Genetic Polymorphism of *CSN2* Gene and its Association with Milk Yield Traits in Crossbred Anglo-Nubian Dairy Goats

Catherine A. Buyan[1*], Trixia S. Macalam[1], Eloise Ann Kristine B. Makasiar[1], Reshe M. Pontevedra[1], Jessah P. Trillo[1], Olive A. Amparado[1], Mark Anthony I. Jose[1], and Carlo Stephen O. Moneva[12]

[1]College of Science and Mathematics
[2]Premier Research Institute of Science and Technology
Mindanao State University – Iligan Institute of Technology
Iligan City, 9200 Philippines
[*]*catherine.buyan@g.msuiit.edu.ph*

Date received: October 8, 2023
Revision accepted: September 16, 2025

Abstract

CSN2 gene is one of the closely linked genes coding for one of the proteins present in milk: casein. Studies on the CSN2 gene have shown an association with increased milk yield in sheep and protein content in cows. This study aims to check the presence of a single nucleotide polymorphism (SNP) in the CSN2 gene of the crossbred Anglo-Nubian dairy goats pooled from three different farms and evaluate its association with milk yield. 101 crossbreed Anglo-Nubian dairy goats were collected with hair follicles, and available data on the milk yield of these individuals were utilized. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to identify the presence of SNP in the CSN2 gene. The effect of genotypes on milk yield was evaluated using ANOVA via a two-way factorial (2x4) randomized block design (RCBD). A 360bp amplicon was produced, and digestion with SspI resulted in 240 bp and 120 bp fragments, indicating the presence of a SNP. Two genotypes were recorded in the population: the homozygous AA with 0.81 frequency and the heterozygous AA^1 with 0.19 frequency. The studied population is in Hardy-Weinberg equilibrium (χ^2=0.18). Significant difference between the average milk yield of the AA and AA^1 genotypes was found, with the AA genotype yielding a 90-day average milk yield of 0.87±0.048 L while AA^1 only averaged to 0.65±0.03 L. The result shows the association of SNP present in the CSN2 gene on milk yield of crossbred Ango-Nubian dairy goats which suggests breeding of goats with the AA genotype.

***Keywords**:* caprine, *CSN2*, genotype, milk yield, PCR-RFLP, SNP

1. Introduction

The number of dairy goats raised is increasing due to rising demand for milk. Caprine milk has been recommended as an alternative for patients allergic to cow milk protein (Park and Haenlein, 2021). Researchers, producers, and policymakers work together and share information to help expand the goat sector and meet the growing market needs (Miller and Lu 2019). In the country, the National Dairy Authority was created to promote sustainable dairy production.

One of the main proteins in goat milk is casein (Martin *et al*., 2002). Among these casein proteins, β-casein, encoded by the CSN2 gene, is the most abundant in goat milk, accounting for up to 50% of the total casein content (Tortorici *et al*., 2016). According to a review by Marletta *et al*. (2007), the casein genes in goats show significant polymorphism that influences milk composition and quality. The CSN2 gene alone has ten known alleles: $CSN2^{A, A1, B, C, C1, D, E, F, 0, 01}$ (Consenza *et al*., 2023).

The main dairy goat breeds raised on farms in the country are Anglo-Nubian, followed by Saanen, and then Alpine (Manalili *et al.*, 2020). Two studies conducted by Moneva *et al*. (2020, 2022) examined single-nucleotide polymorphisms in the growth hormone (GH) gene and the Leptin gene of crossbred Anglo-Nubian dairy goats. Results showed a link between milk yield and various genotypes within each gene.

In 2005, Consenza *et al*. identified a SNP within the CSN2 gene where there is a C to T transition at the 180th nucleotide of the 9th exon; specifically, the $CSN2^{A}$ and $CSN2^{A1}$ alleles. As part of the growing effort to expand knowledge about which gene might influence milk yield, this research aims to determine the presence of the aforementioned SNP within the CSN2 gene in crossbred Anglo-Nubian dairy goats and to evaluate its association with milk yield.

2. Methodology

2.1 Sample, Milk Production, and Preparation of Materials

Field sampling was no longer conducted in the study. Instead, available hair samples for DNA analysis and data on daily milk production from the study

of Moneva *et al*. (2020, 2022) were used. These samples came from a total of 101 crossbred Anglo-Nubian dairy goats from three farms—two located in Barangay Awang, Opol, Misamis Oriental, and the third in Barangay Talay, Dumaguete City, Negros Oriental. The number of samples from each farm was 33 for Opol A, 34 for Opol B, and 34 for Dumaguete.

Daily milk collection was carried out regularly twice daily, from 8:30 AM to 9:00 AM and from 3:30 PM to 4:00 PM. Milk produced by the animal was measured using a pitcher (in mL). Milk yield from the first to at least the fourth parity was standardized over 90-day and 140-day milking periods for analysis (Moneva *et al*., 2020, 2022). Average daily milk yield and total milk production for both the 90-day and 140-day periods were calculated.

2.2 gDNA Isolation

Isolation of genomic DNA from the hair follicles of the crossbred Anglo-Nubian dairy goats was performed at the Animal Ecology and Oceanography laboratories of the Premier Research Institute of Science and Mathematics (PRISM) located within MSU-IIT.

At least five hair follicles were trimmed 5-10 mm from the root tip and placed in a 1.5 mL microcentrifuge tube. The DNeasy Blood and Tissue extraction kit (QIAGEN) was used to extract the gDNA from the hair follicles. The protocol provided with the kit was followed with slight modifications (Zheng, 1988).

Agarose gel electrophoresis was performed to verify successful gDNA extraction. The extracted gDNA was visualized using the blueGel Electrophoresis System (MiniPCR Bio, USA).

2.3 PCR Amplification and Genotyping

Amplification of samples was followed by a 25-µl reaction mix containing 12.5µl of 2X ViRed Taq Master Mix (Vivantis), 1.25µl of forward and reverse primers (Integrated DNA Technologies), 8µl of sterilized water, and 2µl of the extracted gDNA. The primers and amplification protocol were based on the study by Consenza *et al*. (2005).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotyping. 10 U of *SspI* endonuclease (Vivantis) was

diluted following the protocol in the manual. 1 U of the restriction enzyme was used to digest 5 µL of each amplified PCR product for one hour at 37°C, followed by inactivation at 65°C for 20 minutes. The amplicons and digested products were analyzed using a 1.4% agarose gel stained with 2.0 µL gel green in 1X TBE buffer in the BlueGel Electrophoresis System.

2.4 Statistical Analysis

Genotypic and allelic frequencies were calculated. The goat population was tested for Hardy-Weinberg equilibrium (HWE) using the chi-squared (χ^2) values computed using the built-in function in the Popgen32 software. ANOVA via two-way factorial (2×4) in a randomized complete block design was used to determine the association between genotypes and milk yield performance. The main factors considered were parity and genotypes, while the farm was set as the blocking factor. A general linear model in the Statistical Analysis Software (SAS) package was used for the association analysis. The model used to test for the effect of *CSN2* genotypes on milk was $Y_{ijkl} = \mu + G_i + P_j + F_k + e_{ijkl}$ (Moneva *et al*., 2020).

3. Results and Discussion

3.1 Amplification of CSN2 Gene and Genotyping

A fragment of the *CSN2* gene was amplified from the extracted genomic DNA of crossbred Anglo-Nubian dairy goats. The PCR product included 60 bp of the flanking regions and 300 bp of exon 9 (Consenza *et al*., 2005). As shown in Figure 1, the PCR product produced the expected 360-bp fragment.

The presence of a SNP in the 180th nucleotide of the exon 9 characterized by a C to T transition, created a splice in the PCR product after it was digested using *SspI* endonuclease, as shown in Figure 2. These same fragments were observed in the *CSN2* gene of the goat population from Naples, Italy. Figure 2 shows the expected fragments: the 360-bp fragment for the presence of the AA genotype, and the fragments 360-bp, 240-bp, and 140-bp characterized by the AA^1 genotype.

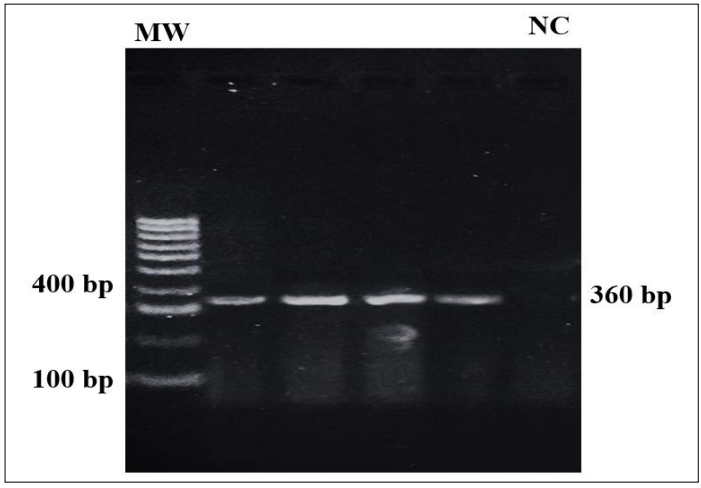

Figure 1. 360-bp amplicon viewed using 1.4% AGE
MW - 100 bp plus DNA ladder; NC - negative control

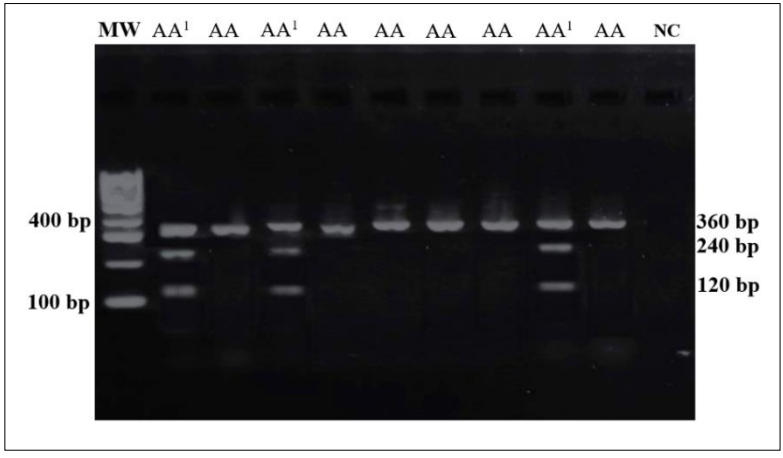

Figure 2. Fragments from digestion of amplicon using the *SspI* restriction enzyme
MW - 100 bp plus DNA ladder; NC - negative control

3.2 Genotypic and Allelic Frequencies, Heterozygosity, and HWE

The RFLP analysis has shown two genotypes (AA, AA^1), while A^1A^1 genotype was not present in the total sampled population (Table 1). The frequency of the homozygous genotype AA (0.81) was found to be higher than the heterozygous genotype AA^1 (0.19), while the allele frequency of A (0.91)

was higher than the A^1 allele (0.09). Lower A^1 allele was also observed in African goat breeds (Chessa *et al.*, 2008) as well as the breeds: Red Sokoto, West African Dwarf Nigerian, West African Dwarf Cameroon, and Born from West Africa (Caroli *et al*., 2007). Absence of A^1A^1 genotype was also found in the study of Tortorici *et al*. (2014) in a population of the Girgentana goat breed. The higher frequency of the A allele can be due to the gene's ancestral condition, since the presence of cytosine in the location was also found in other ruminant species (Consenza *et al*., 2005).

Table 1 also shows both expected (He) and observed (Ho) heterozygosity of the crossbred Anglo-Nubian dairy goats. According to Ilham *et al*. (2016), heterozygosity signifies the level of polymorphism and is described as the average fraction of heterozygous individuals in a population. The difference between these two values gives an insight into whether the study population will conform to the Hardy-Weinberg equilibrium (Sharma *et al*., 2016). A high Ho value with respect to the He suggests that the population sampled has high genetic variability and is in HWE. A study by Sharma *et al*. (2016) reinforces this claim, wherein the population deviates from the HWE and the reported Ho is lower than the reported He value. In the current study, the Ho is higher than the He, meaning the population has high genetic variability.

Table 1. Frequencies of genotype and allele for sequence polymorphisms in the CSN2 from crossbred Anglo-Nubian dairy goats

Farm	N	Genotype Frequency		Allele Frequency		Heterozygosity		χ^2 (HWE)
		AA	AA^1	A	A^1	Expected	Observed	
Opol A		0.70	0.30	0.85	0.15	0.26	0.30	0.27
Opol B		0.88	0.12	0.94	0.06	0.11	0.12	0.10
Dumaguete		0.85	0.15	0.93	0.07	0.13	0.15	0.17
Total	101	0.81	0.19	0.91	0.09	0.17	0.19	0.18

N – total number of sampled goats; HWE – Hardy Weinberg Equilibrium

Moreover, results from the χ^2 tests revealed that the population of dairy goats from the three farms conforms to HWE ($p>0.05$). This result implies that the population did not experience any disruptive mechanism such as non-random mating, natural selection, genetic drift, mutations, and gene flow. This also means that the same genetic variation will be observed in the next generations if the enlisted disruptive mechanisms will not be experienced (Lachance, 2016).

3.3 Association Analysis

Table 2 illustrates the effect of parity and CSN2 genotypes on milk yield in crossbred Anglo-Nubian dairy goats. The interaction between these two factors is not significant ($p>0.05$). Results from the studied population show a decline in average milk yield as parity increases. Generally, the peak milk yield occurs at the second parity, then begins to decrease from the third parity onward. A significant decline starting at the third parity is observed in both 140-day average milk yield and total milk production. This finding aligns with the study by Phoya *et al*. (2003) in Malawi goats, where milk production decreased linearly with increasing parity.

The overall increasing trend observed from the first parity to the second parity can be explained by the study of Lerias *et al*. (2014), which shows that multiparous goats have a higher proportion of developed alveoli from previous lactations, leading to increased secretory parenchyma and udder volume compared to primiparous goats. The same is true in the study of Guney *et al*. (2006), where milk production increases with parity until it is interrupted by age. Aging tends to slow down milk production, and increasing parity is associated with advancing years. Additionally, decreases in the third (140D AMY & 140D TMP) and fourth (90D-140D AMY & TMP) parity can be attributed to lactation persistency, one of the three main factors affecting milk yield (Arnal *et al*., 2018). Furthermore, this is supported by the results of the study by Zamuner *et al*. (2020) in Australian dairy goats, where increasing initial and peak milk yields are observed with increasing parity, but at the same time, persistence decreases (Gipson and Grossman, 1990; Leon *et al*., 2012; Arnal *et al*., 2018).

Table 2 also shows a significant difference in milk yield performance between the two *CSN2* genotypes, AA and AA^1. This variation in milk yield may be due to the SNP found in exon 9, which is part of the 3' untranslated region of the *CSN2* gene. A study by Xu *et al*. (1997) noted that sequences within the 3' UTR can influence mRNA deadenylation and degradation. Additionally, 3′UTRs also regulate mRNA localization, stability, and translation (Mayr, 2019). This finding was supported by the study of Hou *et al*. (2014), in which a SNP within the 3'-UTR of the goat gene PRLR in Xinong Saanen and Guanzhong breeds was significantly associated with milk production traits. The detection of a 4bp indel in the 3'UTR of Sox9 in Shaanbei white cashmere (SBWC) goats was also significantly associated with body measurements (He *et al*., 2020).

At present no known study has been conducted directly associating milk yield with the two *CSN2* genotypes.

Table 2. Effect of parity and CSN2 genotypes on milk yield (mean ± SEM) in crossbred Anglo-Nubian dairy goats

Parity	Milk yield traits[1] (L)			
	90D ADMY	140D ADMY	90D TMP	140D TMP
1st	0.91±0.042[a]	0.98±0.044[ab]	81.10±3.88[a]	133.87±6.29[ab]
2nd	0.97±0.046[a]	1.08±0.075[a]	85.68±4.07[a]	151.54±11.30[a]
3rd	0.85±0.041[a]	0.84±0.040[b]	74.67±3.53[a]	117.12±5.46[b]
≥4th	0.58±0.046[b]	0.60±0.050[c]	50.53±3.81[b]	83.09±6.95[c]
CSN2 genotypes				
AA	0.87±0.048[a]	0.92±0.059[a]	76.99±4.21[a]	128.46±8.45[a]
AA[1]	0.65±0.033[b]	0.68±0.035[b]	57.76±3.02[b]	93.71±4.90[b]

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

4. Conclusion and Recommendation

In conclusion, this study revealed the presence of a SNP within the *CSN2* gene of a pooled population of crossbred Anglo-Nubian dairy goats and evaluate its association with milk yield. The data analysis revealed a significant difference between the two genotypes, with the AA genotype showing higher milk yield compared to the AA[1] genotype. Future studies involving a larger population using the same gene and trait should be conducted for confirmation of the obtained results.

5. Acknowledgement

The author would like to thank the people who in one way or another contributed to the completion of the study. To the Department of Science and Technology - Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP) for funding this research.

6. References

Arnal, M., Robert-Granie, C., & Larroque, H. (2018). Diversity of dairy goat lactation curves in France. *Journal of Dairy Science, 101*(12), 11040–11051. https://doi.org/10.3168/jds.2018-14980

Caroli, A., Chiatti, F., Chessa, S., Rignanese, D., Ibeagha-Awemu, E.M., & Erhardt, G. (2007). Characterization of the casein gene complex in West African goats and description of a new αs1-casein polymorphism. *Journal of Dairy Science, 90*(6), 2989–2996. https://doi.org/10.3168/jds.2006-674

Consenza, G., Pauciullo, A., Gallo, D., Berardino, D.D., & Ramunno, L. (2005). A SSPI PCR-RFLP detecting a silent allele at the goat CSN2. *The Journal of Dairy Research, 72*(4), 456-459. https://doi.org/10.1017/s0022029905001342

Consenza, G., Albarella, S., D'Anza, E., Iannuzzi, A., Selvaggi, M., Pugliano, M., Galli, T., Saralli, G., & Peretti, V. (2023). A new AS-PCR method to detect CSN201 allele, genotyping at ca-sensitive caseins loci and milk traits association studies in autochthonous Lazio goats. *Animals, 13*(2), 239. https://doi.org/10.3390/ani13020239

Gipson, T.A., & Grossman, M. (1990). Lactation curves in dairy goats: A review. *Small Ruminant Research, 3*(4), 383–396. https://doi.org/10.1016/0921-4488(90)90019-3

Guney, O., Torun, O., Ozuyanik, O., & Darcan, N. (2006). Milk production, reproductive and growth performances of Damascus goats under Northern Cyprus conditions. *Small Ruminant Research, 65*, 176-179. https://doi.org/10.1016/j.smallrumres.2005.07.026

He, L., Bi, Y., Wang, R., Pan, C., Chen, H., Lan, X., & Qu, L. (2020). Detection of a 4 bp mutation in the 3' UTR region of goat Sox9 gene and its effect on the growth traits. *Animals, 10*(4), 672. https://doi.org/10.3390/ani10040672

Hou, J.X., Fang, F., An, X.P., Yan, Y., Ma, T., Han, P., Meng, F.X., Song, Y.Y., & Cao, B.Y. (2014). Polymorphisms of PRLR and FOLR1 genes and association with milk production traits in goats. *Genetics and Molecular Research, 13*(2), 2555-2562. https://doi.org/10.4238/2014.january.24.1

Ilham, F., Rachman, S.D.A., & Dagong, M.I.A. (2016). Genetic polymorphisms of growth hormone (GH) gene in Kacang goat population based on polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) identification. *Livestock Research for Rural Development, 28*(9). https://www.lrrd.org/lrrd28/9/ilha28167.html

Lachance, J. (2016). Hardy–Weinberg equilibrium and random mating. *Encyclopedia of Evolutionary Biology, 2*, 208-211. https://doi.org/10.1016/B978-0-12-800049-6.00022-6

Leon, J.M., Macciotta, N., Gama, L., Capote, C.B., & Delgado, J.V. (2012). Characterization of the lactation curve in Murciano-Granadina dairy goats. *Small Ruminant Research, 107*(2-3), 76–84. https://doi.org/10.1016/j.smallrumres.2012.05.012

Lerias, J.R., Hernandez-Castellano, L.E., Suarez-Trujillo, A., Castro, N., Pourlis, A., & Almeida, A.M. (2014). The mammary gland in small ruminants: Major morphological and functional events underlying milk production—A review. *The Journal of Dairy Research, 81*(3), 304–318. https://doi.org/10.1017/s0022029914000235

Manalili, L.L.G., Del Rosario, N.A., Orden, E.A., Cruz, E.M., Garabiles, J.S., & Orden, M.E.M. (2020). Tracing the dairy goat industry: Profiling of dairy goat farms in the Philippines. *International Journal of Education and Development Studies, 1*(1). https://doi.org/10.22137/ijeds.2020.v1n1.01

Marletta, D., Criscione, A., Bordonaro, S., Guastella, A.M., & D'Urso, G. (2007). Casein polymorphism in goat's milk. *Dairy Science and Technology, 87*(6), 491-504. https://doi.org/10.1051/lait:2007034

Martin, P., Szymanowska, M., Zwierzchowski, L., & Leroux, C. (2002). The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reproduction, Nutrition, Development, 42*(5), 433-459. https://doi.org/10.1051/rnd:2002036

Mayr, C. (2019). What are 3' UTRs doing? Cold Spring Harbor Perspectives in Biology, 11(10). https://doi.org/10.1101/cshperspect.a034728

Miller, B.A., & Lu, C.D. (2019). Current status of global dairy goat production: An overview. *Asian-Australasian Journal of Animal Sciences, 32*(8), 1219-1232. https://doi.org/10.5713/ajas.19.0253

Moneva, C.S.O., Vega, R.S.A., Sangel, P.P., Angeles, A.A., & Mendioro, M.S. (2020). Genetic variability in the growth hormone gene (A781G) and its association with milk yield performance in crossbred Anglo-Nubian dairy goats. *Philippine Journal of Science, 149*(3), 503-509. https://doi.org/10.56899/149.03.04

Moneva, C.S.O., Sangel, P.P., Angeles, A.A., Mendioro, M.S., & Vega, R.S.A. (2022). Leptin gene (T117C) polymorphism and its association with milk yield performance in crossbred Anglo-Nubian goats. *Philippine Journal of Science, 151*(1), 333-339. https://doi.org/10.56899/151.01.25

Park, Y.W., & Haenlein, G.F.W. (2021). A2 bovine milk and caprine milk as a means of remedy for milk protein allergy. *Dairy, 2*(2), 191–201. https://doi.org/10.3390/dairy2020017

Phoya, R.K.D., Fullu, W.H.K., & Banda, J.W. (2003). The effect of milking indigenous Malawi goats on kid growth and mortality rates. *Malawi Journal of Agricultural Sciences, 2*(1), 42–48. https://www.cabi.org/gara/mobile/FullTextPDF/2008/20083325275.pdf

Sharma, R., Kumar, B., Arora, R., Ahlawat, S., Mishra, A.K., & Tantia, M.S. (2016). Genetic diversity estimates point to immediate efforts for conserving the endangered Tibetan sheep of India. *Meta Gene, 8*, 14–20. https://doi.org/10.1016/j.mgene.2016.01.002

Tortorici, L., Gerlando, R.D., Mastrangelo, S., Sardina, M.T., & Portolano, B. (2014). Genetic characterisation of CSN2 gene in Girgentana goat breed. *Italian Journal of Animal Science, 13*, 3414. https://doi.org/10.4081/ijas.2014.3414

Zamuner, F., DiGiacomo, K., Cameron, A.W.N., & Leury, B.J. (2020). Effects of month of kidding, parity number, and litter size on milk yield of commercial dairy goats in Australia. *Journal of Dairy Science, 103*(1), 954–964. https://doi.org/10.3168/jds.2019-17051

Zheng, S., Newton, G.L., Gonick, G., Fahey, R.C., & Ward, J.F. (1988). Radioprotection of DNA by thiols: Relationship between the net charge on a thiol and its ability to protect DNA. *Radiation Research, 114*(1), 11–27. https://pubmed.ncbi.nlm.nih.gov/3127858/