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Association of Dik2670 Microsatellite Marker with Carcass Traits in Crossbred Beef Cattle

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The objectives were to evaluate the allele and genotype frequencies of DIK2670 microsatellite marker and to determine the association between the genotype and carcass traits in fattening crossbred beef cattle. A total of two hundred and one Brahman-Charolais crossbred beef cattle were used as sample. Blood DNA was extracted and amplified using a polymerase chain reaction technique. The alleles of the microsatellite were separated using a denaturing PAGE SSLP technique. Sizes of alleles were read manually and then the allele frequencies were calculated. Six alleles with the sizes of 226, 223, 221, 215, 212, and 210 based pairs (called A, B, C, D, E, and F) appeared. The E allele had the highest frequency, 0.28, while the lowest was the allele F, 0.03. In addition, 19 genotypes were found. The top three genotypes, which had the highest frequencies were BE, BD, and EE, respectively. The genotypes of the microsatellite had not significantly influenced the cold carcass weight, dressing percentage, rib eye area, and rib fat thickness, ($P>0.05$), but it had highly significantly affected the marbling score ($P<0.001$). It meant that the DIK2670 microsatellite marker was associated with the marbling score of the Brahman-Charolais crossbreds. Therefore, the DIK2670 microsatellite was a good marker for improving intramuscular fat in the crossbred population.

Keywords: DIK2670 microsatellite, carcass traits, crossbred beef cattle

Introduction

Beef cattle are important economic livestock in Thailand. The number of cattle population in 2015 was approximately 4.9 millions. About 41 % were raised in the North-Eastern part (Office of Agricultural Economics, 2015). Five percents of total beef in Thailand was high quality beef produced from fattening crossbred cattle (*Bos indicus* x *Bos taurus*). Brahman and their crosses with Native are mostly used as dam, while Charolais, Simental, and Limusine are used as sire. The cattle are raised in intensive system and fed with concentrate and roughage such as rice hay and fresh grass until their final weight reach the required weight.

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Microsatellite markers are widely used in beef cattle as marker for genetic improvement of different traits (Casas *et al*., 1998; DeAtley *et al*., 2011; Kim *et al*., 2012). DIK2670 is a microsatellite marker. This name is a loci name based on MARC Database. The marker is located on bovine chromosome 1, and had 9 alleles with a size range between 204 to 220 based pairs (http://www.marc.gov/genome/ genome.html). There are many overseas reports concerning association of microsatellite markers on chromosome 1 with carcass traits. On the other hand, there are scarce reports in Thailand about microsatellite markers. Report on DIK2670, in particular, has not been found.

The objectives of this study were to evaluate the allele and genotype frequencies of DIK2670 microsatellite marker and to determine the association of the genotype and carcass traits in fattening crossbred beef cattle.

Materials and methods

Carcass traits and blood collection

A total of two hundred and one minimally 50%Charolais fattening crossbred beef cattle from member farms of the Pon Yang Khram Livestock Cooperative located in Sakon Nakhon Province, Thailand, were used as samples. The samples were not from registered cow's offspring at the cooperative. The members bought 1-year-old calves from anywhere then raised them intensively under the cooperative feeding system until their body weight reached the slaughtering weight. Three lots of animals were transported to slaughter house. After slaughtered, each carcass was halves and weighed as hot carcass weight then they were aged at 0 to 4°C for 7 days. On the 7^{th} day, the intramuscular fat in *M. longissimus dorsi* at the 12^{th} to 13^{th} rib of the left half was evaluated for its marbling score by two trained assessors, two controllers, and one observer. The score ranged from 1 to 5 (1 = devoid, 2 = slight, 3 = small, 4 = moderate, 5 = abundant) according to the Thai Agricultural Commodity and Food Standard (National Bureau of Agriculture Commodity and Food Standards, 2004). However, in practice, the cooperative assessors were able to judge the marbling by several more scores such as 1.5, 2.5, 3.5, or 4.5.

At the same muscle position where the marbling was evaluated, an acetate sheet was placed on top of the area and the rib eye area and rib fat thickness were traced on the sheet with permanent ink. The rib eye area was evaluated by using Iowa template, while the rib fat thickness was measured at the ¾ length of *M. longissimus dorsi*. The cold carcass was weighed. All data were recorded and collected. Dressing percentage was calculated by dividing

the cold carcass weight with the live final weight and multiplying by 100. The carcass traits data were analyzed as a descriptive statistic, as shown in Table 1.

During the slaughter, 5 ml whole blood sample from each animal's jugular vein was collected and kept in a sterile plastic tube containing 0.5% EDTA. The sample was stored at 2 to 4 °C to be further used in the step of DNA extraction.

Table 1. Descriptive statistic of the carcass traits (n = 201)

Studied Traits	Minimum	Maximum	Mean	S.D.
Final live weight (kg)	399.00	771.00	557.31	73.33
Cold carcass weight (kg)	212.00	412	306.98	41.02
Dressing percentage	47.53	59.63	55.08	2.39
Marbling score	2.50	3.50	3.00	0.07
Rib eye area (cm^2)	94.50	178.00	129.76	16.05
Rib fat thickness (cm)[1/]	0.10	3.05	0.99	0.55

[1/] n = 200
[2/]S.D. = Standard Deviation

DNA extraction and amplification

DNA from whole blood was isolated using a phenol-chloroform method (Sambrook and Russell, 2001). Briefly, transfer 300 µl of the blood to a microtube then add 0.5 ml lysis solution (4M guanididium thiocyanate, 25mM sodium citrate, 0.5% N-laurylsarcosin), and invert the tube. Add 150 µl phenol and 100 µl chloroform (in fume hood), then vortex-mix the solution for 15 min, after that centrifuge the solution at 13,000 rpm for 5 min. Pipette 600 to 800 µl supernatant and transfer it to a new microtube. Repeat the step of phenol-chloroform and centrifugation for one more time. Then, pipette 400 µl of the supernatant and transfer it to a new microtube, add 600 µl absolute ethanol, and invert the tube. Leave the tube to dry at room temperature for 2 min; centrifuge the supernatant at 13,000 rpm for 10 min; remove the supernatant and wash the pellet with 500 µl of 75% ethanol; centrifuge at 13,000 rpm for 5 min; remove the supernantant and then dry the DNA pellet for 3 hr at room temperature. Add 30 µl of TE buffer and store it at – 20 °C. The quailty of the DNA was measured with a SmartSpec^{TM} plus spectrophotometer (BioRad).

The DNA was amplified by using a polymerase chain reaction (PCR) technique. PCR was conducted to a final volume of 25 µl, including 1.50 µl of 10X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 0.90 µl of 50 mM $MgCl_2$, 0.75 µl of10 mM dNTP, 0.60 µl of each primer (DIK2670_F 5′-CCTAGCACACTAATGTGGCATAAA-3′ and

DIK2670_R 5′ GCAGAGGGATGAGCAAGATT - 3′), 0.15 µl of Taq DNA polymerase, 2.40 µl of DNA template (50 ng/µl), and 8.10 µl of dH_2O). Amplification conditions for PCR products were 4-min denaturation at 94 °C, 35 cycles for 30 sec at 94 °C, 1-min annealing for 59 °C, 45 sec for extending the reaction, and final extension for 5 min at 72 °C. The quality of the PCR product was tested by 1% agarose gel electrophoresis with 0.5 x TAE buffer. Ethidium bromide was used to stain the gel. The bands were read under UV light with GelDoc (Bio-Rad). The PCR product bands of sample 96th to 119th are shown in Figure 1. DNA bands from most of the PCR products clearly appeared, but the 112th band was not observed.

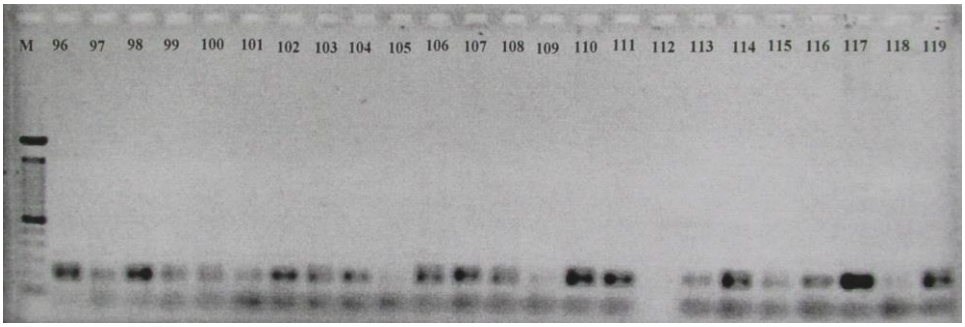

Figure 1. PCR product bands of some samples
M= Ladder 100 marker, Number 96 to 119 = Sample running number

The alleles of the PCR product were separated using 4.5 % Denaturing PAGE SSLP technique. HinfI was used as a standard marker, and the polyacrylamide gel was stained with silver nitrate. The PAGE was run by DNA Technology Laboratory, Kasetsart University Khamphaengsean, Nakorn Pathom Province, as shown in Figure 2. Size of the alleles of each sample was read manually by three experienced assersors and recorded. The size of each allele was calculated based on the standard marker HinfI.

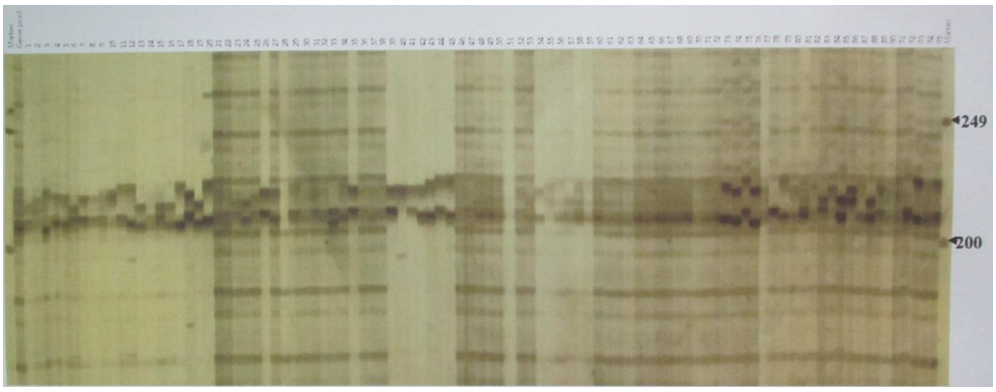

Figure 2. Specific alleles of DIK2670 in an individual sample
Lane 1 and 98 = HinfI marker, Lane 2 = DNA pool, Lane 3 to 99 = Sample 1st to 95th

Statistical analysis

The allele and genotype frequencies of the DIK2670 were calculated. A general linear model (GLM) procedure was used to analyze the association between the marker and the carcass traits. The pdiff option in the GLM procedure in SAS (1996) was used to compare the least squares means of the genotypes. The analyzed model was;

$$y_{ijk} = \mu + SD_i + G_j + \beta(FLW_{ijk} - \overline{FLW}) + \varepsilon_{ijk} \qquad (1)$$

where y_{ijk} (1) is the observation of the ith slaughtering lot and jth genotype for the studied traits, μ is the population mean, SD_i is the fixed effect of slaughtering lot (i = 1, 2, 3), G_j is the fixed effect of the genotypes (j = AA, AB, AC,...., EF), β is the coefficient of regression related to final live weight (FLW), and ε_{ijk} is the random residual error assumed to be normally distributed with a mean of null and a variance of σ^2.

Results

Allele and genotype frequencies

Allele frequency

Six alleles from a total of 193 from the minimally 50% Charolais cattle were detected. Allele from 8 samples was not appeared. The allele frequency of DIK2670 microsatellite marker on bovine chromosome 1 is shown in Table 2.

The size of the allele ranged from 210 to 226 bp. Allele E, D, and B showed the highest frequency at 0.2824, 0.2694, and 0.2306, while the lowest was F (0.0311).

Table 2. Overall allele frequencies of the DIK2670

Allele	Size (base pairs)[1]	Frequency[2/]
A	226	0.0855
B	223	0.2306
C	221	0.1010
D	215	0.2694
E	212	0.2824
F	210	0.0311

[1]/Manually read
[2]/ 193 samples

Genotype frequency

Nineteen genotypes of the DIK2670 marker were found. The number of genotype and the frequency of the DIK2670 genotype are shown in Table 3. The top three genotypes that showed the highest frequencies were BE, BD, and EE, at 0.1554, 0.1503, and 0.1088, respectively. The homozygous genotypes, CC and FF were not found.

Table 3. Overall genotype frequency of the DIK2670

Genotype	n	Frequency	Genotype	n	Frequency
AA	1	0.0052	BF	3	0.0155
AB	1	0.0052	CD	16	0.0829
AC	2	0.0104	CE	15	0.0777
AD	11	0.0570	CF	2	0.0104
AE	13	0.0674	DD	19	0.0984
AF	4	0.0207	DE	8	0.0415
BB	11	0.0570	DF	2	0.0104
BC	4	0.0207	EE	21	0.1088
BD	29	0.1503	EF	1	0.0052
BE	30	0.1554			
Total				**193**	**100**

Assiciation of DIK2670 with the carcass traits

The association of the DIK2670 genotype with the carcass traits was analyzed by using a general linear model. Independent factors were slaughtering lots, genotype, and final live weight was used as a co-variable. Table 4 shows the effect of the independent factors on the carcass traits. It was found that the non-biological factor, the slaughtering lot, had a significantly influence on the dressing percentage ($P<0.05$). The biological factors had an

effect on some carcass traits. Namely, the final live weight, covariate, affected the cold carcass weight, rib eye area, and rib fat thickness (P<0.01), while the genotype, the most important factor for this study, had a highly significantly influence on the marbling score (P<0.01).

Table 4. Factors of slaughtering day, genotype, and final live weight that affected the carcass traits

Studied trait	P-values of independent factors			R^2
	Slaughtering lot	Genotype	Final live weight[1]/	
Cold carcass weight (kg)	0.5476	0.9725	<.0001	0.8776
Dresssing percentage	0.0224	0.7382	0.0958	0.1253
Marbling score	0.0722	<.0001	0.6561	0.2822
Rib eye area (cm^2)	0.2515	0.9261	<.0001	0.3145
Rib fat thickness (cm)	0.8566	0.8129	<.0001	0.1858

[1/] = Co-factor

Figure 3 shows that the dressing percentage of the animals, which were slaughtered in the 3rd lot, was higher than those slaughtered in the 2nd lot, 55.604 and 54.288, respectively, but both were not different from the 1st lot, 54.833.

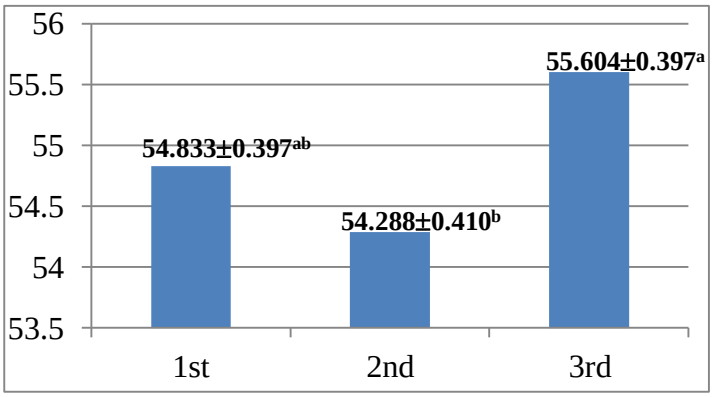

Figure 3. Least squares means and standard error of dressing percentage in each slaughtering lot

[a,b] :Different letters in the chart denote significant difference (P<0.05).

The least squares means and standard error of the marbling score for each genotype is shown in Table 5. EF genotype showed the highest marbling score, 3.485, which is highly statistically different from those of the other genotypes.

Table 5. Least squares means and standard error of marbling score for different genotypes

Genotype	n	LSM of marbling score[1/]	Standard Error
AA	1	3.004[b]	0.065
AB	1	3.002[b]	0.065
AC	2	3.011[b]	0.047
AD	11	3.005[b]	0.020
AE	13	2.994[b]	0.018
AF	4	2.994[b]	0.032
BB	11	3.004[b]	0.020
BC	4	3.001[b]	0.032
BD	29	3.004[b]	0.012
BE	30	3.000[b]	0.012
BF	3	2.996[b]	0.038
CD	16	3.002[b]	0.016
CE	15	3.000[b]	0.017
CF	2	2.982[b]	0.047
DD	19	3.026[b]	0.015
DE	8	3.010[b]	0.024
DF	2	2.984[b]	0.046
EE	21	2.989[b]	0.015
EF	1	3.485[a]	0.065

[1/]: [ab] Different letters in the same column denote highly significant difference (P<0.01).

Discussion

The number of the DIK2670 alleles in Cattle Genomen Database (http://www.marc.gov/genome/ genome.html) was higher than what we found in this study, 9 and 6 alleles, respectively. The reason for this might be that the cattle breed such as Eupean or tropical cattle was an important factor. Most of quantitative trait locus (QTL) mapping experiments were done in taurine (Kűhn *et al*. 2005). The size of the marker from our study ranged from 210 to 226, while those from MARC database was 204 to 220 based pairs. The reason might be that in our study, the bands were run by manual electrophoresis and they were manually read by three experienced assessors then the sizes were calculated by comparing to a standard marker, HinfI, meanwhile for those in the database, the microsatellites were isolated using microsatellite-enriched libraries and were amplified by means of PCR using fluorescent-labeled primers. The sizes of allele were calculated by software package (Ihara *et al*., 2004).

Some factors affecting the carcass traits, such as gender, slaughter age, percent of Charolais blood were not included in the studied model, because, as mentioned above, the samples in this study were not from registered cow's offspring at the cooperative, therefore, these factors were not available.

However, the slaughtering lot, which was one of pre-slaughter factors, had effect on the dressing percentage. The dressing percentage trait in the slaughtering the 3rd lot was higher than those in the 2nd lot ($P<0.05$). The reason might be in the 3rd most of animals had higher levels of Charolais blood and higher slaughter age than those in the 2nd lot.

The study of microsatellite markers on bovine chromosome 1 (BTA1) associated with carcass traits was reported by Kim *et al.* (2012). They found that *UPK1B*, *HRG*, and *MAGE* polymorphisms residing between BM1312 and BMS4048 were significantly associated with growth and carcass traits in the studied population. The DIK2670 microsatellite marker also located on BTA1 between RM194, BMS4028, DIK4331, and DIK5127, was used for our study. No paper reported about DIK2670, while our result found that there was a highly significant difference in the marbling scores according to the DIK2670 genotype. It meant that the DIK2670 microsatellite marker was not associated with most traits except with the marbling score of the Brahman-Charolais crossbred beef cattle. It can be concluded that we can use this marker as MAS for selecting crossbred cattle for their good marbling trait.

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