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Full Length Article

Polymorphisms in the Caprine *Ghrelin* Gene Associated with Milk Production Traits in Crossbred Anglo-Nubian Dairy Goats

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Abstract

Despite rising demand for goat meat and milk, the Philippines' goat production lags behind other livestock species. Genetic polymorphisms, such as those present in *ghrelin* (*GHRL*) gene, might be useful in increasing goat milk production. *Ghrelin* is a potent hunger stimulant that also aids in energy balance. The purpose of this study is to investigate potential correlations between the GHRL G375A genotype and milk perfromance parameters (average daily milk yield and total milk production) in crossbreed Anglo-Nubian dairy goats. One hundred goat hair follicle samples from three different farms in Misamis Oriental and Negros Oriental were collected for DNA extraction, amplification, and genotyping using the PCR-RFLP. A 0.91 and G 0.09 allele frequencies were observed, while AA 0.81 and GA 0.19 were the genotypic frequencies. To examine how genotypes affect milk yield, a two-way factorial experiment (2 × 4) in randomized complete block design (RCBD) was used. Different *GHRL* genotypes were observed to be statistically associated with milk production performance, where dairy goats having *GHRL* AA genotype produced consistently higher milk yield in the studied population.

Keywords: Milk production; Anglo-nubian goats; *Ghrelin;* Polymorphism; Genotyping

Introduction

The Philippines imports all of the milk it needs since local supply is low. The local milk market's budget is set, but the issue is an insufficient supply of milk. The budget for the local milk market is ready, but the lack of enough milk supply is the problem. Due to goats' ability to produce high-quality food in a variety of environmental conditions and thrive in flora that isn't necessarily incompatible with human nutrition, goat farming expanded (Norris *et al.* 2011). Production and processing of goat milk is a thriving and expanding industry that has a significant impact on the economies of many nations across the world (Ulusoy 2015).

In comparison to other nations, the Philippines produces less milk. According to several sources, the United States and the United Kingdom both produce 30 L of milk per day and 20 L per day, respectively, compared to the eight (8) L average daily milk output per animal in the Philippines. The Philippines' yearly consumption rate is 22 kg, whereas that of Thailand is 26 kg, and the United States is 287 kg. The demand for milk and dairy products grows as the economy grows and the population rises (Ang 2020).

The need for increased milk production led researchers to become interested in goat milk production in addition to cow and carabao milk production because it is a nourishing, healthful, and useful food. One way of potentially increasing the country's milk production is through genetic development of dairy animals. Genetic polymorphisms in goats are useful in increasing milk production. Additionally, the main goal of genetic development and breeding for goats is to increase production *via* the use of precise, trustworthy, healthy, and reliable selection methods. One of the ways in which this can be made possible is through determining the genotype of the animal in relation to its performance (Moneva *et al.* 2022).

A digestive hormone called ghrelin was found in the stomachs of rats (Kojima *et al.* 1999). It is a 28-amino acid peptide with the hydroxyl group on serine-3 altered to an octanoic state, which is necessary for ghrelin to release growth hormone (Bednarek *et al.* 2000). According to Kojima *et al.* (1999), the heart, kidneys, placenta and central nervous system are also small-scale producers of ghrelin. As one of the most significant galactopoietic hormones, growth hormone, affects the nutrient distribution for the production

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of milk in nursing cows (Sejrsen *et al.* 1999). Ghrelin binds to the growth hormone secretagogue receptor 1a (GHS-R1a), which is widely distributed in the brain and hypothalamus. Ghrelin is a versatile hormone that affects gastrointestinal functions, tissue growth and development, reproduction, and energy balance, according to more research (Korbonits *et al.* 2004; Kojima and Kangawa 2010).

With this hormone stimulating hunger and releasing the growth hormone, it has the potential to lessen the symptoms of anorexia in newborn calves, promote body weight gain, and enhance resilience to pathological and environmental challenges during early life. Body mass index and early life health status are the best indicators of future reproductive and productive performance. It has been shown that the levels of the ghrelin hormone in human breast milk and the blood of neonates who have been nursed correlate positively (Ilcol and Hizli 2007). To our knowledge, the effect of *ghrelin* in dairy goats has not yet been studied.

This study's goal is to determine how genetic variation in the ghrelin gene affects the performance of milk production in Anglo-Nubian dairy goat crossbreeds. The objectives of the study are to (1) isolate dairy goats' *ghrelin* gene and detect polymorphisms using PCR-RFLP, (2) describe frequencies of genotypes and alleles and (3) ascertain the effect of various *ghrelin* genotypes on milk yield.

Materials and Methods

Data collection

Collection of hair samples and milk production data of crossbred Anglo-Nubian goats were done in three dairy goat farms in Barangay Talay, Dumaguete, Negros Oriental, and Barangay Awang, Opol, Misamis Oriental in the Philippines (Moneva *et al*. 2020).

Genomic DNA isolation

Goat hair was cut 0.5–1.0 cm from the base. Proteinase K, 1M DTT, and 150 uL of buffer ATL were all added before it was incubated for roughly one hour at 56°C. The DNA was extracted according to the instructions included in the Qiagen DNA kit. The product was observed using the 1.4% Agarose gel electrophoresis at 50 V for 30 min.

Amplification and genotyping

Extracted gDNA from the different samples with good reading value were used for PCR amplification. PCR mix included 12.5 µL master mix, 8 µL sdH_2O, 1.25 µL forward primer, 1.25 µL reverse primer and 2 µL gDNA. Forward 5'-GTG GGG ATC TTA AGT TCC CTA-3' and Reverse 3'- AGG GTG GGA GAA CGG ACA GGT -5′ primers was used as suggested from the study of Kowalewska-Łuczak *et al*. (2011). The following PCR conditions were used for the amplification process: initial denaturation at 95°C for 1 min, followed by 30 cycles of denaturation at 95°C for 30 sec, primer annealing at 59°C for 30 sec, primer elongation at 72°C for 30 sec, a cycle of final elongation at 72°C for 7 min and storage at 4°C. In 1.4% Agarose Gel, amplified DNA fragments were separated for 30 min at 50 V.

Genotypic differences in the *ghrelin* gene were checked using the PCR-RFLP techniques. Five (5) μL of PCR product, 12.7 μL of sdH_2O, 2 μL of Restriction enzyme (RE) buffer and 0.3 μL of *FspBI (BfaI)*. Mixed product was incubated at 37°C for 2 h. DNA fragments were observed for 30 min in 1.4% Agarose Gel at 50 V. 0.42 g of agarose, 2 μL of gel green, and 30 mL of 1X TBE were used to make the gel.

Statistical analysis

Calculations were made about genotype and allele frequencies. The POPGENE Software for Population Genetic Analysis was used to calculate the chi-square values, and this analysis was utilized to determine which traits were most or least common in the population. Genotypic variations and parity were the main factors employed to analyze the relationship between various genotypes and milk yield performance, with farm location acting as a blocking factor. The Statistical Analysis Software package's general linear model approach was used.

Results

Amplification and genotyping

A 187-bp DNA fragment was generated by the ghrelin gene's PCR amplification in crossbred Anglo-Nubian goats, as illustrated in Fig. 1.

Fig. 2 displays the various DNA fragments produced by each genotype, with the AA genotype producing fragments of 187 bp and the GA genotype producing fragments of 187 bp, 167 bp and 19 bp.

Genotypic and allelic frequencies

Frequencies of genotypes and alleles, heterozygosity and Hardy-Weinberg equilibrium of the studied dairy goats are presented in Table 1.

Association analysis

The association between different *ghrelin* genotypes and milk production yield in crossbred Anglo-Nubian goats is shown in Table 2.

Table 1: Frequencies of alleles and genotypes for sequence polymorphisms in the *ghrelin* of the Anglo-Nubian dairy goats

FARM	N	GENOTYPE FREQUENCY		ALLELE FREQUENCY		HETEROZYGOSITY		χ^2 (HWE)
		AA	GA	A	G	expected	observed	
Opol A	33	0.70	0.30	0.85	0.15	0.26	0.30	0.94
Opol B	33	0.88	0.12	0.94	0.06	0.11	0.12	0.10
Dumaguete	34	0.85	0.15	0.93	0.07	0.13	0.15	0.17
TOTAL	100	0.81	0.19	0.91	0.09	0.17	0.19	0.46

*N = number of experimental animals; χ^2 (HWE) = Hardy-Weinberg equilibrium by the χ^2- test, $\chi2 = 3.81$ $P = 0.05$, $\chi2 = 6.63$, $P = 0.01$

Table 2: Influence of different *ghrelin* genotypes on milk yield (Mean±SEM) in crossbred Anglo-Nubian dairy goats.

MILK YIELD TRAITS[1]	*PROLACTIN* GENOTYPES	
(Liters)	AA	GA
90d ADMY	0.87 ± 0.048^a	0.65 ± 0.03^b
140d ADMY	0.92 ± 0.049^a	0.68 ± 0.035^b
90d TMP	76.99 ± 4.21	57.76 ± 3.02
140d TMP	128.46 ± 8.45	93.71 ± 4.90

[a,b] Significant results ($P < 0.05$)

[1] 90 d ADMY = 90 days average daily milk yield; 140 d ADMY = 140 days average daily milk yield; 90 d TMP = 90 days total milk produced; 140 d TMP = 140 days total milk produced

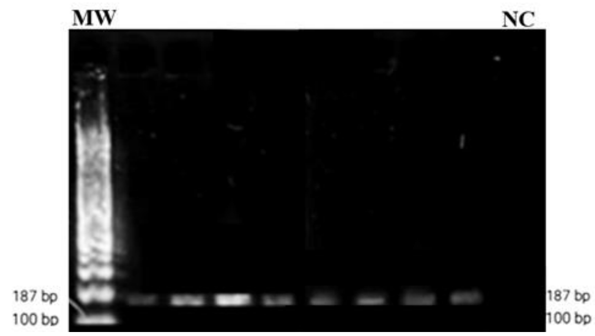

Fig. 1: 1.4% Agarose gel of the 187 bp fragment of *ghrelin* gene from crossbred Anglo-Nubian dairy goats (MW = 100 bp plus DNA ladder; NC negative control)

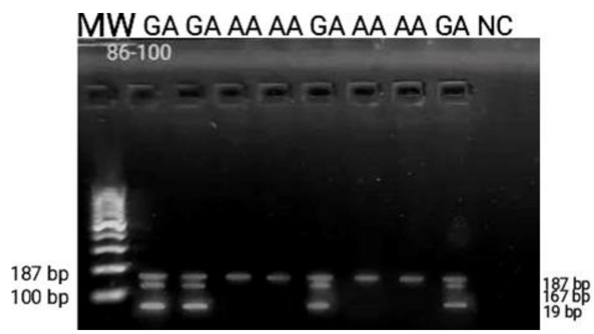

Fig. 2: 1.4% Agarose Gel of digested *ghrelin* gene PCR product from crossbred Anglo-Nubian dairy goats using *FspBI (BfaI)* RE (MW is the 100 bp plus DNA ladder; NC is the negative control

Discussion

The 187 bp PCR product derived from intron 3 of *ghrelin* was visible in 100 crossbred Anglo-Nubian dairy goats from Opol, Misamis Oriental and Dumaguete, Negros Oriental. Two genotypes (AA and AG) and alleles (A and G) were discovered when the *FspBI (Bfal)* restriction enzyme broke down a 187 bp segment of the *ghrelin* gene. Mutation in the sequence could result to the changes in recognizing the specific site. Here, when G base mutate, it changes the sequence from CTAG to CTGG. The mutation resulted to the absence of restriction site that will cause the fragment remains undigested.

AA genotype had the highest frequency of 81% of the studied goat population, whereas the GA genotype was present at a frequency of 19%, while the frequency of the allele A (0.91) is higher than allele G (0.09). The current study's findings were in line with those of Kowalewska-uczak *et al.* (2011), who found that the genotype GG was missing from the dairy cow *ghrelin* gene and that the the A allele was more common than that of the G allele. According to Hu *et al.* (2011), the lack of the homozygous genotype (GG) may be due to the gene, which may induce reproductive disruption, even sterility.

The observed heterozygosity in the population of dairy goats under study was slightly higher than the expected heterozygosity. High genetic diversity is a result of high heterozygosity. Less genetic variety is associated with low heterozygosity. A lower observed heterozygosity value than the expected Heterozygosity will show a divergence from the Hardy-Weinberg equilibrium (HWE) that might indicate inbreeding, according to Sharma *et al.* (2016). Because the observed heterozygosity in the current study is higher than expected heterozygosity, the population of crossbred Anglo-Nubian goat breed from the three farms likely has a relatively high genetic variability.

Chi-square ($\chi 2$) test was used to determine the extent to which variations between observed and expected allele values were caused by chance (Lobo 2008). Crossbred Anglo-Nubian goats from the three farms studied did not depart from the HWE ($P > 0.05$). The HWE predicts that genetic difference in a specific group will persist over generations. This indicates that changes in gene frequency produced by non-random mating and natural selection disrupt the population's balance. Mutations change allele frequencies by introducing new alleles into the population. Genetic drift occurs in small populations when the frequency of alleles changes by chance (Wang *et al.* 2015). Hardy-Weinberg Equilibrium (HWE) makes predictions about the manner in which gene frequencies will be passed down from one generation to the next based on certain sets of assumptions. The following are examples of assumptions: genetic drift, no migration, no mutations, random mating, and random selection of partners by individuals (Andrews 2010). This indicates that genotypic and allelic frequencies will remain consistent throughout generations (Moneva *et al.* 2020).

Parity and genotype interaction effects were not statistically significant at $P > 0.05$. This suggests that parity and GHRL genotypes have a unique impact on milk output. The average daily milk yield and total milk output in the current study increased during the second parity, but the yields sharply declined after the third and fourth parities. The total milk production at 90 days and the average daily milk supply for the fourth parity were considerably lower ($P < 0.05$). As observed from the analysis, average daily milk production and total milk production increased from the first to the second parity at 140 days but significantly decreased in the parities following that ($P = 0.05$). The milk output production increased from the first to the second parity and decreased at the third and fourth parities in the study of Moneva *et al.* (2020), which had similar findings with the current study. According to Lehmann *et al.* (2016), when the parity number rises, fewer lactations for each parity are completed. Additionally, Phoya *et al.* (2003) reported similar findings in Malawi goats, where an increase in parity resulted in a drop in daily milk supply. In addition to parity, litter size, and month of kidding, environmental factors such feed quality, air temperature, relative humidity, rainfall, and sun radiation may have a significant impact on milk production (Zamuner *et al.* 2020).

As for the effect of genotype with milk yield production performance, genotype AA presented higher total milk yield at 90 days and 140 days average daily milk yield than the genotype GA, as shown in Table 2. Similar results were also reported in water buffaloes in the study of Gil *et al.* (2013), where genotype AA presented higher milk yield than genotype GA. These results would increase ghrelin's significance in milk production. According to Nakahara *et al.* (2003), ghrelin is hypothesized to boost milk production *via* increasing blood flow in the mammary gland, which is connected to the development of blood vessels required for the gland's increased blood supply. It has been suggested as a candidate gene for qualities including growth, carcass quality, and milk production of cattle because of its influence on feed intake as a gastrointestinal hormone that acts in the release of growth hormone and influences the body's general metabolism (Gil *et al.* 2013). Ghrelin in the bloodstream increases milk yield indirectly by boosting growth hormone secretion. While growth hormone influences lipid metabolism in adipose tissue, resulting in body weight adjustments during milking period (Roche *et al.* 2008).

Conclusion

Ghrelin (G375A) polymorphism significantly influences the 90 d and 140 d milk yield performance of crossbred Anglo-Nubian goats from the farms of Opol, Misamis Oriental, and Dumaguete City, Negros Oriental, Philippines with AA genotype individuals producing superior milk production traits. Utilization of genetic information of dairy goats derived from the findings of the current study would be helpful in improving milk production performnce. It is recommended to look into the many variants of the caprine *ghrelin* gene and how they relate to other variables like milk quality and other economically-important production parameters.

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Author Contributions

JJA and CSOM planned the study, designed the experiments and analyzed the findings; CSOM acquired the data; JJA drafted the manuscript, and statistically analyzed the data; CSOM, SRMT, MAIJ made critical review and revision of the manuscript.

Conflicts 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 Mindanao State University – Iligan Institute of Technology's Institutional Animal Care and Use Committee's rules and prior approval were followed in carrying out the experimental procedures.

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