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# *Prolactin* gene (*C576A*) polymorphism is associated with milk production performance in crossbred Anglo-Nubian dairy goats

CARLO STEPHEN O MONEVA[1]✉, PERCIVAL P SANGEL[2], AMADO A ANGELES[2], MERLYN S MENDIORO[3] and RENATO S A VEGA[2]

*Mindanao State University–Iligan Institute of Technology, Iligan City 9200 Philippines*

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ABSTRACT

Crossbred Anglo-Nubian goats (Anglo-Nubian × native goat breeds), are valued for exceptional milk production and adaptability. In-depth research on these goats is imperative to propel advancements in dairy goat breeds and enhance milk production efficiency within the region. The present study aims to assess polymorphism in *prolactin* (*C576A*) gene and determine the influence of different *prolactin* (*PRL*) genotypes to milk yield performance in crossbred Anglo-Nubian dairy goats raised from Awang, Opol, Misamis Oriental and Talay, Dumaguete City, Negros Oriental. Genomic DNA was extracted from hair follicles using commercial DNA extraction kit and PCR-RFLP was performed for the genotyping of the *C576A* polymorphism located in exon 5 of goats' *prolactin* gene using *Eco*241 restriction enzyme. Genotypic frequencies of 0.56 for AA, 0.44 for AB, while allelic frequencies of 0.78 for A, and 0.22 for B were recorded. All populations followed the Hardy–Weinberg principle, except for dairy goats from Farm A located in Opol, Misamis Oriental. A two-way factorial (2 × 4) in a Randomized Complete Block Design was used to evaluate the relationship between genotypes and milk yield performance. AB genotype goats produced significantly higher milk yield traits (average daily milk yield and total milk production) than AA genotype, an indication that the polymorphism in the caprine *PRL* (*C576A*) gene influenced milk yield performance in the population of studied crossbred Anglo-Nubian goats. These results have to be validated in other dairy goat breeds.

**Keywords:** Anglo-Nubian, Milk yield, PCR-RFLP, Polymorphism, *Prolactin*

The goat (*Capra hircus*) has contributed to a major source of food through production of meat and milk in developing countries. In 2017, the Philippines produced 21.16 million litres of milk, which is 3.78% higher than 2015's level of 20.39 million litres. Of this total, goat's milk accounted for only 3% of the output (National Dairy Authority 2017). Goat milk is believed to have higher nutritional values and digestibility. It contains proteins, fatty acids, electrolytes, enzymes, vitamins, minerals, and trace elements that can be easily digested and absorbed by the body (Abbas *et al*. 2014). In addition, the milk from goats causes less allergic reactions as it lacks the alpha-s1-casein protein (Idowu and Adewumi 2017).

The prolactin (PRL) or luteotropin or commonly called the luteotropic hormone is released from the anterior pituitary. This peptide hormone is known for its action in the mammary alveoli to stimulate the production and secretion of milk proteins and is primarily involved in the production of all principal components of milk (Ghasemi *et al*. 2009). Prolactin is responsible for the mammary gland's growth and development and for promoting production and secretion of milk. It has been shown to initiate or maintain lactation by interaction with a variety of other hormones, including insulin, corticosteroids, thyroid hormones, growth hormone and estrogens which are necessary to maintain milk secretion (Wallis 1988).

Genetic improvement primarily focussed on selection for quantitative traits. Modern approaches using candidate genes have been effective in the identification of major genes affecting different traits in animals (Al-Samarai and Al-Kazaz 2015). Numerous findings have revealed that polymorphisms in *PRL* gene influenced several milk production traits in cattle (Hart *et al*. 1993, Zhang *et al*. 1994, Chung *et al*. 1996, Alipanah *et al*. 2007) and in buffaloes (Li *et al*. 2017). Despite such findings, no studies concerning the relationship between *PRL* polymorphisms and milk production characteristics have been conducted in dairy goats.

Present address: [1]Department of Biological Sciences, College of Science and Mathematics, Mindanao State University–Iligan Institute of Technology, Iligan City 9200 Philippines. [2]Animal Physiology Division, Institute of Animal Science, College of Agriculture and Food Sciences, University of the Philippines Los Baños College, Laguna 4031 Philippines. [3]Genetics and Molecular Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños College, Laguna 4031 Philippines. ✉Corresponding author email: carlostephen.moneva@g.msuiit.edu.ph

The general objective of this research was to determine genetic polymorphisms of *prolactin* and to evaluate the influence of different genotypes with milk production performance in crossbred Anglo-Nubian goats.

## MATERIALS AND METHODS

Crossbred Anglo-Nubian dairy goats locally raised in Visayas-Mindanao were surveyed in the study. The study utilized a total of approximately 101 pooled individuals of dairy goats from farms in Awang, Opol, Misamis Oriental and Talay, Dumaguete City, Negros Oriental.

*Analysis of milk production performance:* Goats were hand-milked daily from 8:30 to 9:00 in the morning and 3:30 to 4:00 in the afternoon. The amount of milk produced during the milking process was measured using a pitcher scale in terms of ml. Milk yield from first parity to ≥ fourth parity was standardized to 90 days and 140 days in milk, in agreement with the research conducted by Singh *et al*. (2018).

*gDNA extraction and amplification:* To minimize animal stress during DNA extraction, genomic DNA was isolated from the hair follicles of the experimental animals instead of extracting it from blood or ear tissues, using the QIAGEN DNeasy extraction method. Specific primers (forward: ATT CCT GGA GCC AAA GAG and reverse: TGT GGG CTT AGC AGT TGT) were used to amplify the specific 196 bp *prolactin* localized in exon 5 (Lan *et al*. 2009 and Abdel-Azeim *et al*. 2018). PCR optimization was performed to determine the optimum concentration of PCR components and temperature of the PCR profile that will have good quality PCR products. The PCR reaction was carried out using a thermocycler, and were then separated by using AGE in 0.5× TAE on 1.5% agarose gel.

*Genotyping:* Genotyping of the *prolactin* (*C576A*) were done using PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) in 20 µL final volume with *Eco*241 (Thermo Fisher Scientific) restriction enzymes at 37°C for 2 h. Restriction fragments were analyzed at AGE in 1× TAE on 5% agarose gel.

*Statistical analysis:* To test for the significance of differences in genotype and allele frequencies, chi square test was used. Two-way factorial (2 × 4) in a Randomized Complete Block Design (RCBD) was used (Gomez and Gomez 1984), with parity and genotypes as main factors and farm as the blocking factor. The conduct of data analysis was based on the PROC GLM in SAS.

## RESULTS AND DISCUSSION

*Amplification of prolactin gene and genotyping:* The PCR amplification of *prolactin* (*C576A*) gene in the experimental animals produced a 196 bp DNA fragment as shown in Fig. 1.

The C576A mutation located in exon 5 of the caprine *prolactin* gene is revealed by PCR-RFLP using *Eco*241 restriction enzyme (Lan *et al*. 2009). The alteration of amino acid Proline (CCC) to Threonine (ACC) at position 176 of the protein sequence is caused by the X76049:g.576C>A transversion (Abdel-Azeim *et al*. 2018). Fig. 2 shows the different DNA fragments, where AA genotype produced 196 bp, while genotype AB produced 196 bp and 169 bp.

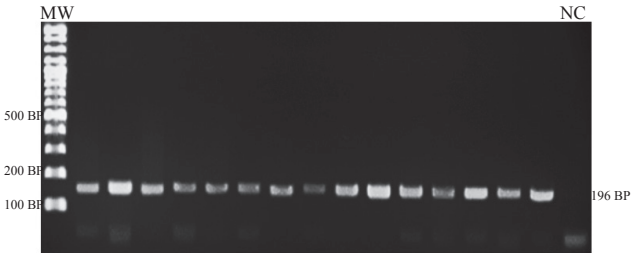


Fig. 1. Agarose gel electrophoresis of specific caprine *prolactin* gene, 196 bp fragment (MW, 100 bp plus DNA ladder; NC, negative control).

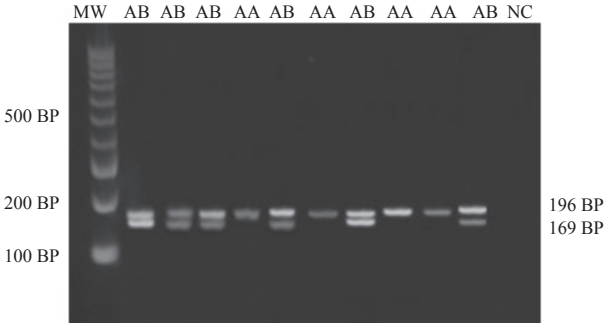


Fig. 2. Agarose gel electrophoresis of digested *prolactin* PCR product using *Eco*241 (MW, 100 bp plus DNA ladder; NC, negative control).

Only two genotypes, AA and AB, were observed in the studied crossbred Anglo-Nubian goats (Table 1). AA genotypic frequency (0.56) was common than AB (0.44), while the frequency of allele B (0.22) was lower compared to allele A (0.78). Contrariwise, a monomorphic pattern of the *PRL* gene was reported by Aravindakshan (2005) in Indian goat breeds (Attappady black, Jamnapari, Malabari and Salem black). Lan *et al*. (2009) documented genetic variations of the *prolactin* gene (exon 5) in Chinese goats through PCR-SSCP and reported three SSCP (CC, CA and AA) banding patterns. Similar to the present study, the genotype BB was found to be non-existent in Damascus goats with lower frequency of BB genotype in Barki goats (0.05) and in Zaraibi goats (0.10) (Abdel-Azeim *et al*. 2018).

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

As shown in Table 1, estimated values of observed heterozygosity were higher than expected heterozygosity except for Opol A, suggesting a high genetic diversity for these populations. In addition, Chi-square tests showed that the observed polymorphisms in *PRL* gene were in Hardy–Weinberg at $p>0.05$, except for the population of dairy goats from Opol A in Opol, Misamis Oriental ($p<0.05$). This imbalance would indicate that dairy goat population from Opol A may have experienced disruptive forces such

Table 1. Frequencies of alleles and genotypes for sequence polymorphisms in the *prolactin* (*C576A*) of the studied dairy goats

| Farm | N | Genotype frequency | | Allele frequency | | Heterozygosity | | $\chi^2$ (HWE) |
|---|---|---|---|---|---|---|---|---|
| | | AA | AB | A | B | Expected | Observed | |
| Opol A | 34 | 0.70 | 0.30 | 0.85 | 0.15 | 0.26 | 0.30 | 0.03 |
| Opol B | 33 | 0.62 | 0.38 | 0.81 | 0.19 | 0.31 | 0.28 | 0.06 |
| Dumaguete | 34 | 0.32 | 0.68 | 0.66 | 0.34 | 0.45 | 0.68 | 0.27 |
| TOTAL | 101 | 0.56 | 0.44 | 0.78 | 0.22 | 0.34 | 0.44 | 0.08 |

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

as genetic drift, mutations, non-random mating, migration or selection. These disruptive mechanisms may alter the balance in the frequencies of genotypes and alleles throughout generations.

*Association analysis:* The effect of different *PRL* (C576A) genotypes to milk production performance in crossbred Anglo-Nubian is shown in Table 2.

Table 2. Influence of different *PRL* (*C576A*) genotypes on milk yield (Mean±SEM**)** in crossbred Anglo-Nubian dairy goats.

| Milk yield traits[1] (Litres) | *Prolactin* genotypes | |
|---|---|---|
| | AA | AB |
| 90d ADMY | 0.60±0.034[b] | 1.05±0.046[a] |
| 140d ADMY | 0.70±0.057[b] | 1.03±0.047[a] |
| 90d TMP | 53.61±3.03[b] | 94.52±4.19[a] |
| 140d TMP | 94.97±8.27[b] | 146.61±6.55[a] |

[a,b] Significant results (p<0.05); [1] 90d ADMY, 90 days average daily milk yield; 140d ADMY, 140 days average daily milk yield; 90d TMP, 90 days total milk produced; 140d TMP, 140 days total milk produced.

Effect of interaction between parity and genotypes was statistically insignificant (p>0.05). Average daily milk yield (ADMY)and total milk production (TMP) were significantly different between two *PRL* genotypes (p<0.05). Individuals with AB genotype had considerably greater ADMYand TMP than AA genotype.

There could be several probable reasons on why the heterozygous AB genotype in the goat's *prolactin* gene displayed considerably greater average daily milk yield and total milk production than the homozygous AA genotype. Some potential explanations include: (1) gene interaction, AB genotype may involve a gene interaction (epistasis) that enhances milk production when both alleles are present (Pizarro Inostroza *et al*. 2020). This could result in increased prolactin hormone levels, which stimulate milk production; (2) genetic diversity, where heterozygosity often brings genetic diversity, which can lead to improved traits (Vellend *et al*. 2005). The combination of alleles from both parents might result in a more favorable genetic makeup for milk production; (3) hybrid vigor (heterosis), in which crossbreeding or having heterozygous genotypes can sometimes lead to hybrid vigor, where offspring exhibit superior traits compared to their parents (Getahun *et al*. 2019).

Numerous studies have reported that genetic variation in the *prolactin* gene affected several milk production characteristics. Using the RFLP method, Chung *et al*. (1996) revealed a significant effect of *PRL- Rsa*I locus on milk fat percentage and production of milk in cattle. Moreover, Alipanah *et al*. (2007) described that cows with BB genotype in the *PRL- Rsa*I location produced higher milk fat yield and milk yield, while cows having AB genotype had high milk fat content. Recently, Li *et al*. (2017) described the connection between genotypic variability in the *PRL* gene and milk characteristics in Italian Mediterranean buffalo. Polymorphism in *PRL* intron 1 significantly influenced the milk protein content, milk yield and peak milk yield with TT genotype individuals having inferior performance than those with CC and CT genotypes. While, the polymorphism in *PRL* exon 2 significantly affected the milk fat content, wherein TT genotype buffaloes showed greater total milk fat content than CT genotype buffaloes. In the present study, significant associations were found between milk yield performance and *C576A* polymorphisms in the goat *PRL* gene. This would strongly suggest that *prolactin* (*C576A*) is a possible candidate genetic marker for the improvement of goats' genetic potential for milk production.

*PRL* (*C576A*) gene had significant influence on milk yield. Thus, the said polymorphism can possibly be considered as potential candidate gene to enhance milk yield potential in crossbred Anglo-Nubian goats. As a recommendation, continued monitoring for the milk production traits of crossbred Anglo-Nubian goats having AB genotypes in the *PRL* (*C576A*) must be done for further validation. In addition, the polymorphisms association to other milk production traits, such as milk quality and composition, should also be given emphasis. Furthermore, evaluation of *prolactin* gene polymorphisms in other goat breeds must be explored.

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