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Effect of Genetic Polymorphism of Bovine Growth Hormone Gene on Preweaning Growth Traits in a Thai Multibreed Beef Population

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ABSTRACT

Growth hormone (GH) gene was investigated as a candidate gene for birth weight (BW) and weaning weight (WW) in a population of multibreed beef cattle of three crossbreds. Beef cattle involved consisted of Charolais, Brahman and Thai local native with different breed fractions. Screening polymorphisms within bovine GH gene was performed by using a single strand conformation polymorphism (SSCP) method and the result was confirmed by a sequencing analysis. To identify polymorphic sites within the bovine GH gene, primers were designed revealing 6 PCR fragments (GH1 to GH6). Four single nucleotide polymorphisms (SNPs) were found in the bovine GH gene of this cattle population. Two SNPs were found within the GH1 and GH2 fragments at nucleotide position 303 and 218 bp. The other two SNPs were located at position 208 and 388 bp of the GH5 fragment. No polymorphisms were found in the GH3, GH4 and GH6 fragments, respectively. The genotypic frequencies of GH1 were found to be 5.40, 85.40 and 9.20 percents for A_1A_1 A_1B_1 and B_1B_1, respectively. The genotypic frequencies of GH2 were found to be 90.00, 8.46 and 1.54 percents for A_2A_2, A_2B_2 and A_2C_2, respectively. The GH5 were found to be 2.31, 30.77, 17.69, 13.85 and 35.38 percents for A_5A_5, A_5B_5, B_5C_5, B_5D_5 and C_5D_5 genotypes, respectively. Considering haplotype of four polymorphic sites, only 17 haplotypes were identified in this population. The 212 and 215 haplotypes were found at a high frequency. Effect of four SNPs in each region genotype regions of GH for BW and WW indicated that GH1 influenced both BW and WW. However, the GH5 influenced only for BW ($P<0.01$). Animals with B_1B_1 of GH1 had the highest BW and WW but animals with A_1A_1 had the lowest of both traits ($P<0.05$). Animals with C_5D_5 of GH5 had the highest BW but animals with A_5A_5 had lowest BW ($P<0.05$). Moreover, haplotype of four SNPs were analysed and the results showed the animals with 313 and 325 haplotypes obtained high BW and WW, respectively ($P<0.05$). However, animals with 111 and 234 haplotypes obtained low BW and WW, respectively ($P<0.05$). Therefore, the producers should select animals with B_1B_1 and C_5D_5 genotypes to obtain high BW and WW. Moreover, mating system should be considered because intra- and inter-genic polymorphic regions or haplotypes has an effect on BW and WW.

Key words: cattle, bovine growth hormone gene, birth weight, weaning weight

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INTRODUCTION

The bovine growth hormone is a 22 kDa single chain polypeptide produced in the anterior pituitary gland. The encoding gene is 2,206 base pair (bp) and consists of five exons separated by four intervening sequences (Hediger *et al*., 1990). It is well known to play an important role in several biological processes such as growth, metabolism regulation and mammary development (Jiang and Lucy, 2001). Therefore, it is a promising candidate gene marker for improving growth, meat and milk production in cattle. Several authors reported that polymorphism of growth hormone gene occurred at promoter and fifth exon (Moody *et al.,* 1996; Reis *et al*., 2001; Kim *et al.,* 2004). Lucy *et al*. (1998) reported that a substitution of cytosine (C) for guanine (G) at position 2,141 caused an amino acid change from leucine to valine at residue 127. However, study on polymorphism of the entire length of bovine growth hormone gene is not yet undertaken. In this research, single strand conformation polymorphism (SSCP) was used to identify point mutations of growth hormone gene. The objectives of this research were: (1) to screen the entire length of growth hormone gene for sequence variation in a multibreed beef population (2) to estimate genotype and allelic frequencies of this gene and (3) to evaluate the effect of polymorphic region of bovine growth hormone gene on birth weight (BW) and weaning weight (WW).

MATERIALS AND METHODS

Animals: The animals selected for this study were raised at Buffalo and Beef Production Research and Development Center (BPRDC), Kamphangsaen campus, Nakhon Prathom and at Kasetsart University. Complete information on the preweaning growth traits of each animal was recorded. The total number of 130 animals used in the present study were born during the years 1987 to 2004 and passed the performance test. Live weight and body measurements were taken at birth and at weaning at 7 months old. The crossbreds were obtained from crossing Charolais (CH), Brahman (BR) and Thai local native (NA) cattle.

DNA samples: DNA was obtained from peripheral blood leukocytes collected from 130 animals. Approximately 10 ml blood was withdrawn from the jugular vein of each animal and genomic DNA was isolated by using Proteinase K and phenol: chloroform: isoamyl alcohol method according to the procedure of Sambrook *et al*. (1989).

DNA amplification with polymerase chain reaction (PCR): To amplify DNA fragments of bovine GH gene, primers were designed based on the bovine GH sequence (GenBank accession number J0008) by using the Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi) as shown in Table 1. Six PCR fragments of the bovine GH gene (GH1 to GH6)

Table 1 Primers used for screening polymorphisms in the bovine GH gene.

Fragment	Primer forwards	Primer reverse	$Tm^{1/}$ (°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

$Tm^{1/}$ = melting temperature.

were amplified with 254 to 419 bp (Table 1). The PCR was performed in a 0.9 µl reaction mixture containing 20ng DNA, 5 µM of each primers, 5 mM of each dNTPs and 1 unit of Taq DNA Polymerase in 1XPCR buffer. The PCRs were performed at 94 °C for 3 min, followed by 40 cycles of 94 °C for 50 second, 59-63 °C (Table 1) for 50 sec, 72 °C for 1 minute and final extension at 72 °C for 7 min. The PCR products were kept at -20 °C.

SSCP analysis: SSCP was carried out with a Biorad "Sequi-gen® GT nucleic acid vertical gel electrophoresis". Two microliter of PCR product was diluted with 2 µl of a loading dye of SSCP. The mixture was denatured at 94 °C for 7 min and then, snap cooled on ice. Subsequently, they were loaded on a 10% non- denaturing polyacrylamide gel. Electrophoresis was performed in 1X TBE buffer using 10 watts for 8 hours at 4°C. The gel was stained using the Silver Staining System. Every difference pattern was confirmed by sequencing.

Classification of genotypes: The following alphabetical symbols of genotype are used in this study: the A_1A_1, A_1B_1, B_1B_1 represent polymorphic of growth hormone gene of GH1, the A_2A_2, A_2B_2 and A_2C_2 represent GH2 and the A_5A_5, A_5B_5, B_5C_5, B_5D_5 and C_5D_5 represent GH5. The following numerical symbols were used for the growth hormone haplotypes: the first number represented GH1 genotype, the second number represented GH2 genotype and the third number represented GH5 genotype. For example, the haplotype 111 symbolizes genotype A_1A_1 of GH1, A_2A_2 of GH2 and A_5A_5 of GH5.

Statistical analysis: The effect of SNPs and haplotypes on BW and WW were analysed by least square method. Other effects were breed regression of CH, BR and NA, sexes, contemporary groups (year-season of birth). Season in each year was identified as cold (November to February), hot (March to June) and raining (July to October) (Thai Meteorological Department, 2002). The mean comparisons among SNPs and haplotypes were carried out using option PDIFF as applied in the general linear model (GLM) procedure of SAS (1996). The statistical models for the study were:

The first model:

$$y_{ijklmn} = \mu + GH1_i + GH2_j + GH5_k + CG_l + Sex_m + b1\left(CH - \overline{CH}\right)_{ijklmn} + b2\left(BR - \overline{BR}\right)_{ijklmn} + b3\left(NA - \overline{NA}\right)_{ijklmn} + e_{ijklmn}$$

The terms in the models were:

y_{ijklmn} = BW and WW of n^{th} of animal in i^{th} of GH1 fragment, j^{th} of GH2 fragment and k^{th} of GH5 fragment in l^{th} of contemporary groups and m^{th} sex,

μ = the overall means,

$GH1_i$ = the fixed effect of genotypes of GH1 fragment growth hormone gene ($i = A_1A_1$, A_1B_1 and B_1B_1),

$GH2_j$ = the fixed effect of genotypes of GH2 fragment growth hormone gene ($j = A_2A_2$, A_2B_2 and A_2C_2),

$GH5_k$ = the fixed effect of genotypes of GH5 fragment growth hormone gene ($k = A_5A_5$, A_5B_5, B_5C_5, B_5D_5 and C_5D_5),

CG_l = the fixed effect of contemporary groups (year-seasons of birth) ($l = 1, 2, \ldots, 33$),

Sex_m = the fixed effect of sex (m = male, female),

$b1\left(CH - \overline{CH}\right)_{ijklmn}$, $b2\left(BR - \overline{BR}\right)_{ijklmn}$, $b3\left(NA - \overline{NA}\right)_{ijklmn}$ = the fixed effect of breed regression on Charolais, Brahman and Thai local native breed fractions, and

e_{ijklmn} = random residual error associated with the ijklmnth observations (e_{ijk} ~ NID (0, σ^2_e)).

The second model:

$$y_{ijkl} = \mu + H_i + CG_j + Sex_k + b1\left(CH - \overline{CH}\right)_{ijkl} + b2\left(BR - \overline{BR}\right)_{ijkl} + b3\left(NA - \overline{NA}\right)_{ijkl} + e_{ijklmn}$$

The terms in the models were:

y_{ijkl} = birth weight and weaning weight of lth of animal in ith of haplotypes in jth of contemporary groups and kth sex,

μ = the overall means,

H_i = the fixed effect of haplotypes (i = 1, 2, …, 23),

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),

$b1\left(CH - \overline{CH}\right)_{ijkl}$, $b2\left(BR - \overline{BR}\right)_{ijkl}$, $b3\left(NA - \overline{NA}\right)_{ijkl}$ = the fixed effect of breed regression on Charolais, Brahman and Thai local native breed fractions, and

e_{ijkl} = random residual error associated with the ijklth observation (e_{ijk} ~ NID (0, σ^2_e)).

RESULTS AND DISCUSSION

Genotypic and allelic frequencies of SNPs

The single strand conformation polymorphism (SSCP) method detected a DNA polymorphism when the electrophoresis mobility of DNA bands was reproducibly different. Six growth hormone fragments (GH1-GH6), which covered almost the entire length of the bovine GH gene, were amplified. A PCR product was subjected to SSCP analysis to discover the DNA sequence variation. The position and length of the fragments were illustrated in Figure 1.

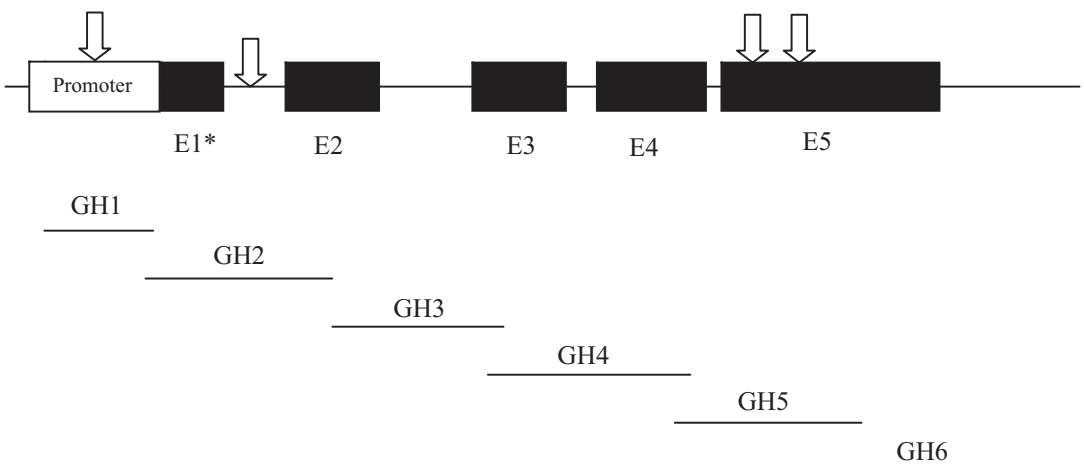


Figure 1 Map of growth hormone gene. The position and length of the first to fifth exons were illustrated as the black block (E1 to E5). The arrowheads indicated the locations of polymorphism. The solid lines (GH1 to GH6) indicated the sizes and locations of the first to sixth PCR fragments.

The GH3, GH4 and GH6 were detected as monomorphic under electrophoretic conditions (data not shown). However, analyses of the GH1, GH2 and GH5 fragments did reveal polymorphism. The analysis of 419 bp GH1 fragment revealed three genotypes (Figure 2). 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 revealed the difference in mobility, which had A-C transversion in the promoter region at nucleotide position 303. The sequence at this position of genotype A_1A_1 was found to be at base A. The genotype B_1B_1 was mutated by substitution from 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. They could be designated as A_1 and B_1 alleles, respectively.

The GH1 fragment was identified from 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. The genotypic frequency of A_1B_1 animals was the highest ($P<0.01$) within the present population. Allelic frequencies of A_1 and B_1 in a population were found to be 48.08 and 51.92 percent, respectively.

The SSCP patterns of GH2 fragment of the gene are illustrated in Figure 3. Three alleles were observed and designated as A_2 B_2 and C_2, respectively. The mutations at nucleotide position 218 were T-G transversion and T-C transition. The A_2A_2 genotype had base T at nucleotide position 218. The A_2B_2 heterozygous genotype occurred as one strand that changed from 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.

The A_2A_2, A_2B_2 and A_2C_2 genotypes were found from 117, 11 and 2 heads of cattle, respectively. Genotypic frequencies were found to be 90.00, 8.50 and 1.50 percents for A_2A_2, A_2B_2 and A_2C_2 genotypes, respectively Allelic frequencies of three alleles were found to be 95.00, 4.23 and 0.77 percents for A_2, B_2 and C_2, respectively. It showed that genotypic frequency of A_2C_2 was the lowest when compared with A_2A_2 and A_2B_2. Allelic frequency of A_2 was higher than other alleles in this population.

The GH5 fragment was identified as five different SSCP patterns (Figure 4). This fragment harboured two mutation points at fifth exon of

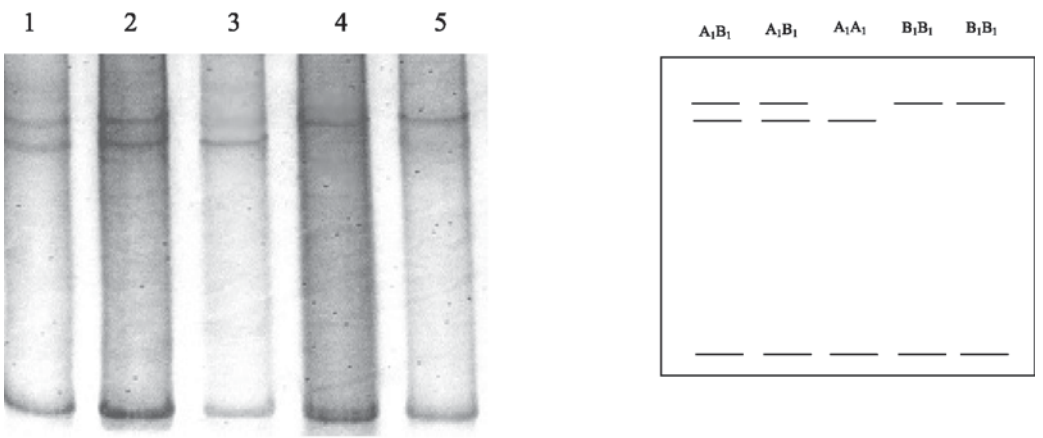


Figure 2 The different SSCP patterns of GH1. Lanes 3 showed pattern 1 (A_1A_1). Lane 4 and 5 showed pattern 2 (B_1B_1) and lane 1 and 2 showed pattern 3 (A_1B_1).

growth hormone gene. The first position of mutation had G - A transition at nucleotide position 208. The second position of mutation had C - G transversion and C-T transition at nucleotide position 388. The genotype A_5A_5 had base G and C at nucleotide position 208 and 388, respectively. The A_5B_5 heterozygous genotype was found to be mutated by the substitution of G by C at nucleotide position 208. 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 208 and 388, 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 208 and 388, respectively. Additionally, the C_5D_5 heterozygous genotype was mutated from C to T at nucleotide position 388. 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 Genotypes frequencies were found to be 2.31, 30.77, 17.69, 13.85 and 35.38 percents for A_5A_5, A_5B_5, B_5C_5, B_5D_5 and C_5D_5 genotypes, respectively. Allelic frequencies of A_5, B_5 C_5 and D_5 genotypes were found to be 17.96, 31.15, 26.54 and 24.62, respectively. In multibreed beef

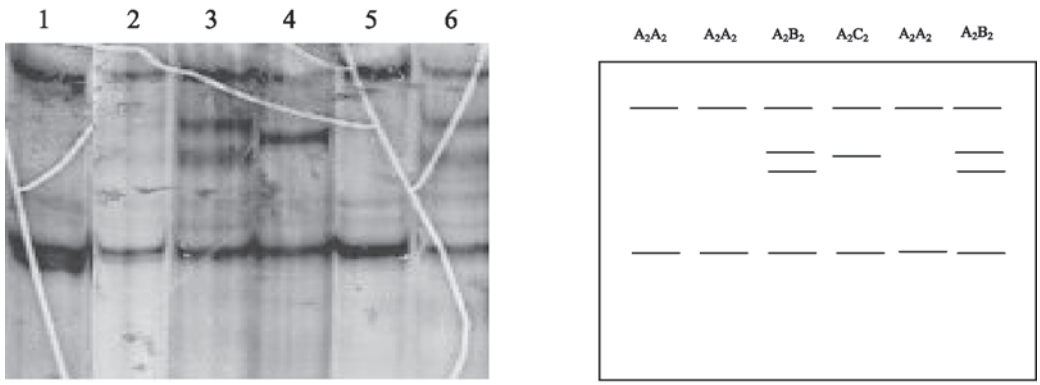


Figure 3 The different SSCP patterns of GH2. Lanes 1, 2 and 5 showed SSCP pattern 1 (A_2A_2). Lanes 3 and 6 showed SSCP pattern 2 (A_2B_2) and lane 4 showed pattern 3 (A_2C_2).

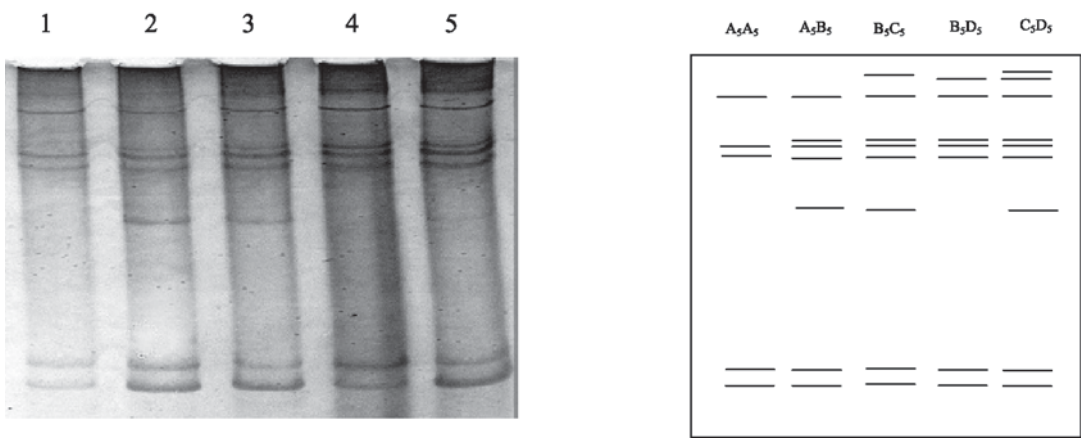


Figure 4 The different SSCP patterns of GH5. Lanes 1, 2, 3, 4 and 5 represented SSCP pattern 1, 2, 3, 4 and 5. They represented with genotypes A_5A_5, A_5B_5, B_5C_5, B_5D_5 and C_5D_5, respectively.

population, the genotypic frequency of C_5D_5 was found to be higher than the others. 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.

The effect of SNPs in growth hormone gene on preweaning growth traits

The effect of SNPs within bovine GH gene on BW and WW is presented in Table 2. Only one polymorphic site (GH1) influenced both BW and WW ($P<0.01$). Moreover, the SNPs of GH5 influenced only BW ($P<0.05$). This result was similar to Schlee *et al*. (1994) and Tambasco *et al*. (2003) who reported that the polymorphisms of GH gene hd some influence on growth hormone levels and growth traits. However, no effect of the SNPs on GH2 with preweaning growth traits was observed in this study. The mean value of BW and WW was significantly higher for B_1B_1 genotype than any other genotypes in the GH1 polymorphism ($P<0.05$). The C_5D_5 genotype on GH5 associated with high BW only ($P<0.05$). However, the A_1A_1 genotype on GH1 was found lowest on both BW and WW ($P<0.05$). The A_5A_5 genotype on GH5 was found lowest on BW only ($P<0.05$). On the other hand, the result on genotypic frequency indicated that genotypic frequencies of B_1B_1 of GH1 were of low values but genotypes frequency of C_5D_5 of GH5 was high. It indicated that C_5D_5 was favored genotype for selection of high BW and WW animals when phenotypic data was taken into consideration. Therefore, selection of B_1B_1 and C_5D_5 animals could increase BW and WW in the population.

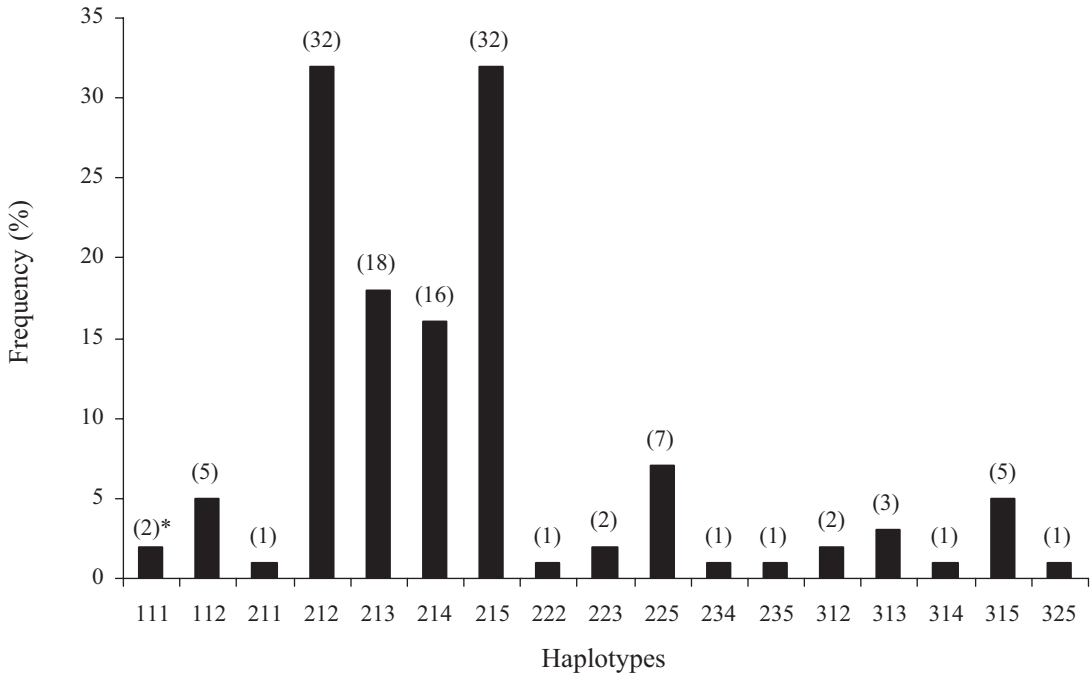
Genotypic frequencies of haplotype

From the total of 45 (3^2x5) haplotypes, 28 haplotypes theoretically possible combinations were not detected. Only 17 haplotypes were identified in this population (Figure 5). Animals with 212 and 215 haplotypes were found at higher than average proportion in this population. Animals with 211, 222, 234, 235, 314 and 315 haplotypes were of low frequencies.

Table 2 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.

Genotypes of SNPs	n (head)	BW (kg)	WW (kg)
GH1[1/]			
A_1A_1	7	22.09±1.35[a]	129.00±19.91[a]
A_1B_1	111	25.75±0.87[b]	159.73±12.85[a]
B_1B_1	12	29.57±1.24[c]	196.86±18.33[b]
GH2			
A_2A_2	117	25.64±0.62	157.73±9.09
A_2B_2	11	25.11±1.19	180.77±17.53
A_2C_2	2	26.66±1.97	147.09±29.05
GH5			
A_5A_5	3	19.05±1.84[a]	138.89±27.05
A_5B_5	40	22.57±1.05[b]	158.28±15.41
B_5C_5	23	25.87±1.10[c]	170.49±16.25
B_5D_5	18	27.92±1.10[d]	165.94±16.19
C_5D_5	46	33.61±0.92[e]	175.72±13.53

[1/] 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 marked with different letter are significantly different at $P < 0.05$



* numbers in the bracket is represented haplotypic frequencies

Figure 5 Haplotypic frequencies of bovine growth hormone gene.

Table 3 Least square means±standard error (SE) of haplotypes for birth weight (BW) and weaning weight (WW) in the multibreed population.

Haplotypes of GH gene	n(head)	BW (kg)	WW (kg)
111	2	15.71±1.81^{a}	100.01±28.97^{ab}
112	5	18.45±1.28^{a}	126.19±20.48^{abc}
211	1	18.33±2.45^{a}	144.76±39.21^{abc}
212	32	22.88±0.62^{b}	151.76±9.90^{abcd}
213	18	25.83±0.71^{c}	174.48±11.31^{abcd}
214	16	28.28±0.73^{d}	169.11±11.73^{abcd}
215	32	32.72±0.52^{e}	167.67±8.32b^{cd}
222	1	22.47±2.39^{ab}	211.42±38.18^{cd}
223	2	24.23±2.09^{c}	123.36±33.50^{abc}
225	7	32.93±1.04^{e}	194.23±16.64^{bcd}
234	1	29.47±2.35^{de}	97.81±37.60^{a}
235	1	34.38±2.35^{e}	214.12±37.62^{cd}
312	2	21.73±1.88^{b}	173.19±30.14^{bcd}
313	3	27.24±1.72^{d}	216.41±27.59^{cd}
314	1	27.16±2.37^{d}	172.91±37.94^{bcd}
315	5	41.51±1.23^{f}	206.99±19.66^{cd}
325	1	36.84±2.63^{f}	266.74±42.02^{cd}

$^{a, b, c, d, e}$ and ^{f} within the same column marked with different letters were significantly different at $P < 0.05$

Effect of haplotypes of growth hormone gene on preweaning growth traits

The least square means and standard errors for BW and WW were presented in Table 3. Some of the haplotypes appeared to have statistically significant effects on both traits ($P<0.01$). The result indicated that animals with 313 and 325 haplotypes attained high BW and WW, respectively. On the other hand, animals having the 111 and 234 haplotypes had the lowest BW and WW ($P<0.05$). This result suggested the existence of preferable intra- and inter-genic polymorphic region within bovine growth hormone gene. From the data on genotypic frequencies, it was found that animals with 212 and 215 haplotypes were in high proportion in this population. Therefore, intra- and inter-genic polymorphic region was considered beneficial in mating system for replacing animals with favorable genes.

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