.

Table 26 Statistics of direct estimated breeding values (EBV_a) and maternal estimated breeding values (EBV_m) of birth weight (BW) and weaning weight (WW)

	EBV_a of BW (kg)	EBV_m of BW (kg)	EBV_a of WW (kg)	EBV_m of WW (kg)
Means ±SD	0.05±1.19	-0.04±0.60	0.38±7.83	-0.05±6.71
Max.	3.30	1.74	30.17	19.59
Min.	-2.81	-2.27	-20.35	-20.67
Sex				
Sires	-0.07±0.21 (-2.14 to 3.30)	0.14±0.10 (-2.27 to 1.12)	-1.14±1.39 (-8.89 to 30.17)	1.25±1.15 (-20.67 to 9.59)
Dams	0.16±0.13 (-2.81 to 3.20)	0.03±0.06 (-1.31 to 1.74)	0.20±0.85 (-20.35 to 24.31)	1.08±0.70 (-15.36 to 19.59)
Range of EBV in each breed group				
1/2CH1/4BR1/4NA[1]	-1.61 to 2.16	-1.27 to 0.70	-5.45 to 14.49	-15.36 to 18.95
1/2CH5/16BR3/16NA	-2.59 to 3.30	-2.27 to 1.28	-13.75 to 30.17	-20.67 to 18.45
1/2CH3/8BR1/8NA	-2.37 to 2.45	-1.07 to 1.74	-15.92 to 22.70	-10.74 to 19.59
CH<1/2	-1.33 to 2.65	-0.82 to 1.40	-20.35 to 22.24	-8.14 to 18.46
CH=1/2	-2.23 to 3.20	-1.31 to 1.27	-17.36 to 24.31	-13.89 to 16.19
CH>1/2	-2.81 to 1.96	-0.34 to 1.56	-10.89 to 17.38	-5.78 to 12.09

Note [1]CH=Charolais, BR=Brahman and NA=Thai native, kg = kilogram, SD=standard deviation, Max. = Maximum and Min. = Minimum, [a, b,] and [c] within the same column values marked with the different letter are significantly different at $P < 0.01$ and the number in the parentheses represent ranges of EBV_a and EBV_m for BW and WW in both sexes.

5. Rank correlation of direct and maternal estimated breeding values (EBV_a and EBV_m) between models with haplotypic effect and without molecular information

The patterns of rank correlation of EBV_a and EBV_m between models with haplotypic effect and without molecular information for BW and WW in each breed group are presented in Table 27 for the whole population and Table 28 for 50 percent of top data.

5.1 All animals in each breed group

The result of rank correlations of all breed groups was of similar patterns. The rank correlation coefficients for EBV_a and EBV_m for BW and WW of whole population between model with haplotypic effect and without molecular information were found positive and slightly high values ($P<0.01$). The rank correlation coefficients for BW between model with haplotypic effect and without molecular information were of 0.52 to 0.73 for EBV_a and 0.49 to 0.79 for EBV_m. The animals with 1/2CH3/8BR1/8NA breed group tended to be highest rank correlation coefficient of EBV_a for BW (0.73) between models with haplotypic effect and without molecular information. However, the animals with 1/2CH1/4BR1/4NA breed group tended to be lowest rank correlation coefficient of EBV_a for BW (0.52) between models with haplotypic effect and without molecular information. The animals with 1/2CH5/16BR3/16NA breed group tended to be highest rank correlation coefficient of EBV_m for BW (0.79) between models with haplotypic effect and without molecular information. However, the animals with CH<1/2 breed group tended to be lowest rank correlation coefficient of EBV_m for BW (0.49) between models with haplotypic effect and without molecular information.

The rank correlation coefficients for WW between model with haplotypic effect and without molecular information were positive and slightly high values ($P<0.01$). The rank correlation coefficients for EBV_a and EBV_m ranged from 0.60 to 0.79 and 0.61 to 0.72. The animals with 1/2CH3/8BR1/8NA breed group tended to be the highest rank correlation coefficient of EBV_a and EBV_m for WW (0.72 and 0.73) between models with haplotypic effect and without molecular information. However, the animals with CH=1/2 and CH<1/2 tended to be the lowest rank correlation coefficient of EBV_a and EBV_m for BW between models with haplotypic effect and without molecular information.

Table 27 The rank correlation coefficients of direct and maternal estimated breeding values (EBV_a and EBV_m) between models with haplotypic effect and without molecular information for birth weight (BW) and weaning weight (WW)

Breed groups	n	BW		WW	
	(records)	EBV_a	EBV_m	EBV_a	EBV_m
1/2CH1/4BR1/4NA[1]	35	0.52	0.62	0.62	0.52
1/2CH5/16BR3/16NA	25	0.70	0.79	0.62	0.70
1/2CH3/8BR1/8NA	14	0.73	0.76	0.72	0.73
CH<1/2	14	0.56	0.49	0.71	0.56
CH=1/2	33	0.65	0.73	0.61	0.65
CH>1/2	9	0.62	0.60	0.66	0.62

Note All estimate rank correlation coefficient are significantly different ($P<0.01$) from zero. [1]CH=Charolais, BR=Brahman and NA=Thai native and n = number of record (records)

5.2 The 50 percent of top all animals in each breed group

The 50 percent of top all animals in each breed group are represented in table 28. Both rank correlation coefficients of EBV_a and EBV_m from 50 percent of whole population showed that both genetic ability of individual animal for BW were of positive and moderate values ($P<0.05$). The rank correlation coefficients of EBV_a and EBV_m for BW ranged from 0.41 to 0.57 and 0.51 to 0.59. The animals with 1/2CH3/8BR1/8NA breed group tended to be highest rank correlation coefficient for EBV_a and EBV_m for BW (0.57 and 0.59) between models with haplotypic effect and without molecular information. However, the animals that had 1/2CH1/4BR1/4NA and other breed group (CH=1/2) tended to be lowest rank correlation coefficient of EBV_a and EBV_m (0.41 and 0.51) for BW between models with haplotypic effect and without molecular information.

The rank correlations for EBV_a for 50 percent of top of animals for WW between with haplotypic effect and without molecular information were of positive and moderate rank correlation coefficient values. This values ranged from 0.39 to 0.52 ($P<0.05$). The rank correlation coefficient of EBV_m for WW was of positive and of moderate values. This value ranged from 0.50 to 0.60 ($P<0.05$). The animals with 1/2CH3/8BR1/8NA breed group tended to be highest rank correlation coefficients of EBV_a and EBV_m for 50 percent of top of animals for WW (0.52 and 0.60) between models with haplotypic effect and without molecular information. However, the animals with other breed (CH=1/2) and 1/2CH1/4BR1/4NA groups tended to be lowest rank correlation coefficient of EBV_a and EBV_m for 50 percent of top of animals for WW (0.39 and 0.50) between models with haplotypic effect and without molecular information.

However, not only rank correlation coefficient for EBV_a but also rank correlation coefficient for EBV_m for BW and WW for 50 percent of top whole population in other breed groups (CH<1/2 and CH>1/2) were non-significant.

Table 28 The rank correlation coefficients for 50 percent of top animals of direct and maternal estimated breeding values (EBV_a and EBV_m) between models with haplotypic effect and without molecular information for birth weight (BW) and weaning weight (WW)

Breed groups	n	BW		WW	
	(records)	EBV_a	EBV_m	EBV_a	EBV_m
1/2CH1/4BR1/4NA[1]	18	0.41	0.58	0.52	0.60
1/2CH5/16BR3/16NA	13	0.51	0.53	0.43	0.50
1/2CH3/8BR1/8NA	7	0.57	0.59	0.47	0.57
CH<1/2	7	-0.14^{ns}	-0.17^{ns}	0.31^{ns}	0.23^{ns}
CH=1/2	17	0.47	0.51	0.39	0.59
CH>1/2	5	0.10^{ns}	0.12^{ns}	0.50^{ns}	0.40^{ns}

Note The estimate rank correlation coefficients are significantly different (P<0.05) from zero. [1]CH=Charolais, BR=Brahman and NA=Thai native, ns = the correlations were non significant (P>0.05) and n = number of record (records)

6. Means comparison of EBV_a and EBV_m from model with haplotypic effect between sort through model with haplotypic effect and without molecular information

Means of EBV_a and EBV_m from model with haplotypic effect for 50 percent of top data for BW were compared with these EBVs that were arranged in order of EBVs from model without molecular information. The result is presented in Table 29.

The result for BW showed that means of EBV_a and EBV_m from model with haplotypic effect by ranking from highest to lowest were higher than means of these EBVs by sort through model without molecular information (P<0.05).

The mean of EBV_a with haplotypic effect model for 50 percent of top data was approximately 57 percent when compared with means of EBV_a of this model by sort through model without molecular information (P<0.05). The difference mean of EBV_a for BW from model with haplotypic effect between sort through model with haplotypic effect and without molecular information tended to be highest in 1/2CH5/16BR3/16NA (65 percent) but it tended to be lowest in CH>1/2 breed group (48 percent).

The mean of EBV_m with haplotypic effect model was approximately 48 percent when compared with means of EBV_m of this model by sort through model without molecular information (P<0.05). The difference mean of EBV_m for BW from model with haplotypic between sort through models with haplotypic effect and

without molecular information tended to be highest in 1/2CH5/16BR3/16NA (58 percent) but it tended to be lowest in 1/2CH3/8BR1/8NA (31 percent).

The result concluded that means of EBV_a and EBV_m for BW from model with haplotypic effect between sorting from this model and model without molecular information were slightly of high difference.

Table 29 Means±standard deviation comparison of direct and maternal estimated breeding values (EBV_a and EBV_m) for birth weight (BW) of 50 percent of top animals with haplotypic effect model between rank through model with haplotypic and model without haplotypic effect

Breed groups	EBV_a^1 (kg)	EBV_a^2 (kg)	EBV_m^1 (kg)	EBV_m^2 (kg)
1/2CH1/4BR1/4NA[1]	2.02±0.17[a]	0.73±0.17[b]	0.09±0.01[a]	0.05±0.01[b]
1/2CH5/16BR3/16NA	1.88±0.32[a]	0.65±0.32[b]	1.15±0.12[a]	0.48±0.12[b]
1/2CH3/8BR1/8NA	2.22±0.29[a]	1.22±0.29[b]	1.02±0.20[a]	0.70±0.20[b]
CH<1/2	2.37±0.31[a]	1.06±0.31[b]	1.01±0.18[a]	0.49±0.18[b]
CH=1/2	2.57±0.25[a]	0.91±0.25[b]	1.04±0.10[a]	0.47±0.10[b]
CH>1/2	1.76±0.33[a]	0.90±0.33[a]	1.16±0.11[a]	0.52±0.11[a]

Note [1]CH=Charolais, BR=Brahman and NA=Thai native, EBV_a^1 and EBV_m^1 = means of direct and maternal estimated breeding values of model with haplotypic effect from highest to lowest rank from this model, EBV_a^2 and EBV_m^2= means of direct and maternal estimated breeding values of haplotypic effect model by sort through highest to lowest rank of EBV_a and EBV_m from model without molecular information, [a] and [b] Within the same row values marked with the different letter between EBV^1 and EBV^2 are significantly different at $P < 0.05$

Means of EBV_a and EBV_m for WW from model with haplotypic effect of 50 percent of top data were compared with these EBVs that were arranged in order of EBVs from model without molecular information. The result is presented in Table 30.

The result for WW demonstrated that means of EBV_a and EBV_m with haplotypic effect model from highest to lowest rank for 50 percent of top data animals in each breed group were higher than means of EBV_a and EBV_m by sort through model without molecular information ($P<0.05$).

The mean of EBV_a from model with haplotypic effect model for WW was approximately 58 percent when compared with this EBV from sort through model without molecular information ($P<0.05$). The difference mean of EBV_a for WW from model with haplotypic effect between sort through models with haplotypic effect and without molecular information tended to be highest in 1/2CH3/8BR1/8NA (77 percent) but it tended to be lowest in CH>1/2 breed group (24 percent).

The mean of EBV_m with haplotypic effect model was approximately 42 percent when compared with means of EBV_m of model with haplotypic effect sort through model without molecular information ($P<0.05$). The difference mean of ranking EBV_m for WW from model with haplotypic effect between sort through models with haplotypic effect and without molecular information for 50 percent of top data tended to be highest in 1/2CH1/4BR1/4NA (65 percent) but it tended to be lowest in CH>1/2 breed group (17 percent).

The result concluded that means of ranking EBV_a and EBV_m for WW with haplotypic effect model between sorted through models with haplotypic effect and without molecular information were slightly of high difference.

Table 30 Means±standard deviation comparison of direct and maternal estimated breeding values (EBV_a and EBV_m) for weaning weight (WW) of 50 percent of top animals with haplotypic effect model between rank through model with haplotypic effect and without molecular information

Breed groups	EBV_a^1 (kg)	EBV_a^2 (kg)	EBV_m^1 (kg)	EBV_m^2 (kg)
1/2CH1/4BR1/4NA[1]	10.88±1.12[a]	7.59±1.12[b]	11.43±0.90[a]	3.92±0.90[b]
1/2CH5/16BR3/16NA	16.09±2.70[a]	5.19±2.70[b]	15.71±1.05[a]	5.25±1.05[b]
1/2CH3/8BR1/8NA	14.42±2.25[a]	3.32±2.25[b]	14.79±2.02[a]	8.36±2.02[b]
CH<1/2	15.37±2.19[a]	3.66±2.19[b]	15.40±2.13[a]	6.05±2.13[b]
CH=1/2	14.53±1.64[a]	3.61±1.64[b]	13.03±1.06[a]	5.58±1.06[b]
CH>1/2	12.50±1.24[a]	9.50±1.24[a]	9.56±1.43[a]	7.86±1.43[a]

Note [1]CH=Charolais, BR=Brahman and NA=Thai native, EBV_a^1 and EBV_m^1 = means of direct and maternal estimated breeding values of model with haplotypic effect by ranking from highest to lowest rank from this model, EBV_a^2 and EBV_m^2= means of direct and maternal estimated breeding values of haplotypic effect model by sort through highest to lowest rank of EBV_a and EBV_m from model without molecular information, [a] and [b] Within the same row values marked with the different letter between EBV^1 and EBV^2 are significantly different at $P < 0.05$

DISCUSSION

Trial I

Genetic polymorphism of growth hormone and growth hormone receptor genes in a multibreed beef population

The benefit for studying genetic polymorphism of growth hormone and growth hormone receptor genes in a multibreed population was this population had several combinations of breed fractions. Therefore, animals with different breed fractions had different gene assortments controlling the desirable traits. Also, the utilization of molecular marker could identify the candidate animals for selection and decision making. In this study, the PCR-SSCP technique was applied because it could obtain a high rate of mutation detection. This method is simple and a reliable technique (Hiyashi, 1991).

The PCR-SSCP technique is based on the assumption that changed in nucleotide sequence of a PCR product affect its single strand conformation (Sunnucks *et al*., 2000). Molecules differing by as little as a single base substitution should have different conformations under non-denaturing conditions and migrate differently. Therefore, those differences can be detected as a shift in the electrophoretic differences (Hiyashi, 1991). However, this technique has limitation such as size of PCR product (less than 500 bp), quantity and quality of PCR product, quality of gel for running, gel length and incomplete denaturation (Sunnucks *et al*., 2000).

The SNPs of growth hormone gene were detected at position -303 of *GH1*, 670 of *GH2*, 2141 and 2354 of *GH5*. However, the *GH5* fragment was an imperfect PCR product. Therefore, the *GH5* fragment would have some problems for polyacrylamind gel electrophoresis. Moreover, the SNP of growth hormone receptor gene was detected at position 1867 of *GHR3*. The SNPs of *GH1* and *GH5* of growth hormone gene are located between promoter to 1st exon and 4th intron to 5th exon, respectively. Schlee *et al*. (1994) and Kim *et al.* (2004) reported that mutation of growth hormone gene was detected on promoter regions by substitution from C to T transition. The polymorphic region of *GH1* was slightly similar with the authors but *GH1* had A to C transversion. Furthermore, Lechniak *et al.* (1999), Ge *et al.* (2003) and Pal *et al.* (2004) reported that the 5th exon was detected as point mutation by substitution from C to G transversion. This region was slightly similar with *GH5* fragment but *GH5* was detected in two point mutations such as G to C at position 2141, G to C and G to T transversions at position 2354. Ge *et al*. (2003) reported the polymorphisms on 10th exon of growth hormone receptor gene. This region was found close to *GHR3* fragment and the region revealed G to A transition. Occurrence of several point mutations of nucleotide sequences would change amino acids of polypeptide chain (Hayes and Goddard, 2001).

The result from the sequencing confirmed that the occurrence of transition and transversion of nucleotide sequences would change amino acid. The amino acid of *GH5* were mutated from valine to leucine in the first point mutation and tryphophane

to cysteine or tryphophane to proline in the second point mutation in peptide chains of growth hormone. The amino acid of *GHR3* was mutated from methionine to isoleucine. These several point mutations would influence the quantity of expression of both genes and the activity of growth hormone and growth hormone receptor. According to Schlee *et al*. (1994) who reported that the point mutation of growth hormone gene on 5^{th} exon influenced concentration of growth hormone in blood samples by radioimmuno-assays.

The genotypic frequencies of A_1B_1 of *GH1*, A_2A_2 of *GH2*, C_5D_5 of *GH5* and X_3Y_3 of *GHR3* were most frequently observed in the population. The animals with 2122 haplotype were found with high genotypic frequency. This result suggested the occurrence of association among genotypes of growth hormone and growth hormone receptor genes. In addition, frequencies of SNPs and haplotypes varied among breed groups studied when it was considered to separate breed groups. The A_1B_1, A_2A_2, C_5D_5, X_3Y_3 genotypes were detected in six breed groups as 1/2CH1/4BR1/4NA, 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA and other breed groups (CH<1/2, CH=1/2 and CH>1/2), respectively. It indicated that these genotypes had relationship among breed groups. Moreover, these genotypes were detected the most frequent in every breed groups.

For the haplotype information, the genotypic frequency of 2122 haplotype was detected with a high frequency in 1/2CH5/16BR3/16NA, 1/2CH3/8BR1/8NA and the other breed groups (CH>1/2 and CH=1/2). The 2153 haplotype was detected with a high frequency in 1/2CH1/4BR1/4NA and other breed group (CH<1/2). No appearance of haplotype that was detected in each breed group. It caused from identify of individual animals by haplotypes was more meticulous than SNPs. Also, the analysis from large number of animals needs to be done to reconfirm the genotypic frequencies of both SNPs and haplotypes of growth hormone and growth hormone receptor genes in each breed group. This population had different SNPs or haplotypes in six breed groups. It indicated that animals in different breed groups as well as animals within breed groups showed the variation of preweaning growth traits.

In the current study, occurrence of genotypic and allelic variation in the regulatory (*GH1*) and structural (*GH2*, *GH5* and *GHR3*) regions of growth hormone and growth hormone receptor genes in this population may influence the diversification of growth. Polymorphism of nucleotide sequences in these regions may influence the gene expression or sequence of product. Moreover, variation in intron (*GH2*) sequences would have a potential usefulness as genetic markers when it had an influence for interested traits.

Genotypic frequencies of SNPs and haplotypes frequencies were detected in this specific population. This study could not be compared with other research works (Moody *et al.,* 1996; Lucy *et al.,* 1998; Andrzej *et al.,* 1999; Reis *et al*., 2001; Ge *et al*., 2003 and Kim *et al.*, 2004) because the method of detecting SNPs, region of both genes for study, breed of cattle and their selection procedure were different compared to present work. Moreover, the sample size of this population was small. Therefore, the present finding needs further confirmation using substantially larger multibreed dataset. However, the mutation positions, genotypic frequencies of SNPs and haplotypes obtain here will serve as a comparison base for more complex future study in a multibreed beef population in Thailand.

Trial II

Effect of genetic polymorphisms of growth hormone and growth hormone receptor genes for preweaning growth traits in a multibreed beef population

Trial I explained about SNPs on *GH1*, *GH2* and *GH5* of growth hormone gene and *GHR3* of growth hormone receptor gene. The point mutations of these regions would then change the sequence of amino acids. Therefore, the polypeptide chain of growth hormone and transmembrane protein of growth hormone receptor would change regulation and activity to stimulate from other hormones (Schlee *et al*., 1994; Andrzej *et al.*, 1999). Many literature reviews reported that the mutation of growth hormone and growth hormone receptor genes influenced for BW and WW (Hale *et al*., 2000; Tambasco *et al*., 2003; Kim *et al*., 2004; Pal *et al*., 2004).

Thus, the trial II was designed which the fixed effects testing for BW and WW demonstrated in this multibreed population. The contemporary group (year-season of birth) of models with SNPs and haplotypic effects influenced for BW ($P<0.01$) but this effect was non-signifinant for WW. It implied that contemporary groups would have a few affect for WW. Nevertheless, maternal ability would have influence for WW because calves were taken care by their dams until weaning age. The breed group of both models influenced for WW ($P<0.05$). It implied that additive and non additive genetic effects of each breed group would influence for WW. The highest WW from models with SNPs and haplotypic effects was found in animals that were 1/2CH5/16BR3/16NA breed group ($P<0.05$). The low WW in these two models was found in other breed (CH>1/2) animals ($P<0.05$). It indicated that the CH>1/2 breed group animals would be prone to environmental stresses and tropical diseases. The 1/2CH5/16BR3/16NA breed group could be detected a high proportion in 2122 haplotype. It implied that the 2122 haplotype would be developed for genetic markers in order to select high WW animals. On the other hand, the animals with CH>1/2 could be detected a high proportion in 2142 haplotype. The producers would consider this haplotype for culling the animals with low WW in this particular multibreed beef population.

The SNPs of *GH1* and *GH5* highly influenced for BW ($P<0.01$) but only *GH1* influenced for WW ($P<0.05$). This result was agreed with Grochowska *et al*. (2001) who reported that the polymorphisms of *GH* gene influence on GH levels and growth traits. The result indicated that the variation in the promoter region (*GH1*) and structural region (*GH5*) sequences has a beneficial effect as genetic markers. In fact, the variation of nucleotide sequences on promoter region influenced for transcription process (Schlee *et al*., 1994). In addition, the changed of nucleotide sequence on exon would influence for polypeptide chain and GH hormone. Therefore, the producers should consider the genetic polymorphism of *GH1* for improvement WW. These SNPs could be applied for genetic marker in order to primarily select by making it possible the identification at birth of interesting genotypes before performance recording and incorporation of maker information in conventional selection. It could save economic loss to the producers for rearing the animals until maturity. However, the *GH2* was non-significant for BW and WW. As a result, the SNPs of *GH1* and

GH5 were located on coding sequences of growth hormone and growth hormone receptor genes but SNP of *GH2* was located at non-coding sequence or at 1st intron. This region was split into RNA sequence when translated from RNA to protein (Sambrook *et al*., 1989). Thus, SNPs of *GH2* would not influence for preweaning growth traits. These SNPs were not importance for genetic marker.

Animals with B_1B_1 genotype of *GH1* performed the highest for both BW and WW ($P<0.01$). However, the animals with A_1A_1 of *GH1* performed lowest in BW ($P<0.01$) and tended to be lowest in WW. It indicated that the B_1B_1 could be a favored genotype for selection of high WW animals. However, the animals would be susceptible to dystocia problem because they were high BW (Kinghorn *et al.* 1999). Therefore, the producers should consider appropriate genotype for future selection in order to achieve optimum progress for BW and WW. Animals with C_5D_5 genotype of *GH5* performed highest BW ($P<0.05$). It indicated that calves at birth with C_5D_5 genotype were heavier than the other genotypes.

The *GHR3* was not found statistically significant. This result agrees with Ge *et al.* (2003). This in contrast with Hale *et al*. (2000) who reported that genetic polymorphism of growth hormone receptor gene influenced growth traits. Therefore, polymorphism of *GHR3* would require large sample size or the populations in future to explore its effect on preweaning growth traits.

The effect of haplotypes of growth hormone and growth hormone receptor genes influenced for both preweaning growth traits. Animals with 3153 and 3133 haplotypes showed highest BW and WW, however, animals with 1111 and 2243 haplotypes showed lowest BW and WW. The results indicated that effects of inter-region or epistatic interaction among loci of both genes are responsible to influence for BW and WW.

The utilization of SNPs and haplotypes for genetic marker should consider effect of polymorphism among point mutation region of these genes because the association among polymorphics would influence for expression of birth weight and weaning weight. However, testing of these effects should be confirmed in other populations to verify the effect of the genetic markers, as well as the effect of the other polymorphisms in growth hormone and growth hormone receptor genes.

The advantage of effect of polymorphic growth hormone and growth hormone receptor genes with economic traits (BW and WW) will help the breeders to search some genetic markers for these economic traits. This may be used as an aid to the selection of parent stock at an early stage. However, the producers should consider other candidate genes because function of some genes would have synergistic or antagonistic effects in nature for expression of BW and WW. Furthermore, the producers should consider pleiotropy of both genes that would affect with other trait such as calving difficulty.

Trial III

Comparison the best fit model among models with SNPs, regression of allelic effect, haplotypic effects and without molecular information

This study was considered the best fit models among SNPs, regression of allelic, haplotypic effects and without molecular information. This study was separated into three sections as follows:

The first section was comparison the best fit model among models with SNPs, regression of allelic effect, haplotypic effects and without molecular information by considering mean square error (MSE), coefficient of determination (R^2) and log likelihood (log L) from PROC GLM and PROC MIXED by SAS (1996). The result indicated that MSE of models with three kind information of molecular information (SNPs, regression of allelic and haplotypic effects) were lower than model without molecular information. In addition, R^2 and log L of these models were higher than model without molecular information. It indicated that accounting the molecular information in the model could increase power of test or could decrease biasness and could be goodness of fit to this multibreed dataset. The model with haplotypic effect for BW and WW had the best fit model when compared among the four models. This model was found to be the lowest of MSE and the highest of R^2 and log L. This model could reduce the residual or unknown effects of affected intra- and inter-polymorphic regions (Andrzej *et al.*, 1999).

The second section was comparison the best fit model among models with SNPs, regression of allelic, haplotypic effects and without molecular information by considering log L and means accuracy of prediction (ACC) from univariate analysis by ASREML software. The result from this section found that the log L of models with three kinds molecular information for BW and WW were higher than model without molecular information. Moreover, the accuracies of prediction direct and maternal breeding values for BW and WW of model with molecular information were higher than model without molecular information in each breed groups ($P<0.05$). This section reported that the model with haplotypic effect tended to be the highest of log L and means ACC of both EBVs for two traits.

The last section was aimed to reconfirm the best fit model between models with and without molecular information by considering of log L and ACC of both EBVs from bivariate analysis. The result from this section confirmed that the log L of model with haplotypic effect was better fit compared to model with SNPs and model with regression of allelic effects. Moreover, log L of model with haplotypic effect was higher than log L of model without molecular information. The means ACC for EBV_a and EBV_m for BW and WW in this multibreed population of model with molecular information were higher than means ACC of model without molecular information. It indicated that adding molecular information in the model for genetic evaluation could increase accuracy of prediction. When considered the different among three kinds of molecular information (SNPs, regression of allelic effect and haplotypic effect), the means of ACC of EBV_a for BW and WW tended to be the

highest model with regression of allelic effect but this model was non significant with model with haplotypic effect. The means of ACC of EBV_m for both traits were the highest in model with haplotypic effect. Therefore, the best fit models among SNPs, regression of allelic and haplotypic effects and without molecular information for genetic evaluation from univariate and bivariate analyses was model with haplotypic effect. This result of this trial indicated that not only using molecular information but also characteristic of molecular information for genetic evaluation would be important for genetic evaluation. Also, the producers should consider appropriate molecular information for genetic evaluation in order to achieve the high accuracy of prediction in this dataset.

In this particular multibreed beef population, the model including haplotypic effect would be useful for genetic evaluation. However, the application of result from this study would be insufficient owing to the availability of limited data. In principle, the data used in this study do not represent almost all data from beef population in Thailand. The utilization of result warrants further confirmation and validation with substantially larger multibreed dataset. Moreover, this model would have some errors from unknown factors such as generation of selected animals and other fixed effects. Therefore, this result from the present study should be applied with caution when used with the similar data structure of other records.

Trial IV

Estimation of genetic parameters for preweaning growth traits with adjusted by molecular information of growth hormone and growth hormone receptor genes and comparison prediction of breeding values for these traits between model with and without molecular information in a multibreed beef population

This study was designed to estimate genetic parameters and to predict breeding value for BW and WW of individual multibreed beef cattle with the model adjusted by haplotypic effect for both the traits in a multibreed beef cattle population. This study was divided into six sections as follows:

The result from the trial III, the model with haplotypic effect was used for estimation of (co)variance components and genetic parameters for BW and WW in the bivariate animal model analysis. The direct genetic variances of both the traits were higher than maternal genetic variances. It implied that variation of direct genetic effects of both traits were higher than variation of maternal genetic effect in this herd. Estimated direct heritabilities for BW and WW were found to be 0.33 and 0.27, respectively. The moderate direct heritabilities for BW and WW indicated that the variation of direct effects of both the traits were of moderate values. It indicated that the producers had a chance to improve direct genetic effect in this herd. Therefore, selection for improving both traits should be considered utilization of BLUP based selection program and molecular information of individual animal. It would increase the consistency of genetic improvement and would decease generation interval. However, estimated maternal heritabilities of BW and WW were low (0.06 and 0.19). It indicated that the mothering ability in term of milking yield and milking ability in this multibreed population was low variation. This result implied that the genetic improvement programs for WW should consider both direct and maternal genetic effects since they had more influence of phenotypic characteristics. Both heritabilities estimates for BW and WW were different from other Thai studies. Differences in the multibreed field datasets used, editing procedures and genetic evaluation models are likely to account for a large portion of these different estimates.

The second section was to investigate the genetic and phenotypic correlation between BW and WW of model with haplotypic effect. The direct-maternal correlation of BW was found moderate and negative (-0.28) and the direct-maternal correlation of WW was found high and negative (-0.69). This implied that selection of dams for high direct genetic values might also get low maternal ability. In fact, if the producers select these traits by considering only direct genetic effect, they would be inferior maternal behavior of dams. The genetic correlations between direct genetic effect for BW and maternal genetic effect for WW and vice versa were found negative and of low to moderate values (-0.21 and -0.07). It indicated that selection of only direct genetic effect for BW may lead to decline in maternal ability for WW. In contrast, selection of only direct effect for WW may lead to slightly decrease in maternal ability for BW. As a result, the producers should consider these antagonistic relationships between direct-maternal genetic effects for within and between traits for selection of the parent stock in order to increase the economical efficiency of this

herd. The direct and maternal genetic correlations between BW and WW were found high and positive (0.88 and 0.81). It implied that further selection to increase birth weight could potentially increase weaning weight. Moreover, the phenotypic correlation was found moderate and with positive value (0.47). The environmental correlation was moderate and of positive value (0.22). It indicated that genetic improvement and improvement in environment conditions will probably work in the same direction. The results implied that the producers could select to improve one of these traits (BW and WW). In doing so, the other trait would also improve in proportion.

The third section was aimed to analyze the coefficient of six breed groups. Breed group effect or genetic group effect in a genetic prediction model can be viewed from a subclass or regression viewpoint in crossbred animals (McDowell *et al*., 1976). However, subclass grouping strategy was better suited to this multibreed dataset than regression grouping strategy because a number of animals in each subclass genetic group model were higher than a number of animals in each breed fraction. Moreover, subclass strategy could give details of descriptive statistical information in the six main breed groups in this population. The coefficient of breed group of model with haplotypic effect was higher than from model without molecular information. The coefficient of breed groups of model with haplotypic effect and without molecular information in 1/2CH5/16BR3/16NA was found to be the highest BW and WW. The coefficient of CH>1/2 breed group was lowest BW and WW from two models. It indicated that this breed group would be the inappropriate mating system. The performance of animals in this breed group would be lower than other breed groups. The coefficient of haplotypes indicated the superiority for BW of 3153 haplotype. Therefore, the 3153 animals in this breed group would be susceptible to dystocia syndrome. In contrast, the animals in this breed group that acquired 3133 haplotype were considered superior for WW in this multibreed beef population. It indicated that this haplotype would be the best set of genotypes of growth hormone and growth hormone receptor genes.

The fourth section was devised to estimate direct and maternal breeding values (EBV_a and EBV_m) from model with haplotypic effect. Both EBVs were taken from estimation of (co)variance information with mixed model equation in bivariate analysis by Best Linear Unbiased Prediction (BLUP). The result from this section confirmed that the EBV_a and EBV_m of BW and WW in this population had a variation for selection of best candidate animals. Besides, this section reported that the range of both EBVs for BW and WW of dams were wider than sires. It indicated that dams had high direct and maternal genetic variabilities. In addition, the wide range of both EBVs of dams would reflect the accuracy of genetic prediction of dams that would be lower than sires. The 1/2CH5/16BR3/16NA breed group had the wide range of EBVs for both traits than other breed groups. It indicated that this breed group had high direct and maternal genetic variabilities. As a result, the chance for improvement of direct and maternal genetics for BW and WW of this breed group was found better than other breed groups. Therefore, if the producers would like to improve both direct and maternal genetic abilities together for BW and WW, they should consider 1/2CH5/16BR3/16NA breed group.

The fifth section was intended to calculate rank correlation coefficients of both EBVs between model with haplotypic effect and without molecular information. This section revealed that ranking between models with haplotypic effect and without molecular information of all data was positive and slightly high rank correlation coefficient for EBVs in both traits in each breed group. It ranged from 0.52 to 0.73 for EBV_a for BW, 0.49 to 0.79 for EBV_m for BW and 0.60 to 0.79 for EBV_a for WW, 0.61 to 0.72 for EBV_m for WW, respectively ($P<0.01$). The result indicated that rank correlation coefficients of direct and maternal genetic abilities of individual animal for BW and WW were quite similar between model with haplotypic effect and without molecular information in each breed group. Indeed, the EBV_a and EBV_m from model with haplotypic effect of individual animal of all data were positive correlated with both EBVs for these traits of model without molecular information. Both rank correlation coefficients of EBV_a and EBV_m for 50 percent of top all animals for BW and WW in each breed group were positive and of moderate values. It ranged from 0.41 to 0.57 for EBV_a for BW and 0.51 to 0.59 for EBV_m for BW and 0.39 to 0.52 for EBV_a for WW, 0.50 and 0.60 for EBV_m for WW, respectively ($P<0.05$). Nonetheless, rank correlation coefficient for EBV_a and EBV_m for BW and WW for 50 percent of top dataset in other breed groups (CH<1/2 and CH>1/2) were non-significant. The result indicated that rank correlation coefficients for 50 percent of top animals of direct and maternal genetic abilities of individual animal of BW and WW were positive correlated with their breeding values for both traits of model without molecular information, except other breed groups (CH<1/2 and CH>1/2). Therefore, the ranking for 50 percent of top animals of EBV_a and EBV_m for BW and WW were quite similar from model without molecular information in each breed group. However, the ranking for 50 percent of top animals of EBV_a and EBV_m for BW and WW of CH<1/2 and CH>1/2 were different from model without molecular information.

The last section was dealed with the means of EBV_a and EBV_m from model with haplotypic effect by rank through model with haplotypic effect and model without molecular information. The result for BW and WW in each breed group showed that means of EBV_a and EBV_m for both traits with haplotypic effect model by rank through from highest to lowest in each breed group were higher than means of EBV_a and EBV_m when sort through model without molecular information ($P<0.05$). They were approximately 50 percent for both EBVs. It indicated that genetic progress of both traits in this particular multibreed beef population would be increase, if the producers select the animals for parent stock by consideration haplotypic effect for genetic evaluation. In fact, this result implied that using molecular genetic informations to select candidate animals would be worthwhile to improve the direct and maternal genetic ability for BW and WW. Moreover, the finding further confirmed that selection for both EBVs from haplotypic effect model could have chance to increase direct and maternal genetic abilities for BW and WW in this population.

Therefore, the improvement of genetic progress for BW and WW in the population, the producers should consider both molecular information and solution getting from BLUP as a selection tool to improve genetic progress for preweaning growth traits from this multibreed beef population. The accounting molecular information could increase genetic progress for both traits (about 50 percent) and could increase accuracy of prediction (about 20 percent). However, the utilization of molecular information linked to conventional breeding for selection of candidate animals would not be appropriate for small beef farms because of high cost involvement in breeding program and insufficient technicians. However, the applicable finding requires careful consideration of economic aspects and business risks. In addition, strategies should be developed to estimate gene effects at the commercial level for nucleus breeding programs, in particular if they involve crossbreeding. This also opens opportunities to use markers to capitalize on nonadditive effect and assignment of specific mating.

CONCLUSION

The general conclusions are drawn from three research studies. In Trial I, growth hormone gene was found to be four single nucleotide polymorphisms (SNPs) at three polymorphic regions (*GH1*, *GH2* and *GH5*). Two SNPs were found within *GH1* and *GH2* fragments at position -303 and 670 bp, respectively. The other two SNPs were located at position 2141 and 2354 bp of *GH5* fragment. The SNP of growth hormone receptor gene was found within *GHR3* fragment at the position 1867. The A_1B_1, A_2A_2, C_5D_5 and X_3Y_3 genotypes of *GH1*, *GH2*, *GH5* and *GHR3* were found with highest frequency in the multibreed population. The A_1A_1, A_2C_2, A_5A_5 and X_3X_3 genotypes of *GH1*, *GH2*, *GH5* and *GHR3* were found with lowest frequency in the multibreed population. Considering haplotypes of four regions, only 23 haplotypes were identified in the blood sample of animals. The 2122 and 2153 haplotypes were found to be with high frequency in this population. Moreover, the A_1B_1 genotype of *GH1*, A_2A_2 and A_2B_2 genotypes of *GH2*, C_5D_5 genotype of *GH5* and X_3Y_3 and Y_3Y_3 genotypes of *GHR3* were found in each breed group. However, the indistinct haplotypes were found in each breed group. Therefore, this trial concluded that the genetic polymorphisms of growth hormone and growth hormone receptor genes in a multibreed beef population had four point mutations and five SNPs. The total genotypes of both genes were 14 genotypes and 23 haplotypes of growth hormone and growth hormone receptor genes

The trial II demonstrated the effect of SNPs and haplotypes for BW and WW. The two SNPs of growth hormone gene (*GH1* and *GH5*) influenced only on BW ($P<0.01$). Only *GH1* influenced for WW ($P<0.05$). No effect of *GH2* was observed in this research. However, growth hormone receptor gene (*GHR3*) did not influence for BW and WW. Animals with B_1B_1 genotype of *GH1* and C_5D_5 genotype of *GH5* performed the highest BW. Animals with A_1A_1 genotype of *GH1* and A_5A_5 genotype of *GH5* performed the lowest BW and WW animals. The haplotypic effect influenced for BW ($P<0.01$) and WW ($P<0.05$). The animals with the 3153 and 3133 haplotypes performed high BW and WW, respectively ($P<0.05$). In contrary, animals with 1111 and 2243 haplotypes performed low BW and WW, respectively ($P<0.05$). Moreover, other fixed effects that affected for BW and WW were contemporary groups for BW ($P<0.01$) and breed groups for WW ($P<0.05$) for the model with SNPs and haplotypes effect. Therefore, trial II concluded the SNPs of *GH1* and *GH5* influenced for BW ($P<0.05$) but only SNPs of *GH1* influenced for WW ($P<0.01$). The haplotypic effect affected both preweaning growth traits in a multibreed beef cattle population.

The finding from trial III indicated the best fit model among models with molecular information (SNPs, regression of allelic and haplotypic effects) and without molecular information. The mean square error (MSE) of model with haplotypic effect for both traits was lower than other models. Moreover, the coefficient of determination (R^2) and log likelihood (log L) of model with haplotypic effect for two traits were higher than the other models. In addition, the highest of log likelihood (log L) from univariate analysis was model with haplotypic effect. This model tended to be higest means accuracy of prediction of both estimated breeding values (EBVs) for BW and WW. The results of means of accuracy of prediction (ACC) in each

breed group were similar with the total population. The highest of log L from bivariate analysis was model with haplotypic effect. Furthermore, the result reported that the means ACC of EBV_a for BW and WW tended to be the highest model with regression of allelic effect and it was non significant from model with SNPs and haplotypic effects. The means ACC of EBV_m for both traits were the highest in model with haplotypic effect. Therefore, this trial concluded that model with haplotypic effect was more appropriate for genetic evaluation in this multibreed population because this model had lowest MSE and highest on R^2, log L from SAS and log L from univariate and bivariate analyses from ASREML. In addition, model with haplotypic effect tended to be highest on means ACC of EBV_a for BW and WW from univariate analysis and EBV_m for both traits from univariate and bivariate analyses than other models.

The last trial, the model with haplotypic effect was used for estimation of (co)variance, genetic parameters, direct and maternal estimated breeding values (EBV_a and EBV_m) for BW and WW and analysis of ranking comparison between model with hapltypic effect and without molecular information. The direct heritabilities for BW and WW were found to be moderate values (0.33 and 0.27). The maternal heritabilities were found to be low for BW and WW (0.06 and 0.19). The direct-maternal genetic correlations of BW and WW were found to be negative and moderate to high values (-0.28 for BW and –0.69 for WW). The direct-maternal genetic correlations between traits were found to be -0.21 for BW and WW and –0.07 for WW and BW. The direct, maternal and phenotypic correlations between BW and WW were found positive and high values (0.88, 0.81 and 0.47, respectively). The coefficient of breed groups of model with haplotypic effect and without molecular information in 1/2CH5/16BR3/16NA was the highest BW and WW. The EBV_a for BW and WW ranged from –2.81 to 3.30 kilograms and from –20.35 to 30.17 kilograms, respectively. The EBV_m for BW and WW ranged from –2.27 to 1.74 kilograms and from –20.67 to 19.59 kilograms, respectively. The range of EBV_a and EBV_m for BW and WW of dams were wider than bulls. Moreover, The range of EBV_a and EBV_m for BW and WW of 1/2CH5/16BR3/16NA were wider than the other breed groups. The rank correlation coefficients of EBV_a and EBV_m of whole population for BW between model with haplotypic effect and without molecular information were found positive and slightly high values in each breed group. It ranged from 0.52 to 0.73 for EBV_a and 0.49 to 0.79 for EBV_m ($P<0.01$). Moreover, the rank correlation coefficients of EBV_a and EBV_m of whole population for WW between model with haplotypic effect and without molecular information were found positive and slightly high values in each breed group. It ranged from 0.60 to 0.79 for EBV_a and 0.61 to 0.72 for EBV_m ($P<0.01$). The rank correlation coefficients of EBV_a and EBV_m for 50 percent of top animals for BW were positive and moderate values in each breed group ($P<0.05$), except in the other breeds (CH<1/2 and CH>1/2). It ranged from 0.41 to 0.57 for EBV_a and 0.51 to 0.59 for EBV_m. In addition, the rank correlation coefficient of EBV_a and EBV_m for 50 percent of top animals for WW were positive and of moderate values in each breed group, except in the other breed (CH<1/2 and CH>1/2) ($P>0.05$). It ranged from 0.39 to 0.52 for EBV_a and 0.50 to 0.60 for EBV_m. Means of EBV_a and EBV_m of model with haplotypic effect for BW and WW in each breed group between sort through model with haplotypic effect and

without molecular information were highly different ($P<0.05$). Therefore, this trial concluded that the direct heritabilities for BW and WW from model with haplotypic effect were moderate value but the maternal heritabilities for BW and WW were low value. The direct genetic, maternal genetic and phenotypic correlations were high and positive values but the direct-maternal genetic correlations within and between traits were negative values. The estimated breeding values for both traits in each breed group and in each sex were slightly wide. Rank correlation coefficients between two models (with haplotypic effect and without molecular information) of all data and 50 percent of data in each breed group were positive. The different means of both EBVs for both traits between sort through model with haplotypic effect and model without molecular information were highly different.

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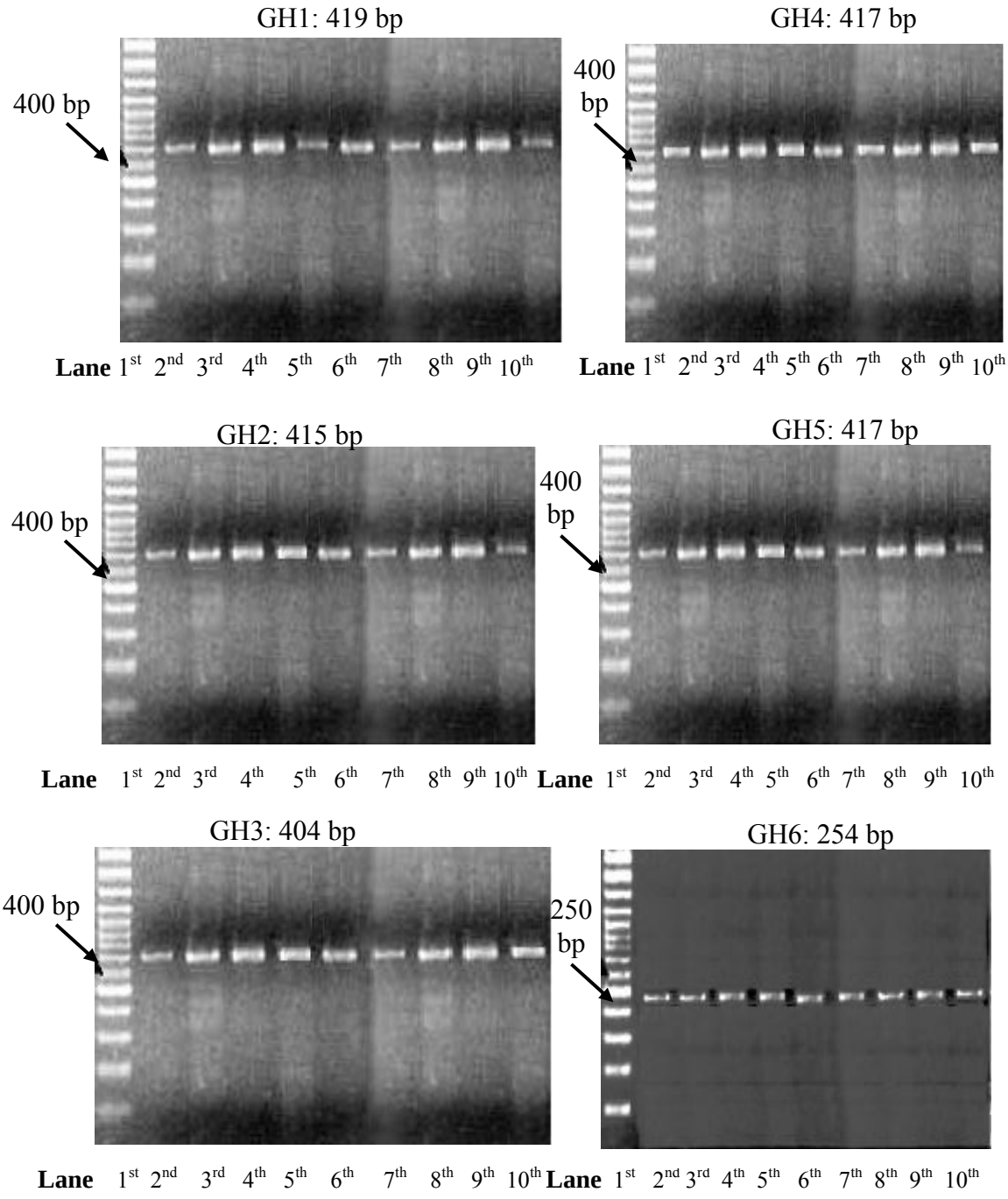
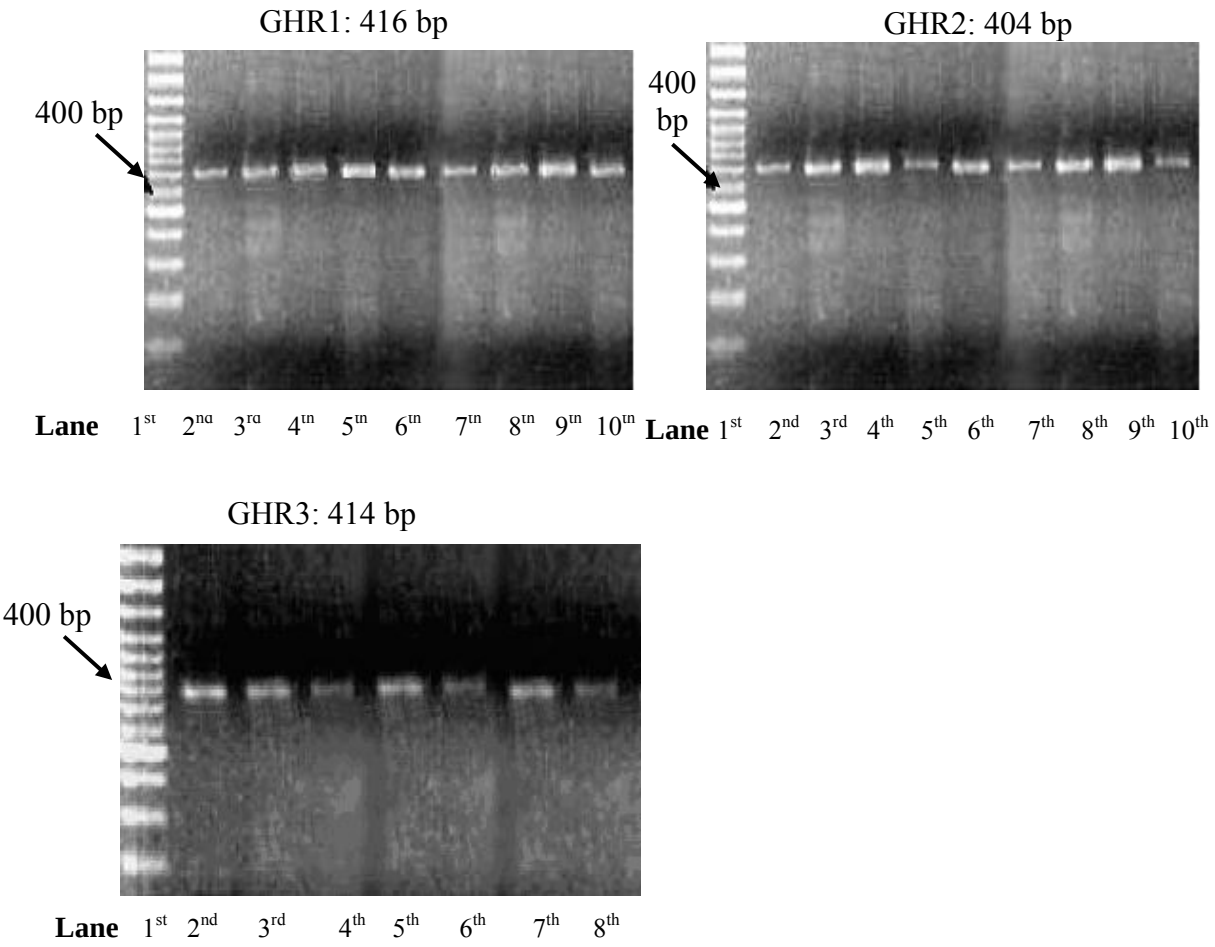
APPENDIX



Appendix Figure 1 The six fragments PCR products of growth hormone gene (*GH1* to *GH6*)

Note The first lane represent 50 bp marker, The second to tenth lanes represent samples of the animals.



Appendix Figure 2 The three fragments PCR products of growth hormone receptor gene (*GHR1* to *GHR3*)

Note The first lane represent 50 bp marker, The second to tenth lanes represent samples of the animals

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Query1: 43    catagacagcagcccaccaggtccccgtccctgggattctccaggcaagaacattattg  102
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 43    catagacagcagcccaccaggtccccgtccctgggattctccaggcaagaacattattg  102
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 43    catagacagcagcccaccaggtccccgtccctgggattctccaggcaagaacattattg  102
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 83     catagacagcagcccaccaggtccccgtccctgggattctccaggcaagaacattgttg  142

Query1: 103   gagtgggttgccatttcctcctccaatgcatgaaagtgaaaagtgaaagtgatcagtgg  162
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 103   gagtgggttgccatttcctcctccaatgcatgaaagtgaaaagtgaaagtgatcagtgg  162
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 103   gagtgggttgccatttcctcctccaatgcatgaaagtgaaaagtgaaagtgatcagtgg  162
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 143    gagtgggttgccatttcctcctccaatgcatgaaagtgaaaagtgaaagtgatcagtgg  202

Query1: 163   tgtctcactcagttgtgtccgaccctcagcgaccccatggactgcagccttccctcaga  222
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 163   tgtctcactcagttgtgtccgaccctcagcgaccccatggactgcagccttccctcaga  222
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 163   tgtctcactcagttgtgtccgaccctcagcgaccccatggactgcagccttccctcaga  222
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 203    tgtctcactcagttgtgtccgaccctcagcgaccccatggactgcagccttccctcaga  262

Query1: 223   atggggtgccattgccttctcctcgcttctgtatagagcacacaggtctacctcccatc  282
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Query2: 223   atggggtgccattgccttctcctcgcttctgtatagagcacacaggtctacctcccatc  282
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Query3: 223   atggggtgccattgccttctcctcgcttctgtatagagcccacaggtctacctcccatc  282
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct: 263    atggggtgccattgccttctcctcgcttctgtatagagcacacaggtctacctcccatc  322

Query1: 283   ctttaaaaagaaaacctatggggtgggctctcaagctgagaccctgtgtgcacagcccg  342
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 283   ctttaaaaagaaaacctatggggtgggctctcaagctgagaccctgtgtgcacagcccg  342
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 283   ctttaaaaagaaaacctatggggtgggctctcaagctgagaccctgtgtgcacagcccg  342
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 323    ctttaaaaagaaaacctatggggtgggctctcaagctgagaccctgtgtgcacagcccg  382
```

Appendix Figure 3 Comparison nucleotide sequences between *GH1* and growth hormone gene from Genbank

Note Query1 represents sequencing the 1st pattern (A_1A_1) of *GH1*, and Query2 represents sequencing the 2nd pattern (A_1B_1) of *GH1*, Query3 represents sequencing the 3rd pattern (B_1B_1) of *GH1*and Sbjct represents growth hormone gene from Genbank

```
Query1: 343   cctctggctggtggcagtggagacgggatgatgacaagcctgggggacatgaccccgct  402
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 343   cctctggctggtggcagtggagacgggatgatgacaagcctgggggacatgaccccgct  402
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 343   cctctggctggtggcagtggagacgggatgatgacaagcctgggggacatgaccccgct  402
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 383    cctctggctggtggcagtggagacgggatgatgacaagcctgggggacatgaccccgct  442

Query1: 403   agagaaggaacgggaacaggatgagtgagaggaggttctaaattatccattagcactc   461
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 403   agagaaggaacgggaacaggatgagtgagaggaggttctaaattatccattagcactc   461
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 403   agagaaggaacgggaacaggatgagtgagaggaggttctaaattatccattagcactc   461
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 443    agagaaggaacgggaacaggatgagtgagaggaggttctaaattatccattagcactc   501
```

Appendix Figure 3 (Continuted)

Note Query1 represents sequencing the 1st pattern (A_1A_1) of *GH1*, and Query2 represents sequencing the 2nd pattern (A_1B_1) of *GH1*, Query3 represents sequencing the 3rd pattern (B_1B_1) of *GH1*and Sbjct represents growth hormone gene from Genbank

```
Query1: 40    ggtccttgcataaatgtatagagcacacaggtgggggaaagggagagagagaagaatc   99
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 40    ggtccttgcataaatgtatagagcacacaggtgggggaaagggagagagagaagaatc   99
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 40    ggtccttgcataaatgtatagagcacacaggtgggggaaagggagagagagaagaatc   99
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 341    ggtccttgcataaatgtatagagcacacaggtgggggaaagggagagagagaagaatc   400

Query1: 100   agccagggtataaaaatggcccagcagggaccaattccaggatcccaggacccagtggg   159
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 100   agccagggtataaaaatggcccagcagggaccaattccaggatcccaggacccagtggg   159
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 100   agccagggtataaaaatggcccagcagggaccaattccaggatcccaggacccagtggg   159
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 401    agccagggtataaaaatggcccagcagggaccaattccaggatcccaggacccagtggg   460

Query1: 160   tcaccagacgactcagggtcctgtggacagctcaccagctatgatggctgcaggtaggc   219
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 160   tcaccagacgactcagggtcctgtggacagctcaccagctatgatggctgcaggtaggc   219
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 160   tcaccagacgactcagggtcctgtggacagctcaccagctatgatggctgcaggtaggc   219
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 461    tcaccagacgactcagggtcctgtggacagctcaccagctatgatggctgcaggtaggc   520

Query1: 220   agctcgctaaaatcccctccattcgcgtgtcctaaaggggtaatgcgggggcccctcga   279
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 220   agctcgctaaaatcccctccattcgcgtgtcctaaaggggtaatgcgggggcccctcga   279
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 220   agctcgctaaaatcccctccattcgcgtgtcctaaaggggtaatgcgggggcccctcga   279
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 521    agctcgctaaaatcccctccattcgcgtgtcctaaaggggtaatgcgggggcccctcga   580

Query1: 280   gccgatggatgtgttcagagctttgggctttagggcttccgaatgtgaacataggtatc   339
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 280   gccgatggatgtgttcagagctttgggctttagggcttccgaatgtgaacataggtatc   339
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 280   gccgatggatgtgttcagagctttgggctttagggcttccgaatgtgaacataggtatc   339
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 581    gccgatggatgtgttcagagctttgggctttagggcttccgaatgtgaacataggtatc   640
```

Appendix Figure 4 Comparison nucleotide sequences between *GH2* and growth hormone gene from Genbank

Note Query1 represents sequencing the 1st pattern (A_2A_2) of *GH2*, and Query2 represents sequencing the 2nd pattern (A_2B_2) of *GH2*, Query3 represents sequencing the 3rd pattern (A_2C_2) of *GH2* and Sbjct represents growth hormone gene from Genbank

```
Query1: 340   atctacacccagacatttggccaagtttaaatgttctcagtccctggagggaaggatta   399
              ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Query2: 340   atctacacccagacatttggccaagttgaaatgttctcagtccctggagggaaggatta   399
              ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Query3: 340   atctacacccagacatttggccaagttcaaatgttctcagtccctggagggaaggatta   399
              ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct: 641    atctacacccagacatttggccaagtttaaatgttctcagtccctggagggaaggatta   700

Query1: 400   gtaggtggggctggcaggagatcaggcgtctagctccctggggccctccgtc          453
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 400   gtaggtggggctggcaggagatcaggcgtctagctccctggggccctccgtc          453
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 400   gtaggtggggctggcaggagatcaggcgtctagctccctggggccctccgtc          453
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 701    gtaggtggggctggcaggagatcaggcgtctagctccctggggccctccgtc          754
```

Appendix Figure 4 (Continued)

Note Query1 represents sequencing the 1st pattern (A_2A_2) of *GH2*, and Query2 represents sequencing the 2nd pattern (A_2B_2) of *GH2*, Query3 represents sequencing the 3rd pattern (A_2C_2) of *GH2* and Sbjct represents growth hormone gene from Genbank

```
Query1: 355    cctgcagttcctcagcagagtcttcaccaacagcttggtgtttggcacctcggacc  414
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 355    cctgcagttcctcagcagagtcttcaccaacagcttggtgtttggcacctcggacc  414
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 355    cctgcagttcctcagcagagtcttcaccaacagcttggtgtttggcacctcggacc  414
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query4: 355    cctgcagttcctcagcagagtcttcaccaacagcttggtgtttggcacctcggacc  414
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query5: 355    cctgcagttcctcagcagagtcttcaccaacagcttggtgtttggcacctcggacc  414
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:2022     cctgcagttcctcagcagagtcttcaccaacagcttggtgtttggcacctcggacc  414

Query1: 415    gtgtctatgagaagctgaaggacctggaggaaggcatcctggccctgatgcgggtg  474
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 415    gtgtctatgagaagctgaaggacctggaggaaggcatcctggccctgatgcgggtc  474
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 415    gtgtctatgagaagctgaaggacctggaggaaggcatcctggccctgatgcgggtc  474
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query4: 415    gtgtctatgagaagctgaaggacctggaggaaggcatcctggccctgatgcgggtc  474
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query5: 415    gtgtctatgagaagctgaaggacctggaggaaggcatcctggccctgatgcgggtg  474
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:2082     gtgtctatgagaagctgaaggacctggaggaaggcatcctggccctgatgcgggtg  2141

Query1: 475    gggatggcgggcaggacccagtgcgttgtgggtcccttccatgtgggggccatgcc  534
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 475    gggatggcgggcaggacccagtgcgttgtgggtcccttccatgtgggggccatgcc  534
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 475    gggatggcgggcaggacccagtgcgttgtgggtcccttccatgtgggggccatgcc  534
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query4: 475    gggatggcgggcaggacccagtgcgttgtgggtcccttccatgtgggggccatgcc  534
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query5: 475    gggatggcgggcaggacccagtgcgttgtgggtcccttccatgtgggggccatgcc  534
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:2142     gggatggcgggcaggacccagtgcgttgtgggtcccttccatgtgggggccatgcc  2201

Query1: 535    cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  594
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 535    cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  594
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 535    cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  594
```

Appendix Figure 5 Comparison nucleotide sequences between *GH5* and growth hormone gene from Genbank

Note Query1 represents sequencing the 1st pattern (A_5A_5) of *GH5*, and Query2 represents sequencing the 2nd pattern (A_5B_5) of *GH5*, Query3 represents sequencing the 3rd pattern (B_5C_5) of *GH5*, Query4 represents sequencing the 4th pattern (B_5D_5) of *GH5,* Query5 represents sequencing the 5th pattern (C_5D_5) of *GH5* and Sbjct represents growth hormone gene from Genbank

```
Query3: 535    cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  594
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query4: 535    cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  594
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query5: 535    cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  594
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:2202     cgccctctcctggcttagccaggagaatgcacgtgggcttggggagacagatccct  2261

Query1: 595    gctctctccctctttctagcagtccagccttgacccaggggaaacctttcccctt  654
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 626    gctctctccctctttctagcagtccagccttgacccaggggaaacctttcccctt  654
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 675    gctctctccctctttctagcagtccagccttgacccaggggaaacctttcccctt  654
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query4: 477    gctctctccctctttctagcagtccagccttgacccaggggaaacctttcccctt  654
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query5: 552    gctctctccctctttctagcagtccagccttgacccaggggaaacctttcccctt  654
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct:2260     gctctctccctctttctagcagtccagccttgacccaggggaaacctttcccctt  2319

Query1: 655    ttgaaacctccttcctcgcccttctccaagcctgtaggggagggtggaaaatggag  714
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Query2: 686    ttgaaacctccttcctcgcccttctccaagcctgtaggggagggtggaaaatggag  714
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Query3: 735    ttgaaacctccttcctcgcccttctccaagcgtgtaggggagggtggaaaatggag  714
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Query4: 537    ttgaaacctccttcctcgcccttctccaagcttgtaggggagggtggaaaatggag  714
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Query5: 612    ttgaaacctccttcctcgcccttctccaagcttgtaggggagggtggaaaatggag  714
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct:2320     ttgaaacctccttcctcgcccttctccaagcgtgtaggggagggtggaaaatggag  2379

Query1: 715    cgggcaggagggagctgctcctgagggcccttcggcctctctgtctctccctccct  772
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 746    cgggcaggagggagctgctcctgagggcccttcggcctctctgtctctccctccct  772
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 795    cgggcaggagggagctgctcctgagggcccttcggcctctctgtctctccctccct  772
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query4: 597    cgggcaggagggagctgctcctgagggcccttcggcctctctgtctctccctccct  772
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query5: 672    cgggcaggagggagctgctcctgagggcccttcggcctctctgtctctccctccct  772
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2380    cgggcaggagggagctgctcctgagggcccttcggcctctctgtctctccctccct  2437
```

Appendix Figure 5 (Continued)

Note Query1 represents sequencing the 1st pattern (A_5A_5) of *GH5*, and Query2 represents sequencing the 2nd pattern (A_5B_5) of *GH5*, Query3 represents sequencing the 3rd pattern (B_5C_5) of *GH5*, Query4 represents sequencing the 4th pattern (B_5D_5) of *GH5*, Query5 represents sequencing the 5th pattern (C_5D_5) of *GH5* and Sbjct represents growth hormone gene from Genbank

```
Query1: 40    acgcacccagaagtggtcacaccctgccaatgcgaggtagacgccaatccctccct  99
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 40    acgcacccagaagtggtcacaccctgccaatgcgaggtagacgccaatccctccct  99
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 40    acgcacccagaagtggtcacaccctgccaatgcgaggtagacgccaatccctccct  99
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1644   acgcacccagaagtggtcacaccctgccaatgcgaggtagacgccaatccctccct  1703

Query1: 100   aaagtacattgccctggcccctcatgtcgaggctgaatcacacgtagatcttggca  159
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 100   aaagtacattgccctggcccctcatgtcgaggctgaatcacacgtagatcttggca  159
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 100   aaagtacattgccctggcccctcatgtcgaggctgaatcacacgtagatcttggca  159
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1704   aaagtacattgccctggcccctcatgtcgaggctgaatcacacgtagatcttggca  1763

Query1: 160   agccaaaccctgccaaaaacattgattttatgcccgctttaaccagaggcttaaa  219
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 160   agccaaaccctgccaaaaacattgattttatgcccgctttaaccagaggcttaaa  219
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 160   agccaaaccctgccaaaaacattgattttatgcccgctttaaccagaggcttaaa  219
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1764   agccaaaccctgccaaaaacattgattttatgcccgctttaaccagaggcttaaa  1823

Query1: 220   gaagacatttacatcaccacagaaagccttaccactacagctgggagggcctagca  279
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||
Query2: 220   gaagacatttacatcaccacagaaagccttaccactacagctgggagggcctagca  279
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||
Query3: 220   gaagacatttacatcaccacagaaagccttaccactacaactgggagggcctagca  279
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct: 1824   gaagacatttacatcaccacagaaagccttaccactacagctgggagggcctagca  1883

Query1: 280   gtaagactgaagggtccggggacagccccagtgttaagaacatgttcaacctggac  339
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 280   gtaagactgaagggtccggggacagccccagtgttaagaacatgttcaacctggac  339
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 280   gtaagactgaagggtccggggacagccccagtgttaagaacatgttcaacctggac  339
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1884   gtaagactgaagggtccggggacagccccagtgttaagaacatgttcaacctggac  1943
```

Appendix Figure 6 Comparison nucleotide sequences between *GHR3* and growth hormone gene from Genbank

Note Query1 represents sequencing the 1st pattern (X_3X_3) of *GH1*, and Query2 represents sequencing the 2nd pattern (X_3Y_3) of *GH1*, Query3 represents sequencing the 3rd pattern (Y_3Y_3) of *GH1*and Sbjct represents growth hormone gene from Genbank

```
Query1: 340   caagttctgagatacctgtcccagattatacctccattcatatagtacttgttatt  399
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 340   caagttctgagatacctgtcccagattatacctccattcatatagtacttgttatt  399
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 340   caagttctgagatacctgtcccagattatacctccattcatatagtacttgttatt  399
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1944   caagttctgagatacctgtcccagattatacctccattcatatagtacttgttatt  2003


Query1: 400   cagtctccacagggcctcgtactcaatgcgactgccctgcccttatacctcc      459
              |||||||||||||||||||||||||||||||||||||||||||||||||||
Query2: 400   cagtctccacagggcctcgtactcaatgcgactgccctgcccttatacctcc      459
              |||||||||||||||||||||||||||||||||||||||||||||||||||
Query3: 400   cagtctccacagggcctcgtactcaatgcgactgccctgcccttatacctcc      459
              |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 2004   cagtctccacagggcctcgtactcaatgcgactgccctgcccttatacctcc      2057
```

Appendix Figure 6 (Continued)

Note Query1 represents sequencing the 1st pattern (X_3X_3) of *GH1*, and Query2 represents sequencing the 2nd pattern (X_3Y_3) of *GH1*, Query3 represents sequencing the 3rd pattern (Y_3Y_3) of *GH1*and Sbjct represents growth hormone gene from Genbank

Appendix Table 1 Frequency of breed groups in this multibreed population

Breed group			n	Percent
BG1	1/2CH1/4BR1/4NA		34	26.20
BG2	1/2CH5/16BR3/16NA		25	19.20
BG3	1/2CH3/8BR1/8NA		15	11.50
BG4	CH<1/2			
		7/16CH3/8BR3/16NA	2	1.50
		3/8CH5/16BR5/16NA	1	0.80
		3/8CH3/8BR1/4NA	3	2.30
		3/8CH1/2BR1/8NA	1	0.80
		1/4CH5/16BR7/16NA	1	0.80
		1/4CH7/16BR5/16NA	1	0.80
		1/4CH1/2BR1/4NA	2	1.50
		1/4CH5/8BR1/8NA	1	0.80
		1/4CH11/16BR1/16NA	1	0.80
BG5	CH=1/2			
		1/2CH1/8BR3/8NA	6	4.60
		1/2CH5/32BR11/32NA	1	0.80
		1/2CH3/16BR5/16NA	5	3.80
		1/2CH7/32BR9/32NA	8	6.20
		1/2CH9/32BR7/32NA	10	7.70
		1/2CH11/32BR5/32NA	3	2.30
BG6	CH<1/2			
		5/8CH1/4BR1/8NA	4	3.10
		5/8CH13/64BR11/64NA	1	0.80
		19/32CH9/32BR1/8NA	1	0.80
		19/32CH5/16BR3/32NA	1	0.80
		9/16CH1/4BR3/16NA	1	0.80
		9/16CH5/32BR9/32NA	1	0.80
		9/16CH9/32BR5/32NA	1	0.80

Note [1]CH=Charolais, BR=Brahman and NA=Thai native

Appendix Table 2 Least square means±standard error of sex and breed groups in SNPs and haplotypic models

Factors	Model with SNPs effect		Factors	Model with haplotypic effectl	
	BW	WW		BW	WW
Sex	**ns**	**ns**	**Sex**	**ns**	**ns**
Male	25.56±1.08	163.49±15.42	Male	27.08±0.58	169.11±7.99
Female	25.27±0.99	159.08±14.19	Female	26.61±0.44	165.25±6.07
Breed groups	**ns**	*****	**Breed groups**	**ns**	*****
1/2CH1/4BR 1/4NA	25.98±1.11	175.31±15.51[a]	1/2CH1/4BR 1/4NA	27.02±0.81	173.11±8.87[a]
1/2CH5/16BR 3/16NA	26.63±1.09	183.92±15.76[a]	1/2CH5/16BR 3/16NA	27.89±0.65	184.39±7.92[a]
1/2CH3/8BR 1/8NA	26.09±1.29	160.52±21.11[b]	1/2CH3/8BR 1/8NA	27.09±0.58	164.34±7.83[b]
CH<1/2	25.21±1.13	147.94±16.09[c]	CH<1/2	26.78±0.57	161.04±13.82[c]
CH=1/2	24.68±1.23	152.25±18.38[b]	CH=1/2	26.47±0.80	162.22±11.14[b]
CH>1/2	23.91±1.48	147.77±17.57[c]	CH>1/2	25.83±1.01	157.97±11.00[c]

Note * = P<0.05, ** = P<0.01, ns = non significant (P>0.05), [1]CH=charolais, BR=Brahman and NA=Thai native

Appendix Table 3 ANOVA table of model with SNPs for birth weight (BW)

Source	**df**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	48	3,691.6641	76.9097	13.17	0.0001
Error	81	473.1128	5.8409		
Corrected Total	129	4,164.7769			

R-Square	**C.V.**	**Root MSE**	**BW Means**
0.8864	8.53064	2.4168	28.3308

Source	**df**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
GH1	2	871.7566	435.8783	74.63	0.0001
GH2	2	57.2206	28.6103	4.90	0.0098
GH5	4	2,358.8223	589.7055	100.96	0.0001
GHR3	2	26.3284	13.1642	2.25	0.1115
CG	32	338.3805	10.5744	1.81	0.0171
SEX	1	1.6289	1.6289	0.28	0.5989
BG	5	45.1053	9.0210	1.54	0.1853

Source	**df**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
GH1	2	126.8955	63.4477	10.86	0.0001
GH2	2	1.1739	0.5870	0.10	0.9045
GH5	4	391.7638	97.94010	16.77	0.0001
GHR3	2	18.5066	9.2533	1.58	0.2114
CG	32	299.6004	9.66453	1.65	0.0376
SEX	1	1.3347	1.3347	0.23	0.6339
BG	5	45.1053	9.02107	1.54	0.1853

Note BG = breed groups, GH1, GH2 and GH5 = first, second and fifth of PCR product fragments of growth hormone gene, GHR3 = third of PCR product fragment of growth hormone receptor gene and CG = contemporary groups (year-season of birth)

Appendix Table 4 ANOVA table of model with haplotypic effect for birth weight (BW)

Source	**df**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	59	3,871.4320	65.6174	15.26	0.0001
Error	70	300.9237	4.2989		
Corrected Total	129	4,172.3557			
	R-Square	**C.V.**	**Root MSE**	**BW Means**	
	0.9279	7.3194	2.0733	28.32692308	
Source	**df**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Haplotype	23	3,554.2932	161.5588	37.58	0.0001
CG	30	286.0225	9.2265	2.15	0.0043
SEX	1	0.7142	0.7142	0.17	0.6848
BG	5	30.4020	6.0804	1.41	0.2298
Source	**Df**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Haplotype	23	2,332.5416	111.0734	25.84	0.0001
CG	30	270.7802	9.0260	2.10	0.0057
SEX	1	0.5295	0.5295	0.12	0.7267
BG	5	30.4020	6.0804	1.41	0.2298

Note BG = breed groups and CG = contemporary groups (year-season of birth)

Appendix Table 5 ANOVA table of model with SNPs for weaning weight (WW)

Source	df	Sum of Squares	Mean Square	F Value	Pr > F
Model	48	96,749.7292	2,015.6194	1.69	0.0183
Error	81	96,513.6939	1,191.5271		
Corrected Total	129	193,263.4231			

R-Square	C.V.	Root MSE	BW Means
0.5006	20.7510	34.5185	166.3462

Source	df	Type I SS	Mean Square	F Value	Pr > F
GH1	2	25,221.1579	12,610.5789	10.59	0.0001
GH2	2	6,154.8768	3,077.4384	2.58	0.0817
GH5	4	11,355.8909	2,838.9727	2.38	0.0581
GHR3	2	2,429.7698	1,214.8849	1.02	0.3651
CG	32	34,774.7066	1,086.7095	0.91	0.6040
SEX	1	467.3629	467.3629	0.39	0.5328
BG	5	16,383.8995	3,276.7799	2.75	0.0240

Source	df	Type III SS	Mean Square	F Value	Pr > F
GH1	2	7,466.4041	3,733.2021	3.13	0.0489
GH2	2	4,594.7766	2,297.3883	1.93	0.1520
GH5	4	2,936.4076	734.1019	0.62	0.6523
GHR3	2	2,354.1156	1,177.0578	0.99	0.3768
CG	32	35,799.5271	1,154.8235	0.97	0.5237
SEX	1	274.1805	274.1805	0.23	0.6327
BG	5	16,383.1431	3,276.6286	2.75	0.0240

Note BG = breed groups, GH1, GH2 and GH5 = first, second and fifth of PCR product fragments of growth hormone gene, GHR3 = third of PCR product fragment of growth hormone receptor gene and CG = contemporary groups (year-season of birth)

Appendix Table 6 ANOVA table of model with haplotypic effect for weaning weight (WW)

Source	**df**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	59	112,453.1394	1,905.9854	1.65	0.0222
Error	70	80,795.4631	1,154.2209		
Corrected Total	129	193,248.6026			
	R-Square	**C.V.**	**Root MSE**	**BW Means**	
	0.5819	20.4235	33.9738	166.34630769	
Source	**df**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Haplotype	23	61,879.3590	2,812.6981	2.44	0.0026
CG	30	39,477.8388	1,273.4787	1.10	0.3587
SEX	1	472.0901	472.0901	0.41	0.5246
BG	5	10,623.8515	2,124.7703	1.84	0.1161
Source	**Df**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Haplotype	23	45,896.3380	2,185.5399	1.89	0.0249
CG	30	37,071.7010	1,235.7233	1.07	0.3966
SEX	1	306.8276	306.8276	0.27	0.6078
BG	5	10,623.8515	2,124.7703	1.84	0.0161

Note BG = breed groups and CG = contemporary groups (year-season of birth)

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