Kasetsart J. (Nat. Sci.) 48 : 605 - 610 (2014)

Association of SNP Marker in the *IGF*1 Gene with Carcass Traits in Crossbred Cattle among Thai Native, Brahman and Charolais

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ABSTRACT

The objectives of this study were to estimate the allele and genotype frequencies of insulin-like growth factor 1 (*IGF*1) polymorphisms as well as to assess the association between these polymorphisms and carcass traits in crossbred cattle among Thai Native, Brahman and Charolais (n = 238). A single nucleotide polymorphism (SNP) of the *IGF*1 gene (c.512C < T) was determined by polymerase chain reaction-restriction fragment length polymorphism assay using the *SnaBI* restriction enzyme. The results showed that the frequency of the B allele was higher than that of the A allele (0.82 and 0.18, respectively). The *IGF*1 genotype and average effect of allele substitution were not associated with hot and cold carcass weights, rib eye area, backfat thickness, marbling score and dressing percentage ($P > 0.05$). The SNP of the *IGF*1 gene was not associated with carcass traits. Therefore the SNP of the *IGF*1 gene could not be used as a marker for improving carcass traits in this crossbred cattle population.

Keywords: beef cattle, carcass trait, *IGF*1 gene, PCR-RFLP, SNP

INTRODUCTION

Carcass traits are economically important traits in the beef cattle industry; these traits are affected by multiple genes and can display variation among individuals and breeds (Stone *et al.*, 1999; Casas *et al.*, 2000). To improve these traits, selection based on the estimated breeding value can be employed. The estimated breeding value has been used to select cattle to be sires and dams, which would in many cases result in offspring with improved phenotypic values compared to the previous generation. However, recent studies have focused on molecular genetics to increase the accuracy of genetic selection of young animals especially in carcass traits (Bertrand *et al.*, 2001).

Insulin like growth factor 1 (*IGF*1) is a peptide hormone that has an essential role in various aspects of animal growth, development and metabolism (Hossner *et al.*, 1997). The *IGF1* gene was mapped on bovine chromosome 5 at 73.5 cM (Grosse *et al.*, 1999). Some studies reported that the quantitative trait loci regions of the *IGF*1 gene are associated with the fat level and carcass traits in beef cattle (Casas *et al.*, 2000; Li *et al.*, 2004). In addition, other studies revealed that the *IGF*1 gene is considered to be a candidate gene for predicting growth and meat quality (Machado *et al.*, 2003; Andrade *et al*., 2008). Moreover, previous research has shown that a single nucleotide polymorphism (SNP) of the

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Received date : 11/11/13 Accepted date : 10/07/14

*IGF*1 gene in the promoter region (*IGF*1/*SnaBI*) of Genbank accession number AF01713 is positively associated with growth and carcass traits in beef cattle (Curi *et al*., 2005; Islam *et al*., 2009).

With regard to the function and association of the *IGF*1 gene with carcass traits, this study focused on crossbred populations. The objectives of this study were to identify the allele and genotype frequencies of SNP of the *IGF*1 gene and to determine the association between genotypes and carcass traits in Thai Native, Brahman and Charolais crossbred cattle.

MATERIALS AND METHODS

Animal and DNA extraction

The study involved 238 crossbred beef cattle progeny from crossing Thai Native, Brahman, and Charolais. The animals were born during 2009 to 2011 at the Buffalo and Beef Production Research and Development Center and on three commercial farms. Combinations between farm, year and season at birth were considered as contemporary groups (Jeanmas *et al.*, 2008). The meat samples and carcass data were collected from Kasetsart University Kamphaeng Saen Campus Beef Producer Corporative Ltd., Nakhon Pathom Province, Thailand. The pre-slaughter live weight was recorded before slaughtering. The hot carcass weight, cold carcass weight, rib eye area, backfat thickness, marbling score and dressing percentage were recorded. The marbling score and backfat thickness were measured at the 12th and 13th rib interface. Marbling was scored (5 = abundant, 4 = moderate, 3 = small, 2 = slight, 1 = devoid) according to the Thai Agricultural Commodity and Food Standard (National Bureau of Agriculture Commodity and Food Standards, 2004). General descriptive statistics for those traits are shown in Table 1. Meat samples were collected and stored at -80 °C before DNA extraction. Genomic DNA from meat was extracted using the phenol-chloroform method (Sambrook *et al.*, 1989) and stored at -20 °C until use.

Amplification and genotyping using polymerase chain reaction-restriction fragment length polymorphism

The polymorphisms of the *IGF*1 gene were identified using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). The genotyping of the *IGF*1 c.512C>T SNP marker (Genbank accession number AF01713) was performed as described by Ge *et al.* (2001). PCR was performed in a reaction volume of 25 µL constituted with 50 ng of genomic DNA, 10 pmol of each primer (*IGF*677F 5′-ATTACAAAGCTGCCTGCCCC-3′

Table 1 Descriptive statistics for carcass traits of crossbred animals among Thai Native, Brahman and Charolais.

Trait[1]	Number	Mean	SD	Minimum	Maximum
HCW (kg)	198	307.40	29.00	251.00	394.50
CCW (kg)	198	301.05	28.02	246.10	386.80
DRP[2] (%)	185	59.51	2.14	54.50	65.20
REA (cm^2)	197	84.35	11.85	55.50	120.10
BFT (cm)	198	0.73	0.40	0.10	2.30
MBS[3]	196	1.93	0.67	1.00	4.00

[1] = HCW = Hot carcass weight; CCW = Cold carcass weight; REA = Rib eye area; BFT = Backfat thickness.

[2] = DRP = dressing percentage = $\frac{\text{cold carcass}}{\text{pre-slaughter live weight}} \times 100$

[3] = MBS =Marbling score: 5 = Abundant, 4 = Moderate, 3 = Small, 2 = Slight, 1 = Devoid.

and *IGF*897R 5′-ACCTTACCCGTATGAAAG GAATATACGT-3′), 2.5 µL 10×PCR buffer (Bio-Rad), 200 µM dNTP, 1.5 mM $MgCl_2$ and 1.0 unit *Taq* polymerase (iTaq; Bio-Rad). The amplification conditions of the *IGF*1 gene were 94 °C for 2 min followed by 31 cycles of 94 °C for 1 min, 61 °C for 45 s and 72 °C for 1 min, with a final extension at 72 °C for 5 min. For RFLP analysis, the PCR products of the *IGF*1 gene were digested using *SnaBI* restriction enzyme (TAC/GTA) (Promega Co., Madison, WI) and incubated at 37 °C overnight. The digestion products were separated on a 1.8% agarose gel electrophoresis with 0.5×TAE buffer and stained using ethidium bromide. The genotype bands were visualized under ultraviolet light.

Statistical analysis

Genotypes of individual animals were recorded by direct counting of the bands. The allelic and genotypic frequencies were calculated. The association between the genotype of the *IGF*1 gene and carcass traits was analyzed using a general linear model (GLM) procedure and least square means of the genotypes were compared using the Tukey test (SAS, 1996). The model in Equation 1 was used:

$$y_{ijk} = \mu + G_i + F_j + b(A_{ijk} - \overline{A_{ijk}}) + e_{ijk} \quad (1)$$

where y_{ijk} is the observation of carcass traits, µ is the overall mean of population, G_i is the fixed effect of *IGF*1 genotypes (i = AA, AB, and BB), F_j is the fixed effect of contemporary groups (farm-year-season) of birth (j = 1, 2, 3, …, 30), b is the regression coefficient associated with age at slaughter (A_{ijk}) and e_{ijk} is the random of residual error assumed as a normal distribution with a mean of 0 and a variance of σ^2.

The average effects of allele substitution of the carcass trait were analyzed by estimating the regression coefficients. The fixed genotypic effects were replaced with coding values of 0, 1 and 2 for genotype BB, AB and AA, respectively. As described by Falconer and Mackay (1996), the regression coefficient estimates the average effect of allele substitution, or the average effect of replacing the B allele with the A allele.

RESULTS AND DISCUSSION

Two hundred and thirty eight DNA samples of crossbred Thai Native, Brahman and Charolais cattle were genotyped for the *IGF*1 gene using the PCR-RFLP method. Each PCR product (246 bp) was digested using *SnaBI* (TAC/GTA) restriction enzyme. Genotype AA was characterized by the presence of two restriction fragments of 220 and 26 bp, while genotype BB was determined by the presence of a single 246 bp. Heterozygous genotype AB showed three fragments of 246, 220 and 26 bp (Figure 1). The 220 bp fragment and the whole fragment (246 bp) were clearly visible, while the 26 bp fragment migrated to the base of the gel. The sequence data of the *IGF*1 gene was submitted as GenBank accession number KF202095.

The allelic and genotypic frequency of the *IGF*1 gene was determined as shown in Table 2. The *IGF*1 gene had a high frequency of the BB

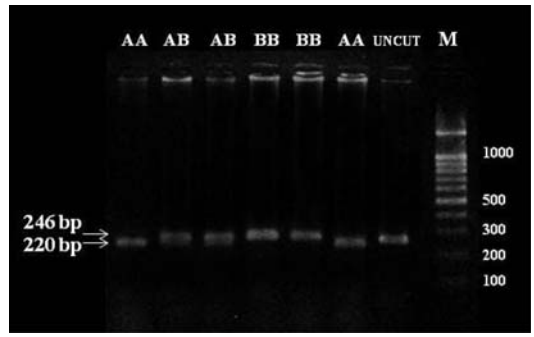

Figure 1 *IGF*1 gene fragments digested with *SnaBI* and submitted to electrophoresis on 1.8% agarose gel. From left to right: lane 1 and 6 are AA genotype, 220 bp; lane 2 and 3 are AB genotype, 220 and 246 bp; and lane 4 and 5 are BB genotype (246 bp); lane 8 is molecular size marker.

genotype (0.65) and a very low occurrence of the AA genotype (0.01). The frequency of the B allele (0.82) was higher than that of the A allele (0.18). Curi *et al.* (2005) and Akis *et al.* (2010) reported similar results. The frequencies of the B allele were almost fixed in South Anatolian Red (0.82), East Anatolian Red (0.87) and Beefmaster population (0.97) and fixed in Nellore population (1.00). The B allele is a main characteristic in *Bos indicus* or Zebu cattle. Thus, the high frequency of the B allele in the current population could have resulted from the breed compositions of Thai Native and Brahman.

Associations between the *IGF*1 gene and carcass traits in crossbreds among Thai Native, Brahman and Charolais are shown in the Table 3. In this study, the genotypic effects and average effects of allele substitution of the *IGF*1 gene were not significant for all carcass traits in this population ($P > 0.05$). Similarly, Islam *et al*. (2009) reported that the SNP of the *IGF1* gene was not significantly associated with ultrasound backfat, ultrasound rib eye area, slaughter weight, carcass weight, average backfat, lean meat yield and marbling score in Charolais and the hybrid population but was significantly associated with ultrasound backfat, average backfat and lean meat yield in the Angus population. In addition, Chang *et al.*, (2009) reported that the SNP of the *IGF*1 gene was associated with carcass and meat weights in Limousin and Jersey populations. In several studies, the effect of the *IGF*1 gene has been generally determined on growth traits and for carcass traits, the effects of *IGF*1 gene were significant in some populations (Curi *et al.,* 2005; Andrade *et al.,* 2008; Akis *et al.,* 2010). Due to

Table 2 Allelic and genotypic frequency of *IGF*1 gene in crossbred Thai Native, Brahman and Charolais cattle.

Number of animals	Allelic frequency		Genotypic frequency[1]		
	A	B	AA	AB	BB
238	0.18	0.82	0.01 (3)	0.34 (81)	0.65 (154)

[1] = Numbers in parentheses are the number of animals in each genotype.

Table 3 Least squares means (LSM), SE and estimates of allele substitution effects of *IGF*1 gene for carcass traits.

Trait[1]	Genotype						*P*–value	Allele substitution		*P*–value
	AA		AB		BB					
	LSM	SE	LSM	SE	LSM	SE		Effect	SE	
HCW (kg)	294.09	23.59	312.70	5.29	307.97	4.09	0.47	3.38	4.25	0.42
CCW (kg)	288.05	22.81	306.37	5.12	301.69	3.96	0.45	3.36	4.11	0.41
DRP [2] (%)	60.24	1.62	59.75	0.37	59.79	0.28	0.95	-0.01	0.30	0.97
REA (cm^2)	1.18	0.57	1.92	0.12	1.86	0.09	0.47	0.02	0.10	0.23
BFT (cm)	0.24	0.35	0.89	0.07	0.78	0.06	0.06	0.07	0.06	0.25
MBS[3]	1.18	0.57	1.92	0.12	1.86	0.09	0.40	0.02	0.10	0.80

[1] = HCW = Hot carcass weight; CCW = Cold carcass weight; REA = Rib eye area; BFT = Backfat thickness.

[2] = DRP = dressing percentage = $\frac{\text{cold carcass}}{\text{pre-slaughter live weight}} \times 100$

[3] = MBS = Marbling score: 5 = Abundant, 4 = Moderate, 3 = Small, 2 = Slight, 1 = Devoid.

the rare genotype AA in the current study, the standard errors of least square means and average allele substitution effects were high. Although the least square means of the genotypes AA, AB and BB were not significantly different ($P > 0.05$), the least square means of the heterozygous genotype (AB) were not within the ranges of the homozygous genotypes (AA and BB) for hot carcass weight, cold carcass weight, rib eye area, backfat thickness and marbling score. The results in this study indicated that carcass traits were not under the control of the additive single gene effect but the dominant effect of the *IGF*1 gene might be important for those traits.

CONCLUSION

This study showed the B allele of *IGF*1 gene polymorphism (c.512C > T) was the characteristic in these Thai Native, Brahman and Charolais crossbred cattle. The genotypic effect and average effect of allele substitution of *IGF*1 were not associated for all carcass traits in this population. The SNP of the *IGF*1 gene would not be a good marker for carcass traits in this crossbred beef population.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to the Kasetsart University Research and Development Institute (KURDI) for financial support and to Kamphaeng Saen Campus Beef Producer Corporative Ltd., for providing the meat samples and carcass data. The authors also thank the Integrative Research Center for Animal Science, Aquaculture and Animal Health (ASA-AH) of Kasetsart University under the National Research University Project of Thailand and the Department of Animal Science, Faculty of Agriculture for providing the facilities for this study.

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