**The Influence of Genotypic Variability in the *Stearoyl-Coenzyme A Desaturase 1* Gene on Milk Yield Performance in Crossbred Anglo-Nubian Dairy Goats**

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**Novelty statement**

The research paper assesses the polymorphisms in the*stearoyl-coenzyme A desaturase 1* (*SCD1*) gene and its influence in milk production performance of crossbred Anglo-Nubian goats. The study’s purpose is to provide additional knowledge to the few existing studies on enhancing milk yield performance by associating gene polymorphism with milk yield performance in goats. Improving the milk yield of livestock such as goats using specific genes that can enhance milk yield and composition will help amplify the total profit of small farmers and boost local and national economies. Furthermore, the findings of this study will help supplement future research on the SCD1 gene as potential genetic markers for milk production traits in goats.

**Abstract**

The present study was carried out to assess the polymorphism in the *SCD1* gene (G931T) and investigate whether *SCD1* genotypes have something to do with milk yield performance in the 101 crossbred Anglo-Nubian dairy goats from Awang, Opol Misamis Oriental, and Talay, Dumaguete City, Negros Oriental, Philippines. Hair follicles from goats were collected to obtain genomic DNA and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the 101 gDNA was carried out using *Rsa*I as the restriction enzyme (RE). Two genotypic and allelic frequencies of 0.71 for GT, 0.29 for TT, 0.64 for T and 0.36 for G were obtained. All farms did not deviate Hardy-Weinberg Equilibrium (p > 0.05). The association between genotypes and milk yield performance was assessed using a two-way factorial (2 x 4) in a Randomized Complete Block Design. Goats with GT genotype were observed to have noticeably higher milk yield performance than goats with TT genotype. The results of this study suggest that polymorphism identified in the SCD1 gene affects milk performance of the Anglo-Nubian goats and can be ideal markers for milk production traits in Anglo-Nubian dairy goats.

Keywords: Anglo-Nubian, *Stearoyl-Coenzyme A desaturase*, milk yield, PCR-RFLP, polymorphisms.

**Introduction**

 Fat and protein yield and milk composition are traits of great economic importance in the dairy goat industry. Although ruminants are considered of little importance in animal production, it plays a relevant role for smallholders in ecologically marginal areas such as drylands and mountains (FAO, 2007; Crepaldi). Unlike all other commercially used animal milk on the market, goat milk has more vitamins, is richer in minerals and easier to digest, and has lesser allergic proteins (Lad et al., 2017; Sonu, 2020; Brzakova et al., 2021). The only problem with goat milk production is that small-scale farms have low number of goats, and goats produce insufficient milk compared to cows or cattle ( Brzakova et al., 2021). The advancement of molecular genetics can help future research improve economically important milk traits of almost all animals. Choosing the suitable gene or desirable genotype of an animal, such as goats, can be used as a genetic marker to improve milk production traits (Alim et al., 2012).

The Stearoyl-CoA desaturase (SCD), also known as delta-9 desaturase, is a complex multifunctional enzyme that plays an essential role in the biosynthesis of fatty acids and lipid metabolism. Oleic acid is the major unsaturated fatty acid found in most ruminants' adipose tissues. In milk, the enzyme stearoyl-CoA desaturase (SCD) synthesizes oleic (cis9 C18:1), palmitoleic (cis9 C16:1), and the central part of conjugated linoleic acids (cis9, trans11 C18:2) in the mammary gland ( Bernard et al., 2001).

In recent years, it was reported that caprine SCD nucleotide sequence shares 98% and 95%t similarity with those of sheep and cows ( Yahyaoui et al., 2003). The degree of similarities of the dairy animals might explain the almost similar findings with the association of their SCD gene and their respective milk yield performances.

Although minimal, the association between caprine SCD and milk yield performance has been previously studied. In 2013, Crepaldi et al. administered a study on the association of ACACA, SCD, and lipoprotein lipase genes with dairy traits in alpine goats. Effects of genotypes of a sample of 59 Alpine bucks on phenotypes of their 946 daughters raised in 75 flocks were investigated. Their results show that the TGT deletion located on the untranslated region of the SCD gene had caused a decrease in milk and protein daily yield of about 0.5 L/d and 16g/d. This showed a significant effect on average milk and protein yields. Even if they used a small number of goat samples in their study, their findings suggested that there was indeed a correlation between the SCD gene and the milk yield performance of the alpine goats. The same findings were found with the Czech dairy goats. Together with other biochemically essential genes, the polymorphism of the SCD gene showed a significant association with milk traits within the sampled population (Brzakova et al., 2021). In a study conducted by Yahyaoui and his team (2003), polymorphism was detected within a 447-bp-long PCR amplification product of the goat stearoyl coenzyme A desaturase (SCD) gene, which contains the complete exon 5 sequence (239 bp). They noticed that the substitution of one allele to another (G to T) resulted in the disappearance of the *Rsa*I recognition site. This allows them to conduct rapid RFLP and screen the genetic variation of the SCD gene. Thus, in the present study, polymorphism in the exon 5 of the SCD 1 gene was screened using *Rsa*I as the restriction enzyme to assess the genotypic and allelic frequencies on crossbred Anglo-Nubian dairy goats.

The main objective of this study was to assess the influence of *stearoyl-CoA desaturase 1* on the milk yield performance of the crossbred Anglo-Nubian dairy goats. Specifically, the study aims to (1) detect the genetic polymorphism within the SCD1 gene in crossbred Anglo-Nubian dairy goats using PCR-RFLP; (2) estimate genotypic and allelic frequencies in the SCD1 gene; and(3) determine the effects of polymorphism in the SCD1 gene on goats' milk yield.

**Materials and Methods**

**Experimental Animals**

One hundred and one hair follicles of crossbred Anglo-Nubian dairy goats were acquired from three selected dairy farms in the Philippines (OB=34; OA=33; D= 34). Two of the three selected farms were located at Barangay Awang, Opol, and Misamis Oriental, while the third farm was situated in Barangay Talay, Dumaguete City, Negros Oriental. The two farms in Opol used roughage (Napier grass hay or corn silage) as feedstuff, while both concentrate (mix of spent grain, corn bran, copra meal) and roughage (80% super Napier and 20% legumes) were used in Dumaguete. All farms practiced ad libitum feeding. The selection of farms was based on the availability of goat samples and the response of the farm owners on the request to conduct a study on their farms. All samples were already available in the laboratory and were ready for DNA extraction (Moneva et al., 2020).

**gDNA isolation and PCR amplification**

Hair follicles were trimmed to 5-10mm of the proximal (root) end and were placed in a microcentrifuge tube (1.5mL). The extraction of gDNA was carried out using the QIAGEN DNeasy extraction method following a modified protocol based on the package manual. Extracted gDNA from the 101 goat samples were viewed in a 1.4% agarose gel electrophoresis (AGE) using 1X TBE buffer. A 447-bp fragment located in exon 5 was amplified using the forward primer (AGTGTAGAAGGGACAGCCCAGC) and the reverse primer (GTGGAATGACACATGGAGAGGG) referred from the study of Yahyaoui et al. (2003). PCR amplification of all the 101 samples was accomplished in a 25 µL final reaction volume containing 2 µL of goat genomic, 12.5µL of 1X PCR master mix (Vivantis, Malaysia), 1.25 µL of each primer (Forward & Reverse), and 8µL of nuclease-free water. The amplification was performed using this PCR condition: initial denaturation of 95 C for 4 min, 30 cycles of 94 C for 30 s, —C for 30 s, 72 C for 40s, followed by a final extension at 72 C for 10 min. Amplified DNA fragments were separated using a 1.4% agarose gel in 1X TBE and viewed in the agarose gel electrophoresis (Yahyaoui et al., 2003; Kgwatalala et al., 2009; Moneva et al., 2020).

**Genotyping**

Protocol for rapid genotyping of the polymorphic site was developed using PCR-RFLP. All PCR product with a final volume of 20µL was digested at 37°C overnight using *Rsa*Ias the restriction enzyme. The digested products were separated on a 1.4% agarose gel in an agarose gel electrophoresis. The emergence of bands with specific sizes after complete digestion was used to determine a genotypic variation within the SCD1 gene (Yahyaoui et al., 2003; Kgwatalala et al., 2009.

**Statistical Analysis**

Genotypic and allelic frequencies were calculated, and the chi-squared (χ2) test was utilized using PopGen32 Software to test the Hardy-Weinberg equilibrium (HWE) of the crossbred Anglo-Nubian goat population studied. A two-way factorial (2 x 4) in an RCBD was used to determine the association between genotypes and milk yield performance. The general linear model procedure in the Statistical Analysis Software package was used in the statistical analysis. In the SAS systems software, the farm category was set as the blocking factor to eliminate the effect of the different animal care practices used on each farm. Parity and genotypes were considered the main factors. The model used to test for the effect of SCD1 genotypes on milk yield was Yijkl = µ + Gi + Pj + Fk + eijkl (Moneva et al., 2020).

**Results**

**PCR Amplification:** The PCR amplification of *stearoyl-coenzyme A desaturase 1* (G931T) gene in the experimental animals produced a 447-bp DNA fragment as shown in Figure 1.

**Genotyping:** Figure 2 shows the different DNA fragments, where TT genotype produced 349-bp and 98-bp, while genotype GT produced 349, 238, and 98/101 bp, respectively

**Genotypic and allelic frequencies:** Table 1 shows the frequencies of alleles and genotypes, heterozygosity, and Hardy-Weinberg equilibrium of the diary goat population studied.

**Association analysis:** The effect of different *SCD1* (G931T) genotypes to milk production performance in crossbred Anglo-Nubian is shown in Table 2.

**Discussion**

**Amplification of *SCD1* gene and genotyping**

A 447-bp fragment of *Stearoyl Coenzyme A desaturase 1* gene was amplified from the gDNA extracted from the hair follicles of the crossbred Anglo-Nubian dairy goats from Opol, Misamis Oriental, and Dumaguete City, Negros Occidental. The polymorphism was observed at the G931T locus in exon 5 (Accession number: AF339909). But this resulted in no amino acid change (Yahyaoui et al.,2003).

**Genotype and Allele frequencies**

After conducting SCD-*Rsa*I digestion to all hair follicle samples using RFLP, it generated a SNP of G and T alleles. The homozygous T (or TT as genotype) allele gives off only two fragments of 349 and 98 bp. In comparison, the heterozygous genotype for both G and T alleles (G/T) produces three fragments, namely, 349, 238, and 98/101 bp products, respectively. The absence of the homozygous G allele (238, 111, and 98bp) may be due to the limited number of sampled animals and the breed of sampled goats (Yahyaoui et al., 2003; Crepaldi et al., 2013). Thus, the present study failed to observe a homozygous G allele in the 98 goat hair samples. Figure 2. shows the SCD-*Rsa*I digested RFLP product with precise band sizes. Homozygous TT has two bands, while heterozygous GT genotype gives off three bands.

In the present study, one undigested fragment of GG homozygous genotype and two genotypes were recognized after a 5h-digestion of the SCD1 PCR amplicons of the goat hair samples. The absence of GG genotype, in the present study, (or certain genotype,) may be caused by breed differences and/or the consequence of sampling fluctuations of populations (Gooki et al, 2018; Moneva et al., 2022). As shown in Table 1, the heterozygous G/T genotype showed higher genotypic frequencies, with an average frequency of 0.78 (0.91, 0.75, and 0.67) from all farms, while the TT genotype only had an average of 0.29 genotypic frequency (0.15, 0.35, 0.36). On the other hand, the allelic frequency of allele T (0.64) was much higher than the G allele (0.36). Similar findings were observed in the work of Crepaldi and colleagues in 2013 with alpine goats.

Table 1 also shows both expected and observed heterozygosity frequencies of heterozygous individuals in the three farms with respect to the SNP. As observed, all observed heterozygous frequencies (Ho) from all farms showed significantly higher estimates (0.85, 0.65, and 0.64) than the expected heterozygous frequencies (0.49, 0.44, and 0.44). Dumaguete farm showed the highest frequency among the three farms, followed by Opol B. The results signify that each farm has high genetic variability (Moneva et al., 2020). Heterozygosity plays a vital role in population studies. The frequencies of Ho and He in a given population sample will tell if there is high genetic variability or low genetic variability in the given population. The heterozygosity frequency represents the genetic polymorphism in the population (Ilham et al. 2016; Moneva et al., 2020). High heterozygosity frequency in a given population means that the genetic variability in that population is high, while low heterozygosity signifies low genetic variability. Suppose the observed heterozygosity (Ho) estimate is lower than the expected heterozygosity (He). It might be due to inbreeding in the population; lower Ho than the He will deviate from the HWE; this may be caused by inbreeding (Moneva et al.,2020).

Hardy-Weinberg equilibrium was observed within the three farms: Dumaguete, Opol B, and Opol A conform to the Hardy- Weinberg expectations (p>0.05). This was proven by conducting a chi-square test on all three farms. The results may indicate that selection pressure was insignificant with genotypic frequencies (Alim et al., 2012). This means that the present study's sample population met all the five requirements of the Hardy-Weinberg Equilibrium test: random mating, the occurrence of mutation is absent, no gene flow, and no natural selection should occur. Like heterozygosity frequency, Hardy-Weinberg Equilibrium plays a crucial part in population genetics. According to the rules of HWE, "genotype frequencies in a population remain constant between generations in the absence of disturbance by outside factors" (Edwards, 2008). Hardy-Weinberg Equilibrium (HWE) is used in population genetics to measure the number of homozygous and heterozygous carriers by looking at the allele frequency on the population/s that are not evolving (Abramovs *et al.,* 2020).

**Association Analysis**

The effect of parity and SCD1 genotypes on milk yield performance was tallied in Table 2. Parity of the goats on the three farms was used to assess the milk yield performance since it is an important attribute in the life of a ruminant animal. Many studies have claimed that parity influences the reproductive and productive traits in farm animals, especially in dairy cows and goats (Ilham et al. 1970; Lee et al. 2006; Zamuner et al. 2020). When both genotype and parity were tested for any relevant connection with milk yield performance, PAR\*SCD1 (p= 0.72) (p >0.05) had no significant effect on milk yield production of the crossbred Anglo-Nubian dairy goats. However, both significantly affected milk yield performance when the two factors were tested separately. This aligns with the work of Moneva *et al*., 2020. In their study, the interaction effect between parity and *GH* genotypes was also insignificant at p > 0.05. Least significant difference (LSD) test was also conducted on the study to check whether each factor (e.g levels of parity and genotype variability) was significantly different from each other. It can be observed in Table 2 that as parity of the crossbred Anglo-Nubian goats progressed, the milk yield performance of each goat decreased. During the 2nd parity, goats tend to produce the highest milk yield with an average daily milk yield of 0.97 L/d and 1.08 L/d (90d and 140d milking data) and average total milk production of 85.68 ± 4.07a L and 151.5 ± 11.29a L (90d and 140d milking data), respectively. Based on the data in Table 2 below, the data suggested that after the 2nd parity, successive parities have an inverse effect on milk yield performance. This conforms to the work of Ciappesoni *et al.* (2004) with factors that might affect goat milk yield and composition, their study also noticed that milk yield increased progressively with the parity until the 3rd lactation, and successive parities showed lower milk yield; 11.7%; 15% and –1.5% between 1st and 2nd, 2nd and 3rd and 3rd and 4th and further lactations, respectively. The same findings were observed in the study of Moneva *et al.* (2020). The average daily milk yield and total milk production at 90 d in their study were considerably lower in the ≥ fourth parity (p < 0.05), while at 140 d milking period, average daily milk yield and total milk production increased from the first to second parity and decreased significantly in succeeding parities (p < 0.05). This was also observed in the works of Phoya *et al*, (2003) in Malawi goats and Bemji *et al*, (2007) in West African Dwarf goats and Red Sokoto.

The inverse relationship between milk yield and parity may be caused by the interruption of the mammary alveoli as the goats’ age (Idowu and Adewumi 2017). Although the SCD gene was widely associated with milk fat composition rather than milk yield in bovine and ruminants, the present study results showed in Table 2 that the SCD1 genotype (p<0.05) had a significant effect on milk yield performance in crossbred Anglo-Nubian goats. Furthermore, milk yield performance between SCD1 genotypes was statistically significant (p <0.05). G/T genotype (0.98 ± 0.058 L/d) had the highest daily milk yield average at 140d milking, while the TT genotype only had 0.54L, much lower even when compared to G/T (0.94L) 90-d milk average. The same observation was seen in the total milk production of the Anglo-Nubian goats, wherein G/T genotype produce (82.70L) in the 90d milking period was still higher than the 140tmp (75.32L) produce of TT genotype. The present study suggests that G/T genotype was dominant over the TT genotype in terms of both population and milk yield performance.

A similar observation was reported by Čítek *et al*. (2021), the TT genotype in SCD1 was associated with the lowest milk, protein, and fat yields and with the highest milk protein percentage (p<0.01). The T allele had higher values than the C allele (p<0.05) The same findings were observed in an association study conducted by Pauciullo *et al*. (2013) between milk yield performance and SCD polymorphism. In their study, an association between the g.133A >C SNP was previously found in the promoter region of the SCD gene and daily milk yield in the Italian river buffalo. Another association study between the SCD gene and milk yield stated that a certain TGT deletion at the untranslated region of the SCD gene had significantly affected the average milk and protein yields in Czech dairy goats (Crepaldi *et al*., 2013). According to the study, the deletion of the genotype had caused lower milk and protein yield (0.5 L/d and 16 g/d), respectively, compared with the TGT/TGT genotype. This may be due to the occurrence of conformational change in the mRNA secondary structure after deletion, which promotes the formation of a long-stacked pair terminated in 2 small hairpin loops that affect milk yield performance. Their study suggests that the presence of the TGT genotype enhances the milk yield performance of the Czech dairy goats. Aside from these studies, an assessment of the effect of polymorphisms in the *ABCG2*, *LEPR,* and *SCD1* genes on milk production traits in Holstein cows was carried out by Soltani-*Ghombavani et al* (2016), the polymorphisms in the *SCD1* gene (g.293A>V) and rare homozygous VV showed significantly higher milk yield performance of the Iranian Holstein cows compared to AA genotype were observed on their study. Their results indicated an effect of the SCD1-A293V genotype on 305-day milk yield.

**Conclusion**

It was determined in this study that *SCD1* genotypes significantly affect the milk yield traits of the crossbred Anglo-Nubian dairy goats (p<0.05). This is one of the few studies that associates *SCD1* polymorphisms with milk yield performance in goats. In the present study, due to the limited resources available in the laboratory, it is highly recommended to use a spectrophotometer during DNA extraction to check the purity of the gDNA and to ensure good reads after PCR-RFLP. DNA sequencing of the PCR amplicons and digested RFLP product should also be considered to ensure that the gene of interest’s target sequence was correctly determined. It is also recommended to conduct the same research with a much larger population of goats to validate the results. Furthermore, it would be interesting to conduct this study on our Philippine native goats or other goat breeds found in the Philippines in order to help the breeders to improve our dairy products in the country.

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**Author Contributions**

CSOM designed the study acquired the data; EAKBM planned the experiments, interpreted the results, drafted the manuscript, and statistically analyzed the data; CSOM,CAB, and SRT made critical review and revision of the manuscript.

**Conflict of Interest**

All authors declare no conflict of interest.

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

**Ethics Approval**

The experimental procedures were carried out in accordance with the guidelines and prior approval of the Institutional Animal Care and Use Committee of the Mindanao State University – Iligan Institute of Technology.

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**Figure 1.** 1.4% AGE of the 447 bp fragment of the caprine Stearoyl Coenzyme Desaturase gene from crossbred Anglo-Nubian dairy goats (MW=100 bp plus DNA ladder; NC=negative control).

**MW**

**NC**

**400 bp**

**100 bp**

**447 bp**

**MW GT GT GT TT GT TT**

**Figure 2.** 1.4% AGE of *stearoyl coenzyme desaturase* gene PCR product from crossbred Anglo-Nubian dairy goats using *Rsa*1 RE (MW = 100 bp plus ladder; TT = 349, 98 BP; G/T= 349, 238, 98 bp)

**Table 1.** Frequencies of genotype and allele for sequence polymorphisms in the GH (A781G) from crossbred Anglo-Nubian dairy goats.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Farm** | **N** | **Genotype frequency** | **Allele frequency** | **Heterozygosity** | **(χ2) (HWE)** |
|  | TT | G/T | G | T | Expected | Observed |
| Dumaguete |  | 0.15 | 0.85 | 0.43 | 0.57 | 0.49 | 0.85 | 0.7 |
| Opol B |  | 0.35 | 0.65 | 0.33 | 0.67 | 0.44 | 0.65 | 0.47 |
| Opol A |  | 0.36 | 0.64 | 0.32 | 0.68 | 0.44 | 0.64 | 0.45 |
| **TOTAL** | **101** | **0.29** | **0.71** | **0.36** | **0.64** | **0.46** | **0.71** | **0.45** |

N – number of experimental animals; χ2 (HWE) – Hardy-Weinberg equilibrium by the chi-squared test: χ2 = 3.81 with P = 0.05, χ2 = 6.63 with P = 0.01.

**Table 2.** Influence of parity and *SCD1* genotype on milk yield (mean±SEM) in crossbred Anglo-Nubian dairy goats.

|  |  |
| --- | --- |
| **Parity** | **Milk yield traitsI (L)** |
| 90 ADMY | 140 ADMY | 90 TMP | 140 TMP |
| 1st | 0.91± 0.042a | 0.98± 0.044ab | 81.10± 3.8a | 133.87± 6.29ab |
| 2nd | 0.97± 0.046a | 1.08± 0.075a | 85.68± 4.07a | 151.54± 11.29a |
| 3rd | 0.85± 0.041a | 0.84± 0.040b | 74.67± 3.53a | 117.12± 5.46b |
| ≥4th | 0.58± 0.046b | 0.60± 0.050c | 50.53± 3.81b | 83.09± 6.95c |
| ***SCD 1* genotypes** |
| TT | 0.52± 0.025a | 0.54± 0.027a | 45.92± 2.26a | 75.32± 3.88a |
| G/T | 0.94± 0.047b | 0.98± 0.058b | 82.70± 4.12b | 135.78± 8.33b |

a,b,c Significant results are designated by different superscript letters (p < 0.05). 190d ADMY – 90-d average daily milk yield; 140d ADMY – 140-d average daily milk yield; 90d TMP – 90-d total milk produced; 140d TMP – 140-d total milk produced.