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 (B) 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 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
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 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 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.

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 electrophoresis patterns of P2 were sequenced. The sequence analysis revealed an A → G transition at nucleotide position 5145 in exon3. Genotypic

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Reference sequence</th>
<th>Region</th>
<th>Annealing temperature (°C)</th>
<th>Fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>F:CCAGGTCTATGCCTATCC R:GAGGGTCTGGTGTTTGTAC</td>
<td>AF422167</td>
<td>Exon2</td>
<td>55</td>
<td>491</td>
</tr>
<tr>
<td>P2</td>
<td>F:GTGCCCTGTCTATCCGT R:CATCTCTTCTTGGCCTT</td>
<td>AF422168</td>
<td>Exon3</td>
<td>62.5</td>
<td>362</td>
</tr>
<tr>
<td>P3</td>
<td>F:GCTACGCTAGATTATCCC R:GTATATCTTCCACTCCT</td>
<td>AF422169</td>
<td>Exon5</td>
<td>57</td>
<td>365</td>
</tr>
<tr>
<td>P4</td>
<td>F:TGAAGGCTTCCACAACTA R:GCATCTAAGGGCAGACT</td>
<td>AF422171</td>
<td>Exon 6</td>
<td>60</td>
<td>377</td>
</tr>
<tr>
<td>P5</td>
<td>F:GCACAGGCACTGCATTCTAG R:CAAGCATTGCACGTGCGCT</td>
<td>AF325499</td>
<td>3'UTR</td>
<td>55</td>
<td>290</td>
</tr>
</tbody>
</table>

UTR, untranslated region.
frequencies of AA and GG and allelic frequencies of A and G at this position were 0.81 and 0.19. Sequence analysis of PCR products of P3 showed C\( \rightarrow \)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\( \rightarrow \)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 \( \chi^2 \) (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\( \rightarrow \)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, anti-atherogenic and anti-inflammatory activities.
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 hypercholesterolemia 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.

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


