Indian Journal of Animal Sciences **86** (4): 441–444, April 2016/Article

Genetic polymorphism of caprine stearoyl-coA desaturase (*SCD*) gene and their relationship with blood cholesterol, triglyceride and pre-weaning growth traits of mixed breeds of goats in southern Thailand

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Received: 19 January 2015; Accepted: 1 September 2015

ABSTRACT

The present study attempts to identify genetic polymorphisms of stearoyl-coA desaturase (*SCD*) gene and their relationship with blood cholesterol, triglyceride and pre-weaning growth traits in a goat population in southern Thailand. Genetic variability in caprine *SCD* was analyzed in 290 animals belonging to several types of Thai native (TN), Anglo-nubian (AN), Boer (B) and Saanen (SA) breed crosses by PCR-SSCP and DNA sequencing. Four SNPs were identified in exon3 (A/G), exon5 (C/T), exon6 (C/G) and 3'untranslated region (3'UTR) (TGT deletion). Five haplotypes (A, B, C, D and E) were constructed. Haplotype frequency B was the highest (0.50) but haplotype frequency C was the lowest (0.01). Haplotype effect of *SCD* gene had influenced on blood cholesterol, triglyceride, weaning weight and growth rate. Individuals with haplotype C had significant lowest triglyceride, weaning weight and growth rate ($P<0.05$). Also, this haplotype could be culled for growth improvement in this population.

Key words: Blood cholesterol, Goat, Pre-weaning growth traits, Stearoyl-coA desaturase (*SCD*) gene, Triglyceride

Stearoyl-coA desaturase(SCD) is a rate-limiting enzyme responsible for the conversion of saturated fatty acids into monosaturated fatty acids. It affects the fatty acid composition of membrane phospholipids, triglyceride and cholesterol esters. The *SCD* gene was localized on chromosome 26q21 and consisted of 6 exons and 5 introns. Variations in *SCD* gene have significant associations with meat and milk but they may have association with growth traits and others (Zhang *et al*. 2010, Chen *et al*. 2011). Therefore, the objectives of this study were to determine the genetic polymorphisms of *SCD* gene and their association with blood cholesterol, triglyceride and pre-weaning growth traits of a goat population in Thailand. These outcomes may contribute to the possible use of the caprine *SCD* gene as a genetic marker related to caprine breeding and genetics.

MATERIALS AND METHODS

Animals: Several types of breed crosses of Thai native (TN), Anglo-nubian (AN), Boer (BO) and Saanen (SA) goats were raised in a large commercial farm in southern Thailand. The grazing method was followed by feeding freshcut grasses as well as rotational grazing. Bucks and does were supplemented once per day with concentrate diet containing 20% crude protein approximately 1.5–2% of their body weight. A vaccination program was established by the Department of Livestock Development (DLD) in Thailand. The digital weighing scale was used to obtain live weight of goats at birth and weaning. Growth rate (GR) was calculated as $(((WW-BW)/90)*1,000)/((BW+WW)/2))^{0.75}$ (Prolomkarn*et et al*. 1996). Average of goat age in this study was 592.34±53.44 days and average age of bucks and does were 576.85±37.86 and 594.47±54.95 days, respectively. Animals (290: 36 male and 254 female goats) were included in this study. The blood samples were taken from the jugular vein in the morning after an overnight fast. A sample was separated into the tubes including anticoagulant and a clot activator. They were stored at –4°C.

Chemical analysis and DNA extraction: The 3 ml of blood samples were collected for total serum cholesterol measurement. After an hour, the serum was centrifuged at 3,000 rpm for 15 min. The clear non hemolysed supernatant fresh serum was then carefully transferred to sterilized glass vials. The samples were then immediately analyzed for blood cholesterol and triglyceride determination by enzymatic colorimetric test. Genomic DNA was extracted from 3 ml of whole blood using a commercial genomic DNA kit.

Molecular analysis: The primers were designed based on the reference sequences for the caprine *SCD*

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Table 1. Primers for amplification of caprine stearoyl-CoA desaturase (*SCD*) gene

Primer	Sequence	Reference sequence	Region	Annealing temperature (°C)	Fragment size (bp)
P1	F:CCAGGTCTATGCCTATCC R:GAGGGTCTGGTGTTTGTAC	AF422167	Exon2	55	491
P2	F:GTGCCCTGTCTTATCCTG R:CATCTCCTTCTTGCCTCT	AF422168	Exon3	62.5	362
P3	F:GCTACGCTAGATTTATCCG R:GCTTATCCTCCACTCCC	AF422169	Exon5	57	365
P4	F:TGAGGGCTTCCACAACTA R:GCATCATAAAGGCAGAGT	AF422171	Exon 6	60	377
P5	F:GCACAGGCATTCGCATTCATG R:CAAGCATTGCACTGTCCGCT	AF325499	3'UTR	55	290

UTR, untranslated region.

gene in Table 1

All 290 samples were scanned for point mutations using PCR-SSCP analysis. The PCR reaction was prepared according to Top Taq master mix kit using 50 ng of genomic DNA and 0.5 µM of primer. The cycling protocol was 4 min at 95°C, 35 cycles of denaturing at 94°C for 30 sec, annealing temperature as indicated in Table 1 for 30 sec, extension at 72°C for 1 min, with final extension at 72°C for 7 min. The products from amplification were analyzed by electrophoresis on a 2% agarose gel, visualized with ethidium bromide staining. Amplicons (3 µl) were mixed with 9 µl denaturing solution, heated for 10 min at 98 °C and chilled on ice. The denatured DNA samples were subjected to PAGE (8–12% polyacrylamide/TBE gel in 1 × TBE buffer). Gels were run at 5W for 12 h at 10°C. They were stained with silver nitrate. After the polymorphisms were detected, the PCR products of different electrophoresis patterns were sequenced in both directions. The sequences were aligned among them with ClustalW multiple alignments and were compared with blast tool of NCBI (National Center for Biotechnology Information).

Calculations and statistical analysis: Genotypic and allelic frequencies were obtained by gene counting method. These frequencies, as well as the test for Hardy-Weinberg equilibrium, were calculated using R program in Hardy-Weinberg equilibrium package (R program 2010). The effects in the model were assumed to be fixed effects, except for the residual. The relevant effects composed of sex (male and female), birth type (single kid and twinning), contemporary group (year-season of birth), covariate of age, covariate of breed fraction of TN, AN, BO and SA. Genetic polymorphisms of *SCD* gene were considered in haplotypes of *SCD* gene. Analysis of variances was conducted for each trait using generalized linear model (GLM) procedure of R program (R program 2010). The statistical model for determination of the investigated traits was: $y_{ijklm} = \mu + Sex_i + BT_j + CG_k + b_1(AGE\text{-}\overline{AGE})_{ijklm} + b_2(TN\text{-}\overline{TN})_{ijklm} + b_3(AN\text{-}\overline{AN})_{ijklm} + b_4(BO\text{-}\overline{BO})_{ijklm} + b_5(SA\text{-}\overline{SA})_{ijklm} + HP_l + e_{ijklm}$ where, y_{ijklm} is the investigated trait measured on each of the ijklmth goats, µ is the overall population mean, Sex_i, BT_j and CG_j are the fixed effects due to the ith sex, jth birth type and kth contemporary group. The symbols of $b_1(AGE\text{-}\overline{AGE})_{ijklm}$, $b_2(TN\text{-}\overline{TN})_{ijklm}$, $b_3(AN\text{-}\overline{AN})_{ijklm}$, $b_4(BO\text{-}\overline{BO})_{ijklm}$ and $b_5(SA\text{-}\overline{SA})_{ijklm}$ are the fixed effects associated with covariates of age, breed fractions of TN, AN, BO and SA, respectively. The HP_l in the model is fixed effect due to the lth haplotype of *SCD* gene. The e_{ijklm} is the random error (e_{ijkl} ~NID $(0,\sigma^2_e)$). Covariate of age was excluded in analysis of variances for pre-weaning growth traits. Multiple comparisons between and among significantly fixed effects for blood cholesterol, triglyceride and pre-weaning growth traits were obtained by using Duncan Multiple Range Tests (DMRT) in function muticomp.test of R program.

RESULTS AND DISCUSSION

Descriptive statistics for the investigated traits are presented in Table 2. Physiological ranges of total cholesterol in the blood serum and triglyceride of goats in this study were in accordance to Samardzija *et al*. (2013).

PCR-SSCP analysis was detected in 4 of 5 caprine *SCD* fragments from the primers in Table 1. The PCR products of P2, P3, P4 and P5 showed banding patterns on the polyacrylamide gel (Fig. 1).

They were aligned with ref. BC_022318 (Dong *et al*. 2013). The PCR products of electrophoresis patterns of P2 were sequenced. The sequence analysis revealed an A→G transition at nucleotide position 5145 in exon3. Genotypic

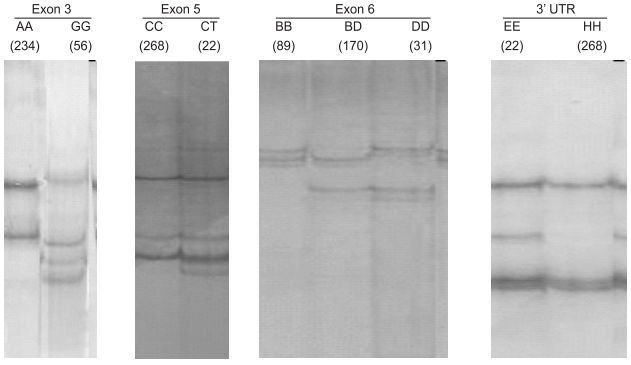

Fig. 1. PCR-SSCP of *SCD* gene and number of observed goats (in parentheses).

Table 2. Descriptive statistics of blood cholesterol, triglyceride, birth weight, weaning weight and growth rate

	Number of observations	Means±SD	Maximum	Minimum
Cholesterol (mmol/L)	290	2.35±0.62	5.92	0.85
Triglyceride (mmol/L)	290	0.15±0.07	0.44	0.03
Birth weight (kg)	290	2.42±0.55	3.70	0.80
Weaning weight (kg)	290	14.30±4.20	25.00	8.00
Growth rate ($g/kg^{0.75}$/day)	290	26.72±3.38	34.79	17.62

SD, standard deviations.

Table 3. Types and haplotype frequencies of caprine *SCD* haplotypes

	Caprine *SCD* haplotypes				
	A	B	C	D	E
Genotypes in exon3	AA	AA	GG	GG	GG
Genotypes in exon5	CC	CC	CC	CC	CT
Genotypes in exon6	BB	BD	BD	DD	BD
Genotypes in 3'UTR	HH	HH	HH	HH	EE
Haplotype frequencies	0.31	0.50	0.01	0.11	0.08

frequencies of AA and GG and allelic frequencies of A and G at this position were 0.81and 0.19. Sequence analysis of PCR products of P3 showed C→T transition at nucleotide position 8594 in exon5. Genotypic frequencies of CC and CT at this position were 0.92 and 0.08. Allelic frequencies of C and T were 0.96 and 0.04. A point mutation of PCR products of P4 occurred in exon6 such as C→G transition at nucleotide position 11475. This could be identified into 3 genotypes. The genotypic frequencies were 0.31, 0.59 and 0.10 for BB, BD and DD, respectively and the allelic frequencies of B and D were 0.61 and 0.39. The numbers of observed and expected genotypes in exon6, as well as χ^2 (14.19) and p-value (0.0002) suggested that the goat population was not consistent with Hardy-Weinberg equilibrium. Genetic polymorphisms in exon3, 5 and 6 were similar to Zhang *et al*. (2010) and Chen *et al*. (2011) who stated that a substitution of A→G in exon3 caused an amino acid change from valine to methionine. Moreover, 3 base pairs (TGT) deletion polymorphism was observed in the 3'UTR of PCR products of P5. The absence of the triplet (TGT) was detected in homozygous EE and presence of triplet bases was homozygous HH. Genotypic frequencies of EE and HH and allelic frequencies of E and H were 0.08 and 0.92. The mutation of the 3'UTR was in accordance to Zidi *et al*. (2010) but it was disagreed with Garcia-Fernandez *et al*. (2009). Besides, the total of 5 possible haplotypes in Table 3 were detected in this population. The lowest frequency of haplotype C consisted of does with low percentage of SA breed fraction.

Fixed effects testing and mean comparisons of investigated traits in each fixed effect are presented in Table 4. This result was similar to that of Supakorn and Pralomkarn (2009), who reported that sex and birth type had influence on growth traits in meat goats in Thailand. Haplotype of *SCD* gene in this study was the significant fixed effects for blood cholesterol, triglyceride, WW and GR. It was in correspondence with Chen *et al*. (2011) who reported genetic polymorphisms of *SCD* gene affecting body size in Chinese goats.

Haplotype effect had significant influence on the investigated traits, except BW. Goats with haplotype C could not be considered for selection of the parent stock in order to increase pre-weaning growth traits in this population. Genetic polymorphisms of *SCD* gene might be hypothesized causing the diet-independent variation in conjugated linoleic acid contents such as anti-carcinogenic,

Table 4. Fixed effect testing and mean comparisons of blood cholesterol, triglyceride, birth weight, weaning weight and growth rate between sex, birth type and among haplotypes of *SCD* gene

	No. (head)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Birth weight (kg)	Weaning weight (kg)	Growth rate ($g/kg^{0.75}$/day)
Sex		**	ns	**	*	*
Male	36	2.04±0.47[a]	0.16±0.06	3.06±0.34[a]	21.97±2.42[a]	31.50±1.91[a]
Female	254	2.39±062[b]	0.15±0.07	2.34±0.51[b]	17.58±2.97[b]	26.06±2.99[b]
Birth type		ns	ns	*	*	*
Single	265	2.34±0.60	0.15±0.07	2.50±0.46	14.81±3.95	27.13±2.41
Twin	25	2.55±0.89	0.13±0.07	1.26±0.21	10.55±2.26	24.69±2.02
Haplotype		**	*	ns	*	*
A	89	2.78±0.17[a]	0.15±0.04[a]	2.41±0.54	14.12±1.79[a]	26.35±2.16[a]
B	145	2.16±0.23[b]	0.15±0.04[a]	2.44±0.56	14.94±1.05[a]	27.11±2.36[a]
C	3	1.95±0.23[b]	0.10±0.05[b]	2.56±0.56	10.67±1.78[b]	21.03±2.55[b]
D	31	2.07±0.14[b]	0.15±0.04[a]	2.38±0.45	14.18±1.01[a]	26.41±2.55[a]
E	22	2.56±0.24[a]	0.17±0.06[c]	2.38±0.48	14.61±1.35[a]	26.85±2.50[a]

* and ** significant difference at P<0.05 and P<0.01. ns, non significant difference (P>0.05) and [a,b]and [c] within the same column marked with different letter are significantly different at P<0.05.

anti-atherogenic, anti-lipogenic and immune-modulating (Zidi *et al*. 2010). The contents in milk could be necessary for WW and GR.

The result of association between genetic polymorphisms of *SCD* gene and blood parameters could be a model for evaluating genetic factors contributing to hyperchole sterolemia and atherosclerosis in other livestock animals or human. However, the development of atherosclerosis is caused by a high concentration of LDL-cholesterol in blood. Also, types of cholesterol and triglyceride in both blood and meat could be considered in the next future research.

ACKNOWLEDGEMENT

This project (MRG5680096) was supported by Walailak University, the office of the higher education commission (OHEC) and the Thailand Research Fund (TRF). Finally, we would like to thank Associate Professor, Dr. Olga R. Kopp from Utah Valley University for proofreading our paper.

REFERENCES

Chen Z, Sun J, Li Z, Lan X, Zhang C, Qu Y, Liu Y, Fang X, Lei C and Chen H. 2011. Novel SNPs in caprinestearoyl-CoA desaturase (SCD) and decorin (DCN) genes that are associated with growth traits in Chinese breeds. *Molecular Biology Report* **38** (5): 3121–27.

Dong Y, Xie M, Jiang Y, Xiao N, Du X, Zhang W, Tosser-Klopp G, Wang J, Yang S, Liang J, Chen W, Chen J, Zeng P, Hou Y, Bian C, Pan S, Li Y, Liu X, Servin B, Sayre B, Zhu B, Sweeney D, Moore R, Nie W, Shen Y, Zhao R, Zhang G, Li J, Faraut T, Womack J, Zhang Y, Kijas J, Cockett N, Xu X, Zhao S, Wang J and Wang W. 2013. Sequencing and automated whole-genome optical mapping of the genome of a domestic goat (*Capra hircus*). *Nature Biotechnology* **31** (2): 135–41.

Garcia-Fernandez M, Gutierrez-Gill B, Garcia-Gamez E and Arranz J J. 2009. Genetic variability of the stearoyl-CoA desaturase gene in sheep. *Molecular and Cellular Probes* **23** (2): 107–11.

R PROGRAM. 2010. *R program version 2.11.1*. Institute for Statistics and Mathematics, GNU General Public License**,** Boston, USA.

Samardzija M, Vince S and Duricic D. 2013. Association of parity, fecundity and body condition score with blood serum concentration of some metabolites during pre and post parturient period in German Improved Fawn goats. *Veterinarski Arhiv* **83** (5): 469–77.

Supakorn C and Pralomkarn W. 2009. Pre-weaning growth of goats for meat raised on a commercial farm in southern Thailand. *Thai Journal of Agricultural Science* **42** (1): 13–19.

Zhang C L, Gao X Y, Shao R Y, Wang Y H, Fang X T and Chen H. 2010. Stearoyl-CoA desaturase (SCD) gene polymorphism in goat breeds. *Biochemical Genetics* **48** (9–10): 822–28.

Zidi A, Fernandes-Cabanas V M, Urrutia B, Carrizosa J, Polvillo O, Gonzalez-Redondor P, Jordana J, Gallardo D, Amills M and Serradilla J M. 2010. Association between the polymorphism of the goat stearoyl-CoA desaturase1 (SCD1) gene and milk fatty acid composition in Murciano-Granadina goats. *Journal of Dairy Science* **93** (3): 4332–39.