



Research article

Milk production and genotypic variants for Prolactin (*PRL*) and Prolactin receptor (*PRLR*) Slick1 genes identified using polymerase chain reaction-restriction fragment length polymorphism on selected dairy cattle farms in the Philippines

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Article Info

Article history:

Received 4 October 2025

Revised 25 May 2026

Accepted 5 June 2026

Available online 15 July 2026

Keywords:

Dairy cattle,
Polymerase chain reaction-restriction fragment
length polymorphism (PCR-RFLP),
Prolactin (*PRL*) gene,
Prolactin receptor (*PRLR*) gene,
Thermotolerance

Abstract

Importance of the work: A locally developed polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) test for the Prolactin (*PRL*) and Prolactin receptor (*PRLR*) Slick1 gene variants is the first to be made available to dairy farms that is comparable to commercial genotyping kits.

Objectives: To test the locally developed PCR-RFLP assay for determining the genotype frequencies of the *PRLR* Slick1 variant and *PRL* genes and to determine whether there are variations in milk production performance of cows according to genotype.

Materials and Methods: Genomic DNA samples of 479 dairy cattle from four farms in the Philippines were genotyped using PCR-RFLP with *PciI* and *RsaI* restriction enzymes for the *PRLR* Slick1 and *PRL* genes, respectively. Genotype and allele frequencies were determined and the 305 d lactation period (305D) milk yield of cows was analyzed according to genotype.

Results: The PCR-RFLP of the *PRL* gene identified three genotypes: *AA*, *AG* and *GG*, with allele frequencies of 0.16 (*A*) and 0.84 (*G*). The PCR-RFLP of the *PRLR* Slick1 gene variant had two genotypes: hetero-slick (*CT*) and non-slick (*CC*), with allele frequencies of 0.94 (*C*) and 0.06 (*T*). The genotypes obtained using the PCR-RFLP assay were consistent with the commercial genotyping method. The main effect of *PRL* was associated with significantly higher 305D milk yield for *GG* genotyped cows. There were no significant differences in the 305D milk yield among the cows with different *PRLR* Slick1 genotypes. The interaction of calving season × *PRL* genotype produced a significantly lower 305D milk yield for the *GG* cows calving during the hot-humid season, whereas no significant difference was observed for the *AG* cows calving regardless of season. However, while the results are preliminary, irrespective of the *PRL* genotype, hetero-slick cows fared better than non-slick cows calving during the hot-humid season.

Main finding: The results from the locally developed PCR-RFLP test for *PRL* and *PRLR* Slick1 mutation were comparable to those from the commercial genotyping method, which should enable local testing for selection and planned breeding of animals carrying the favorable alleles of the *PRL* and *PRLR* Slick1 genes.

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<https://doi.org/10.34044/j.anres.2026.60.4.11>

Introduction

Climate stress and dairy production

Temperature, humidity, precipitation, air movement, radiation, barometric pressure and ionization are all components of climate. The range and duration of ambient temperature largely depend on latitude, with latitudes closer to the equator experiencing conditions increasingly conducive to heat stress (West, 2003). Globally, the potential heat stress experienced by dairy animals has risen, as evidenced by temperature and humidity data (Polsky and Keyserlingk, 2017).

The optimal temperature range for lactating dairy cows is -0.5 to 20°C (Herbut et al., 2018), while the upper critical air temperature is 25 – 26°C (Berman et al., 1985). Several studies have shown that while the reduction in milk yield due to heat stress varies between individuals—suggesting potential for selection—the trait is lowly to moderately heritable (Ravagnolo and Misztal, 2000; Bernabucci et al., 2014). Consequently, response to selection using traditional pedigree-based best linear unbiased prediction genetic evaluation is slow (Fathoni et al., 2022; Sungkhapreecha et al., 2022).

Role of Prolactin and Prolactin receptor genes

Genomic selection offers a faster alternative, with genomic breeding values for heat tolerance now being developed (Nguyen et al., 2016). Central to this research are the Prolactin (*PRL*) gene and its receptor (*PRLR*) gene (Fang et al., 2025). The *PRLR* gene produces a member of the transmembrane cytokine class-1 receptor superfamily, which binds three major ligands: prolactin (*PRL*), placental lactogen (*PL*) and growth hormone (*GH*) (Scotti et al., 2007).

The *PRL-PRLR* interaction plays vital roles in cell growth, survival, reproductive capacity, lactation and stress response (Chu et al., 2007; Zhang et al., 2008; El-Magd et al., 2021; El-Shorbagy et al., 2022). Specifically, the binding of *PRL* to *PRLR* activates signaling pathways that promote milk protein synthesis (Arboccó et al., 2017; Gai et al., 2022). Variations within these genes have been linked to differences in milk yield, fat percentage and other production characteristics (Oğuzkan and Bozkurt, 2019; Al-Obaidi and Al-Janabi, 2025). For example, the *PRL* gene, located in chromosome 23 of the bovine genome, is crucial for mammary gland development and milk secretion (Kumar, 2017; Beishova et al., 2023). The silent G/A-transition in exon 4 of bovine *PRL* gene has been

reported to give rise to a polymorphic site for *RsaI* restrictions enzyme (Setyorini et al., 2023). A study on Russian Red Pied cows using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) reported that cows with the *PRL-RsaI BB* genotype had higher average milk yields than *AA* and *AB* individuals (Alipanah et al., 2007). Prolactin is essential for lactogenesis (milk synthesis) but is also a primary “stress hormone” in cattle rising sharply during heat stress to regulate water balance and sweating; thus, the elevation of *PRL* concentration is a signature of heat stress (Silva et al., 2023; Clarke et al., 2025). Alamer (2011) reported that synthesis and secretion of *PRL* by the anterior pituitary gland are sensitive to changes in ambient temperature; specifically, higher levels of this hormone were observed during summer due to its influence on the regulation of body fluids and its relationship with seasonal hair growth (Tucker and Wettemann, 1976; Gebbie et al., 1999).

The *PRLR* gene on chromosome 20 in cattle is well-known for its association with milk production (Zhang et al., 2008). Recently, it has been identified as a candidate marker for thermotolerance due to the “Slick1” mutation (Cheruiyot et al., 2022). Initially, this mutation was identified in the heat-tolerant Senepol breed of cattle (Olson et al., 2003) and later located in the *PRLR* gene (Littlejohn et al., 2014). The Slick1 mutation involves a deletion of a cytosine residue at position g.1466 of the *PRLR* gene (Littlejohn et al., 2014). This causes a premature stop codon, truncating approximately 120 amino acids at the C-terminal end of the protein (Littlejohn et al., 2014). This mutation does not hinder the general function of *PRLR* but leads to a short, sleek hair coat, lower rectal temperatures and increased sweating and evaporative heat loss (Dikmen et al., 2014). Crucially, introgressing the Slick1 mutation into Holstein and Jersey breeds has shown that lactating cows with the mutation have superior thermoregulation and suffer less reduction in milk yield during summer compared to non-carrier cows (Dikmen et al., 2014; Sánchez-Rodríguez et al., 2023).

In the past, this assumption stemmed from the phenotype of animals having slick coats. In slick animals, body and rectal temperature during heat stress is lower, but milk yield is higher (Dikmen et al., 2014). In addition, slick animals have increased sweating and evaporative heat loss in part due to short and less dense hair coats (Dikmen et al., 2014; Littlejohn et al., 2014). However, not all slick-looking animals contain the identified mutation (Porto-Neto et al., 2018); hence, phenotype cannot be relied upon as an indicator of thermotolerance.

Other reports have suggested that the slick phenotype may be breed-specific and might be caused by a different mutation

along the *PRLR* gene such that to date, a total of six SNPs along the last exon of the gene are being investigated for their association with the said phenotype (Porto-Neto et al., 2018). It is yet to be elucidated whether these SNPs confer both the phenotype and thermotolerance; however, there are already some reports that reveal the importance of the first mutation (Slick1 in the *PRLR* gene) in cattle (Porto-Neto et al. 2018). Hence, selection for this mutation would be favorable, especially for animals raised in tropical and subtropical countries (Porto-Neto et al. 2018; Cheruiyot et al., 2022), where heat stress can greatly impact cattle productivity and welfare.

Direct sequencing, while a common method for mutation detection, can be labor intensive and costly (Soverini et al., 2019). Cheaper and faster methods have been developed for detecting and genotyping Slick1 mutation in cattle (Jannaman et al., 2019). However, some advanced methods can still be cost-ineffective for laboratories with limited facilities due to requirements for resource-intensive heavy computational bioinformatic tools and specialized equipment. Furthermore, genetic testing services using these methods are not available locally. One way to address these challenges is the use of a simpler technique that yields comparable results.

Therefore, the objective of the current study was to test the locally developed PCR-RFLP assay for *PRL* and *PRLR* Slick1 gene markers to determine allele and genotype frequencies and to determine whether there were variations in milk production performance of cows according to genotype from four selected farms in the Philippines, based on similar procedures to studies in other countries.

Materials and Methods

Blood sample collection

In total, 479 Holstein-Jersey dairy cattle samples were collected from four farms in the Philippines between 2020 and 2023. Animals were grazed during the day and were brought back to the animal shed in the evening. Two herds were located in Southern Luzon and the other two were in Mindanao. The Philippines is a tropical country with hot, humid season during the summer extending into the start of the rainy season in June after which it starts to cool down extending into the dry months at the end and beginning of each year (PAGASA, 2026).

The two herds located in Mindanao were in the same province, close to each other; hence, their local climatic conditions

were the same. Similarly, the two herds in Southern Luzon were in the same province and close to each other. Blood and other appropriate tissue samples were collected and processed following protocols adopted by the Philippine Carabao Center (PCC) on genetic material sample collection, processing and handling. All samples were brought to the PCC Molecular Genetics Laboratory for processing.

DNA extraction

Approximately 4.0 mL blood samples were collected aseptically using jugular venipuncture into a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA). A high-salt concentration procedure using a Promega Wizard® Genomic DNA Purification Kit (Promega Corporation; USA) was used according to the manufacturer's protocol for the extraction of genomic DNA from collected whole blood samples. A DNA extraction kit (Qiagen; Germany) was used, with slight modifications, to extract available hair and tissue samples, according to the PCC Molecular Genetics Laboratory protocol. Total genomic DNA was assessed using agarose gel electrophoresis and quantified using a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific; USA). In total, 120 mL of 2% agarose gel with 2.4 µL GelRed nucleic acid stain was prepared and 2 µL of isolated genomic DNA with 2 µL 2X loading dye were run in the gel. Mupid-exU electrophoresis was used to run the samples for 50 min at 100 V. The gel was visualized under ultraviolet light and checked for the integrity of the isolated gDNA. An Invitrogen™ 100 bp ladder (Thermo Fisher Scientific; USA) was used as the molecular ladder. A sample (1 µL) of each isolated gDNA was used to measure DNA concentration and purity. DNA samples with concentrations in the range 30–100 ng/µL were used for succeeding analyses.

Primer design

The primers were designed for the *PRL* and *PRLR* gene markers considering the known location and identification of the target mutation according to the published literature. The online molecular biology database National Center for Biotechnology Information (NCBI) was used to search for deposited *Bos taurus* *PRL* and *PRLR* sequences with the complete sequence and annotation. This information was used manually to design primers flanking the mutation. For the *PRL* gene, the primer pair, forward 5'-CCAAATCCACTGAATTATGCTT - 3' and reverse 5'-ACAGAAATCACCTCTCTCATTCA - 3', targeting the

mutation site has approximately 22–23bp sequence length and a flanking sequence length of approximately 294bp (amplicon size) and is optimal for PCR amplification. The primer pair for the *PRLR* gene, forward 5'-TGTCTCCAGGCCCTCAG-3' and reverse 5'-AAGGGGGTTTTGTCTTGGGG-3', targeting the Slick1 mutation has approximately 18–20bp sequence length, 55–67% GC content, an average melting temperature (TM) of 60°C and a flanking sequence length of approximately 338bp (amplicon size) which is optimal for PCR amplification. Through visual simulation, the primer designs for both genes were expected to have a restriction site that yielded optimal fragment sizes when a restriction enzyme targeted the mutation site.

Polymerase chain reaction conditions

For the *PRLR* gene, PCR reactions were prepared and optimized in a final volume of 10 µL consisting of 1× PCR buffer, 2 mM MgCl₂, 0.15 mM dNTPs, 0.5 µM each of the synthesized forward and the reverse primers; 0.5 units of Taq polymerase; at least 10 ng of template cattle DNA; and nuclease-free water. The amplification reaction was carried out in an Applied Biosystems™ SimpliAmp™ thermal cycler (Thermo Fisher Scientific; USA) with initial denaturation of 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 15 s, annealing at 65°C for 1 min and an extension at 72°C for 1 min. A final elongation at 72°C for 10 min was performed and the PCR products were stored at 4°C before further use.

For the *PRL* gene, PCR amplifications were prepared and optimized in a final volume of 10 µL consisting of 5× PCR buffer, 2.5 mM MgCl₂, 0.15 mM dNTPs, 0.5 µM each of the synthesized forward and reverse primers; 0.1 unit of Taq polymerase; at least 10 ng of template cattle DNA; and nuclease-free water. The amplification reaction was carried out in the Applied Biosystems™ SimpliAmp™ thermal cycler with initial denaturation of 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 15 s, annealing at 64°C for 1 min and extension at 72°C for 1 min. A final elongation at 72°C for 10 min was performed and the PCR products were stored at 4°C before further use.

Genotyping

All PCR products were electrophoresed on a 2% agarose gel in a 1× TAE buffer solution for 35 min at 100 V after digestion. A 50 bp molecular ladder of Invitrogen™ (Thermo Fisher Scientific; USA) was used for standard sizing of the

PCR products. The expected PCR product was digested using *PciI* restriction enzyme for genotyping individual animals. The *PciI* restriction enzyme (New England Biolabs; USA) recognizes the sequence 5'-A[^]CATGT-3' and cuts any nucleotide sequence into fragments at its recognition site. Other genotyping methods do not use *PciI* restriction enzyme such as Kompetitive Allele Specific PCR (He et al., 2014; Alvarez-Fernandez et al., 2021). This is a probe-based method that utilizes a universal fluorescence resonance energy transfer (FRET) reporter system to detect specific alleles wherein specific fluorescent dyes—fluorescein amidites (FAM) or hexachlorofluorescein (HEX)—emit a signal and this is then read by a FRET-capable plate reader or qPCR instrument. Genotyping the *PRLR* slick1 mutation using the *PciI* restriction enzyme is also possible because the Slick1 mutation creates a *PciI* restriction site (A/CATGT) in the target DNA sequence, allowing for differentiation of alleles through PCR and PCR-RFLP analysis and does not require qPCR or a FRET-capable plate reader that are not commonly available locally. The PCR-RFLP mixture consisted of 2 µL PCR product, 0.1 µL restriction enzyme, 1 µL restriction enzyme buffer and 1.9 µL nuclease-free water. Restriction enzyme digestion was carried out in a thermal cycler at 37°C for 2 hrs and subsequently stored at 4°C before genotype visualization using agarose gel electrophoresis.

To validate the PCR-RFLP method, a comparison was made between the genotyping results obtained using PCR-RFLP and the TaqMan® SNP Genotyping Array (Thermo Fisher Scientific; USA), following manufacturer's protocol. Seven control samples were analyzed and their genotypes were determined using both methods. Included in the seven reference samples were frozen semen of two homozygous slick bulls that are available commercially. Furthermore, reported daughters of the two bulls from the two participating dairy herds were sampled, as these could be expected to be hetero-slick. Sire verification was achieved using the PCC-developed Verisire™ parentage verification test (PCC; the Philippines) to confirm the pedigree of the reported daughters.

The targeted sequence base pairs for the *PRL* mutation region was 294bp. All PCR products were electrophoresed on a 2% agarose gel in a 1× TAE buffer solution for approximately 40 min at 100 V after digestion. A 50 bp molecular ladder from Invitrogen™ (Thermo Fisher Scientific; USA) was used for standard sizing of the PCR products. The expected PCR product was digested using the *RsaI* restriction enzyme (New England Biolabs; USA) for genotyping individual animals. *RsaI* recognizes the sequence 5'...GT⁺AC ...3' in the target

DNA sequence, allowing for differentiation of alleles through PCR and PCR-RFLP analysis (Alipanah et al., 2007).

The PCR-RFLP mixture consisted of 1.5 μ L PCR product, 0.2 μ L restriction enzyme, 1 μ L restriction enzyme buffer, 0.2 μ L bovine serum albumin and 3.0 μ L nuclease-free water. Restriction enzyme digestion was carried out in a thermal cycler at 37°C for 30 min and stored at 4°C before genotype visualization using agarose gel electrophoresis.

The cows, heifers and breeding bulls from the participating herds that were sampled were genotyped using the PCR-RFLP method for both the *PRL* and *PRLR* genes. The genotype frequency as a percentage was calculated using Equation 1:

$$\text{Genotype frequency} = \text{Genotype} / N \text{ individuals sampled} \times 100$$

$$\begin{aligned} \text{Genotype frequency} \\ = \text{Genotype} / N \text{ individuals sampled} \times 100 \end{aligned} \quad (1)$$

where *CC*, *CT*, *TT* are the possible genotypes of each individual sampled for *PRLR* gene while *AA*, *AG*, *GG* are the possible genotypes of each individual sampled for *PRL* gene.

Allele frequency was calculated using Equation 2–3:

$$p = (2N_{11} + N_{12}) / kN_{\text{total}} \times 100 \quad (2)$$

$$q = 1 - p \times 100 \quad (3)$$

where N_{11} and N_{12} are the numbers of *CC* or *GG* homozygotes and *CT* or *AG* heterozygotes, respectively, and N is the total number of diploid individuals sampled in the population.

In addition, the phenotype of cows with the Slick1 mutation was evaluated among the local genotyped cows by taking photographs and determining if they had sleek, short hair.

Capillary sequencing

The correct amplification of the target region of the *PRLR* gene was validated based on sequencing of the PCR product from representative samples of non-slick, hetero-slick and homozygous slick animals that were used for concordance genotyping. These products were purified individually using the ExoSAP-IT™ Express PCR Product Cleanup Reagent (Applied Biosystems™, Thermo Fisher Scientific; USA), following the manufacturer's protocol. Then, the purified PCR products were processed using a BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™, Thermo Fisher Scientific; USA). A double-pass sequencing reaction (forward and reverse) per sample was prepared to cover the targeted region of the *PRLR* gene. Next, the products were precipitated using 70 μ L of a solution containing ethanol,

sodium oxaloacetate and EDTA. The mixture was vortexed thoroughly and centrifuged at 14,000 RPM at 4°C for 20 min. The supernatant was discarded and the DNA was washed with 70% ethanol. The resulting pellet was air-dried in a flow hood for 30 min and eluted with 11 μ L highly deionized formamide.

The samples were transferred into genetic analyzer barcoded plates for sequencing using the ABI 3500xl Genetic Analyzer (Applied Biosystems™, Thermo Fisher Scientific; USA). The rapid sequencing analysis template was used to run the sequencing. The resulting sequences were assessed and analyzed using the Geneious Prime® Software version 2023.2.1 (Biomatters, Inc.; New Zealand) consisting of quality control, trimming, contig assembly and sequence alignment. Identities of the contiguous sequences were checked using the nucleotide BLAST online tool (<https://blast.ncbi.nlm.nih.gov>) and compared to the *PRLR* gene sequences of *Bos taurus* available in the NCBI database.

Milk production records

Lactating cows were subjected to monthly milk test day recording to determine individual milk, fat and protein yields. The participating dairy herds had recorded daily milk production but in bulk, rather than on an individual cow basis; hence, there were no available historical individual milk production records. The two herds that participated in the milk recording were from Southern Luzon, close to each other, where the animals were grazed during the day with similar feed resources and local climatic conditions. The monthly milk test day yields from morning and afternoon milking were weighed and recorded. Individual 305 d lactation period (305D) milk yield was calculated from monthly test day yields following the test interval method (Flores et al., 2013). Cows with ongoing lactation that were in their initial stages at the start of monthly test recording were included and backtracking of milk yield records was done. For cows where lactation had already dried off upon returning to the farm for monthly milk test day recording, but without actual dry-off dates, the date of the last test-day record plus 15 d was taken as the dry-off date (ICAR, 2023). Many cows did not have actual dry-off dates, or the interval between test-day records was 3-7 mth, which could possibly have encompassed a new lactation already; hence, only the 305D lactation measure was used to determine lactation performance, rather than the total lactation measure. The cows were all Holstein-Jersey crosses, with an undetermined breed proportion; however, they came from one supplier; hence, all cows were considered to be from the

same genetic group. The effect of the different *PRL* and *PRLR* Slick1 genotypes on milk production was determined using the software/program JMP13.2.1® (JMP, 2016) for statistical analysis using the model in Equation 4:

$$Y_{ijklmn} = \mu + \text{Herd}_i + \text{Year}_j + \text{Season}_k + \text{PRL}_l + \text{PRLR}_m + (\text{PRL} \times \text{PRLR})_{lm} + (\text{PRL} \times \text{Season})_{kl} + e_{ijklmn} \quad (4)$$

where Y_{ijklmn} is the the observed 305D milk yield of an individual cow, μ is the overall population mean for 305D milk yield, Herd_i is the fixed effect of the i^{th} herd (Herd 1, Herd 2), Year_j is the fixed effect of the j^{th} year of calving (2021, 2022, 2023), Season_k is the fixed effect of the k^{th} calving season (hot-humid, cool-dry), PRL_l is the fixed effect of the l^{th} genotype of the Prolactin gene (*GG*, *AG*), PRLR_m is the fixed effect of the m^{th} genotype of the Prolactin Receptor gene (*CT*, *CC*), $(\text{PRL} \times \text{PRLR})_{lm}$ is the interaction effect between the *PRL* and *PRLR* genotypes, $(\text{PRL} \times \text{Season})_{kl}$ is the interaction effect between the *PRL* and calving season and e_{ijklmn} is the random residual error term, accounting for unexplained variation

Due to limited records, the actual parity number for most cows was not determined; hence, all cows were categorized as first- or multi-parity with ages in the range 3–6 yr. As there were substantially fewer hetero-slick cows relative to non-slick cows, only the lactation records of the hetero-slick and non-slick cows calving in the same month-year were included in the analysis. Given this condition, there were no first parity records included in the analysis. Therefore, parity number was not included in the model. After applying the edits on the 309 records, there were 126 lactation records available for analysis, 27 of which were from hetero-slick cows that had calved during 2021–2023. A cow with a specific genotype can only calve in either one of the two defined seasons; hence, to determine the effect of season of calving on milk production, the *PRL* x Season of calving interaction and the *PRL* x *PRLR* interaction were included in the model. Standard least squares

analysis used the Fit Model platform of JMP® 13.2.1 (JMP, 2016) software. In JMP, “Fit Least Squares” finds the best-fit regression line/model by minimizing squared errors. The test outputs included least-squares mean estimates for the different levels of the fixed effects.

Ethics statements

This study was approved by the Ethics Committee of the Philippine Carabao Center, dated 21 July 2023.

Results and Discussion

The aim was to develop a PCR-RFLP method for genotyping because based on DNA sequence analysis, the target region for both the *PRL* and *PRLR* genes can be cleaved by a restriction enzyme. Table 1 summarizes the PCR-RFLP genotypes for the *PRLR* gene and the corresponding sequences at the recognition site for the three possible genotypes.

The gel electrophoresis image (Fig. 1) shows the results of the PCR-RFLP method for detecting the mutation site in cattle samples. The genotypes obtained using the PCR-RFLP method were consistent with the result of the TaqMan® genotyping array for identifying reference homo-slick, hetero-slick and non-slick animals (Table 2).

The same reference samples used for genotyping concordance were subjected to capillary sequencing. Fig. 2A and Fig. 2B illustrate the sequence chromatogram and nucleotide alignment, respectively, at position g.1466 for non-slick, hetero-slick and homo-slick animals, highlighting the deletion at position 1466 for Slick1 (homozygous slick, sample #3 in Fig. 2A) animals and the presence of a restriction site (samples 5 and 6 in Fig. 2B). This indicated that the target mutation site was amplified successfully. The sequencing of

Table 1 Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotypes and sequence comparison at the Prolactin (*PRL*) gene and Prolactin receptor (*PRLR*) Slick1 mutation target region

Genotype call	PCR-RFLP genotype	Amplicon size	PCR-RFLP fragment sizes	Sequence at recognition site
Normal (non-slick)	<i>CC</i>		338bp	ACATGCT
Homozygous (homo-slick)	<i>TT</i>	338bp	204bp, 133bp	ACATG_T
Heterozygous (hetero-slick)	<i>CT</i>		338bp, 204bp, 133bp	ACATGYT
Homozygous (favorable)	<i>GG</i>		294bp	GTGCG
Heterozygous	<i>AG</i>	294bp	294bp, 162bp, 132bp	GTYCG
Homozygous (normal)	<i>AA</i>		162bp, 132bp	GTACG

Table 2 Comparison of genotyping results for Prolactin receptor (*PRLR*) Slick1 gene marker using TaqMan Array and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods

Sample*	Gender	TaqMan array	PCR-RFLP genotype
1	F	Hetero-slick	CT
2	F	Hetero-slick	CT
3	F	Hetero-slick	CT
4	M	Non-slick	CC
5	M	Non-slick	CC
6	M	Homo-slick*	TT
7	M	Homo-slick*	TT

*Sample taken from commercially available frozen semen of homozygous slick bull.

the target region validated the genotyping result, indicating that the PCR-RFLP protocol provided the correct genotype at the Slick1 mutation site of the *PRLR* gene.

In addition, PCR-RFLP genotyping was performed for the *PRL* gene. Digestion of the 294 bp fragment from the PCR-amplified DNA samples with the *RsaI* restriction enzyme resulted in three bands at 294 bp, 162 bp and 132 bp that were visible based on the gel electrophoresis, as shown in Fig. 3. The *GG* genotype had a single 294 bp band (undigested), whereas the *AA* genotype had 162 bp and 132 bp bands and the heterozygous *AG* genotype had 294 bp, 162 bp and 132 bp

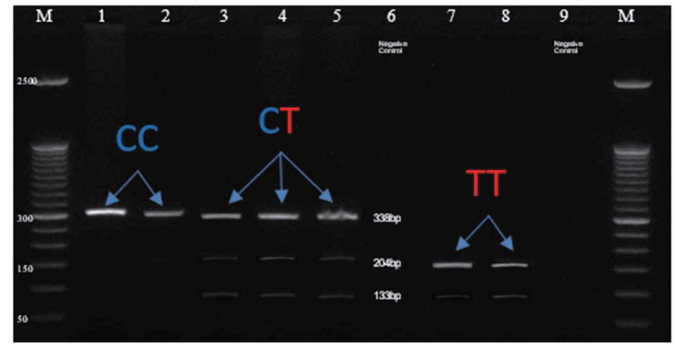


Fig. 1 Agarose gel electrophoresis image displays the different genotypes of the *PRLR* Slick1 gene mutation (*CC*, *CT*, *TT*) based on fragment patterns/sizes, with lane M containing a 50 bp DNA ladder for size reference, lanes 1–2 (non-slick) having homozygous (*CC*) genotype indicated by single band at 338 bp, while Lanes 3–5 (hetero-slick) having hetero-slick (*CT*) genotype indicated by bands at 338 bp, 204 bp and 133 bp, lanes 7–8 (homo-slick) having homozygous slick (*TT*) genotype indicated by bands at 204 bp and 133 bp and lanes 6 and 9 as negative controls, to ensure absence of contamination and validation of results.

bands.

Genotype and allele frequencies

In the screening of the four participating dairy farms for the *PRL* gene, the frequency of the minor allele *A* was low (16%)

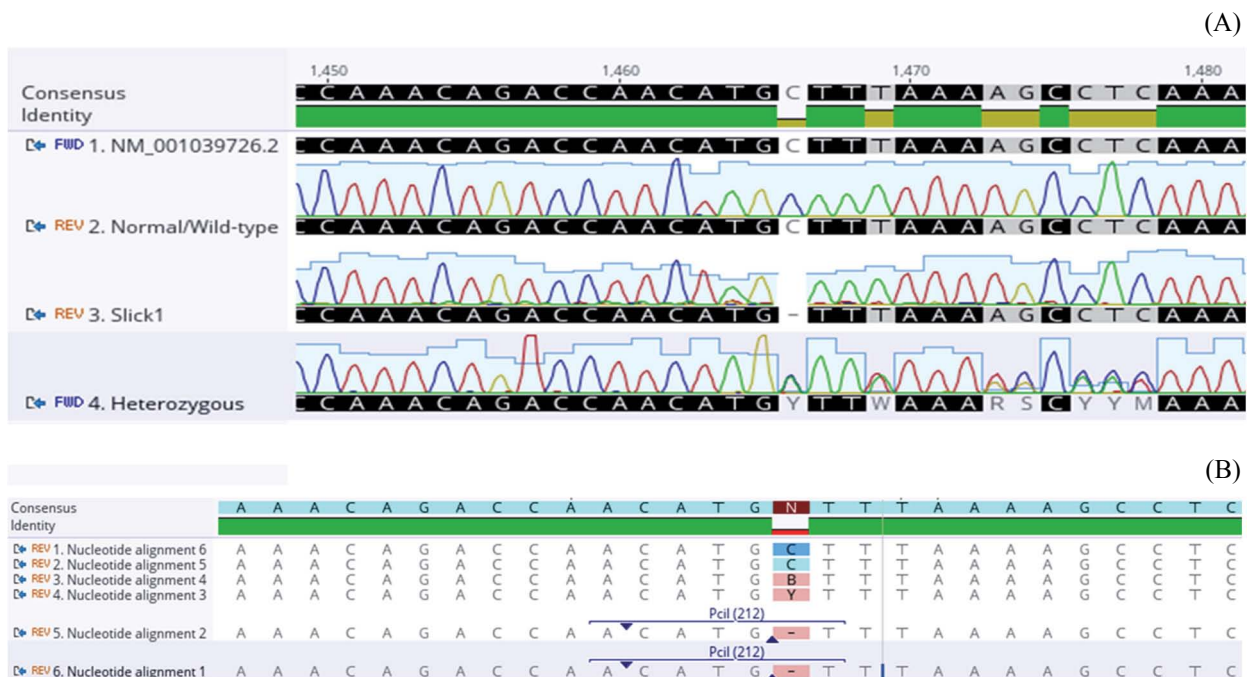


Fig. 2 (A) sequence chromatogram of different genotypes (top to bottom) for normal (non-slick), Slick1 (homozygous slick), heterozygous (hetero-slick); (B) nucleotide alignments showing point mutations at position 1466 and presence of restriction site

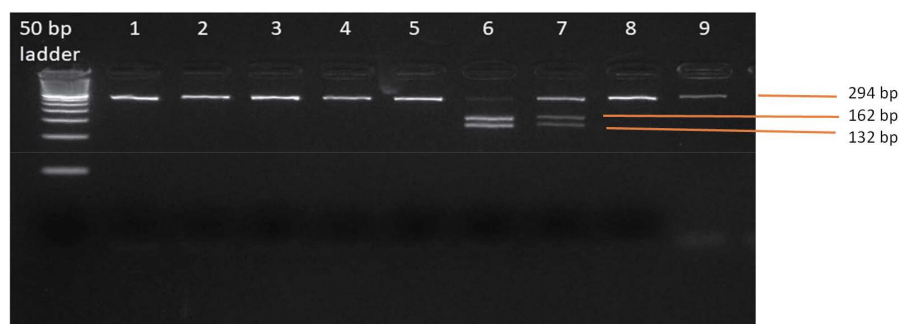


Fig. 3 Agarose gel electrophoresis of representative polymerase chain reaction-restriction fragment length polymorphism results from analyzing amplified 294bp fragment from *PRL* gene using the *RsaI* restriction enzyme, where lanes 1-5 and 8-9 = *GG* genotype (294bp), lane 6 = *AA* genotype (162bp, 132bp) and lane 7 = *AG* genotype (294bp, 162bp, 132bp)

compared to the *G* allele (84%). In fact, only one herd had two *AA* genotyped animals. The *AA* genotype and the *A* allele frequencies for the *PRL* gene marker in the local dairy cattle sampled in the current study were 0.6% and 16%, respectively. The *AA* genotype and *A* allele were low (6% and 25%, respectively), whereas the *AG* and *GG* genotypes comprised 30.1% and 69.3% of sampled animals.

The *PRLR* Slick1 gene mutation among the breeding bulls, cows and heifers had a low frequency of the *CT* genotype (hetero-slick) and favorable *T* alleles (12.5% and 6.0%, respectively), as shown in Table 3. There were no homo-slick individuals screened from the local population, as the sampled animals were mainly offspring of cows inseminated with imported semen of homo-slick or hetero-slick bulls. The absence of local testing, making it difficult to identify

carriers of the *PRLR* Slick1 *T* allele, coupled with low artificial insemination use, were most likely the cause of the low minor *T* allele frequency.

In the current study, there was good concordance between the “slick” phenotype and the *PRLR* Slick1 genotype based on photographs, while a large percentage of non-slick cows were “non-slick looking” and 12.2% were “slick looking” (Table 4), two of which are shown in Fig. 4. The current results supported Littlejohn et al. (2014) and Soverini et al. (2018), who reported that the “slick” phenotype was not a reliable means to identify animals with the *PRLR* Slick1 mutation, highlighting the need for a simple PCR-RFLP method for *PRLR* Slick1 genotyping.

Table 3 Genotype and allele frequencies of Prolactin receptor (*PRLR*) Slick1 and Prolactin (*PRL*) gene markers from sampled local dairy herds

Herd	<i>PRLR</i> Slick1								<i>PRL</i>							
	Genotype						Allele, %		Genotype						Allele, %	
	<i>CC</i>		<i>CT</i>		<i>TT</i>		<i>C</i>	<i>T</i>	<i>AA</i>		<i>AG</i>		<i>GG</i>		<i>A</i>	<i>G</i>
	n	%	n	%	n	%			n	%	n	%	n	%		
Herd A	136	88.9	17	11.1	0	0	94	6	0	0	26	39.4	40	60.6	20	80
Herd B	62	74.7	21	25.3	0	0	87	13	0	0	23	29.5	55	70.5	15	85
Herd C	53	93.0	4	7.0	0	0	96	4	0	0	7	20.6	27	79.4	10	90
Herd D	168	90.3	18	9.7	0	0	95	5	2	1.2	48	28.7	117	70.1	16	84
TOTAL	419	87.5	60	12.5	0	0	94	6	2	0.6	104	30.1	239	69.3	16	84

Herds A and D = from Southern Luzon; Herds B and C = from Mindanao

Table 4 Proportion of “slick-looking” and “non-slick looking” animals according to Prolactin receptor (*PRLR*) slick1 genotype

Genotype	Non-slick looking		Slick looking		Total
	n	%	n	%	
CC	43	87.8	6	12.2	49
CT	0	0	20	100	20

Milk production performance

The least squares mean estimates of 305D milk yield of non-slick (*CC*) and hetero-slick (*CT*) cows from two herds in the southern part of Luzon are shown in Table 5. The statistically significant main effects were Herd ($p = 0.00001$)



Fig. 4 Photographs of representative cows genotyped using polymerase chain reaction-restriction fragment length polymorphism method: (A) hetero-slick; (B) non-slick

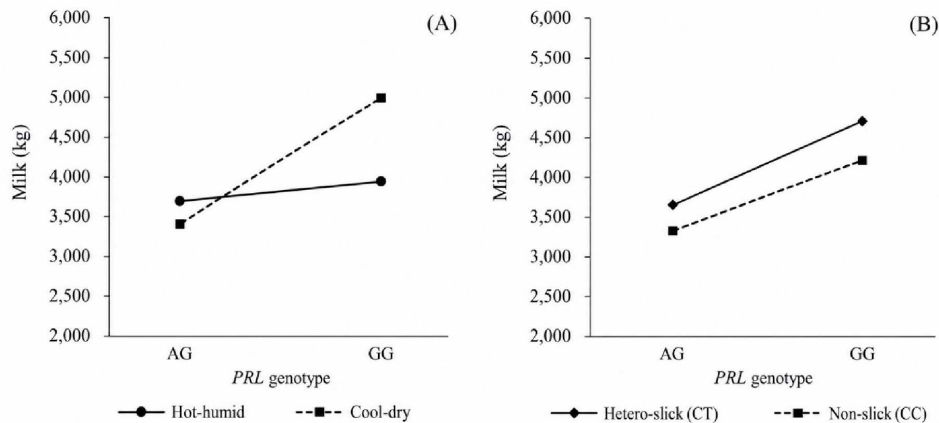


Fig. 5 Milk yield during the 305 d lactation period (305D) of dairy cows classified by PRL genotype. (A) Comparison of 305D milk yield of cows with PRL genotypes AG and GG under hot-humid and cool-dry environmental conditions. (B) Comparison of 305D milk yield between hetero-slick (CT) and non-slick (CC) coat genotypes according to PRL genotype. AG = PRL genotype AG; GG = PRL genotype GG.

and *PRL* genotype ($p = 0.00708$) wherein Herd 2 and the *GG* genotype had higher 305D milk yield estimates, respectively (Table 5). Thus, based on these results, the *G* allele of the *PRL* gene was favorable. The main effect of the *PRLR* Slick1 genotype on 305D milk yield was not significant; however, the interaction between the *PRL* genotype and season of calving was significant ($p = 0.0249$; Fig. 5A). The 305D milk yield of the *GG* genotyped cows calving in the hot-humid season decreased significantly ($p = 0.0249$) relative to cows calving in the cool-dry season. On the other hand, the 305D milk yield

performance of the *AG* cows calving in hot-humid season increased, though not significantly. There was no significant interaction between *PRLR* and *PRL* (Fig. 5B) although the *CT* genotyped cows had higher milk yields across the two *PRL* genotypes, *AG* and *GG*. A separate analysis (Table 6) of the average 305D milk production of cows calving in the cool-dry and hot-humid seasons, broken down by *PRL* and *PRLR* Slick1 genotype, showed that the *CT*/*GG* and *CT*/*AG* cows fared better in the hot-humid season than the *CC*/*GG* and *CC*/*AG* cows. However, this effect was readily evident,

Table 5 Least squares (LS) mean estimates of 305D milk yield of cows according to herd, calving year, genotype and season of calving.

Effect	n	(305D milk yield (kg))			
		Mean	LS mean	±SE	p Value
Herd					
Herd 1	36	3,176.9	3,202.5 ^a	252.2	<i>p</i> < 0.0001
Herd 2	90	4,892.6	4,859.9 ^b	236.4	
Calving year					
2021	74	4,153.5	3,938.3	220.8	
2022	28	3,980.3	4,139.5	331.0	
2023	24	4,208.2	4,015.8	373.2	
PRL genotype					
AG	23	3,446.8	3,565.5 ^a	263.19729	<i>p</i> = 0.0070
GG	42	4,499.7	4,496.9 ^b	236.9	
PRLR SLICK genotype					
CC - non-slick	99	4,141.0	3,871.2	219.7	
CT - hetero-slick	27	4,095.0	4,191.2	291.5	
PRL × Season interaction					
GG × Cool-dry	18	4,490.5	5,029.5 ^a	322.2	<i>p</i> = 0.0249
GG × Hot-humid	23	4,538.6	3,861.56 ^b	280.6	
AG × Cool-dry	10	3,148.9	3,293.9 ^b	411.6	
AG × Hot-humid	13	3,771.8	3,649.6 ^b	363.0	
PRL × PRLR interaction					
GG × CC	46	4,562	4,218.6	240.7	
GG × CT	53	4,097	4,672.3	464.3	
AG × CC	12	4,167	3,393.2	389.6	
AG × CT	15	3,945	3,550.4	421.3	

PRL = Prolactin gene; PRLR = Prolactin receptor gene; n = number of cows; 305D = 305 d lactation period.
Levels with different lowercase superscripts in same column are significantly (*p* < 0.05) different.
Analysis of variance Prob > F= 0.0001, Lack of fit Prob > F = 0.2455

Table 6 Average 305 d lactation period milk yield of cows according to prolactin (PRL) and prolactin receptor (PRLR) Slick1 genotype and season of calving

PRLR Slick1 genotype	PRL genotype							
	AG				GG			
	Cool-dry		Hot-humid		Cool-dry		Hot-humid	
	N (%)	Mean, kg	N (%)	Mean, kg	N (%)	Mean, kg	N (%)	Mean, kg
CC	7 (10.8)	3,255.7	15 (7.7)	2,999.2	15 (23.1)	5,088.5	15 (29.2)	4,018.4
CT	3 (4.6)	2,863.9	8 (12.3)	4,112.3	4 (6.2)	4,945.0	4 (6.2)	4,132.3

as there were only a few CT cows in the sampled animals. However, the Slick1 allele, a mutation within the PRLR gene, has been shown to confer a short and sleek coat phenotype in cattle, enhancing their thermoregulatory capacity and maintaining milk production during heat stress (Cartwright et al., 2023; Zayas et al., 2025). The presence of the “slick” allele is associated with a distinctive short hair coat phenotype which, as exemplified by the phenotype of the local hetero-slick cows and validated by DNA sequencing in the current study, facilitates heat dissipation and thermal regulation in

cattle, thereby improving their ability to withstand heat stress conditions (Xia et al., 2023; Santos et al., 2024). This improved thermoregulation may contribute directly to a reduced decline in milk yield and improved overall welfare during periods of elevated environmental temperatures (Pozzebon et al., 2024). The favorable PRLR Slick1 T allele in the hetero-slick cows probably mitigated the effect of heat stress on these cows, allowing the animals to divert energy away from thermoregulatory maintenance (such as excessive panting) and back toward lactogenesis. The mutation is a dominant allele as

Sosa et al. (2021) reported that there were few differences in phenotype between animals inheriting one or two copies of the allele.

The primary aim of the study was to develop a PCR-RFLP genotyping test to be used locally, being the first to be made available for both the *PRL* and *PRLR* Slick1 gene markers for the various dairy herds. The concordance of the genotyping results with the TaqMan® genotyping array suggested that PCR-RFLP could be a reliable method for genotyping the *PRLR* Slick1 gene mutation in cattle. The locally developed PCR-RFLP assay for the *PRLR* Slick1 and *PRL* genes offers a cost-effective and reliable method for identifying favorable alleles, facilitating marker-assisted selection programs in developing regions (Marshall et al., 2019). In particular, this approach is valuable for improving cattle resilience to climate change, enabling the selection of animals with enhanced adaptation to tropical environments (Camargo et al., 2022; Ngeno, 2023). This genotyping tool should contribute substantially to the genetic improvement of dairy cattle by identifying individuals with desirable genotypes, thereby optimizing milk production and overall animal health under challenging environmental conditions (Camargo and Pereira, 2022). This local genotyping capability not only supports sustainable livestock production, by improving feed efficiency and disease resistance, but also addresses the broader challenges of food security and environmental impact in a changing global climate.

The determination of the effect of the *PRL* and *PRLR* on thermotolerance in dairy cows has been reported elsewhere and so was not the primary objective of the current study. However, there was an opportunity to record, on a preliminary basis, milk production performance of local dairy cows from the use of commercially available semen of slick bulls, once the PCR-RFLP test had been developed. The significant ($p < 0.001$) difference in 305D milk yield between the two herd groups was most likely due to differences in management, considering that not only were the two herds within each location close to each other, but the cows of the two herds came from a single source, as well as having similar feed resource availability and a similar source of frozen semen used for breeding. However, since pedigree records were not available for these crossbred cows, their genetic makeup could not be ascertained. A robust statistical analysis was not possible due to the absence of pedigree and other pertinent records of individual animals, such as parity, and the small, uneven sample sizes of the non-slick and hetero-slick cows with milk records,

The *PRL* *RsaI* polymorphism in the current study supports Alipanah et al. (2007), who reported a significant difference

in milk production performance among cows of different genotypes. Other than the main role of the *PRL* in milk production, the gene has been associated with sweating and water/electrolyte balance, among others, suggesting the hormone has a particular function in relation to response to or mitigation of heat stress (Clarke et al., 2025). Among the effects of heat stress in dairy animals are reductions in lactation length and milk production (Das et al., 2016). The mitigation of heat stress was not very evident in the interaction between the *PRL* gene and calving season due to a significant decrease in milk yield for the *PRL* *GG* genotyped cows calving in hot-humid season. Nevertheless, regardless of *PRL* genotype (*AG* or *GG*), the hetero-slick cows fared better when calving in hot-humid season compared to non-slick cows, suggesting the *T* allele had a mitigating effect. This may have been due to the “slick” phenotype, as reported elsewhere (Dikmen et al., 2014). The limitations of the current study mean there was no clear evidence of the heat stress mitigation effect of the *PRLR* Slick1 *T* allele.

The size of the current dataset of cows with individual 305D milk yield was small. There were not enough records to reach a valid conclusion, rather only suggesting that hetero-slick cows could fare better in hot humid conditions, supporting the study by Dikmen et al. (2014). The current results suggested that the *PRL* gene and the *PRLR* Slick1 mutation could convey better milk production and heat tolerance in the hot-humid season to cows, consistent with reports from other countries (Porto-Neto et al. 2018; Carmickle et al., 2022) that the *PRLR* Slick1 mutation would be favorable to animals raised in tropical and sub-tropical climates. In temperate countries, selection for high-yielding cows can be carried out with minimal attention to heat tolerance. Modifications to the environment, such as installing a mist/fan cooling system, can minimize milk production losses during the summer season. However, in a tropical climate, the hot, humid conditions persist longer and may negatively impact milk production. It could be beneficial to use both the *PRL* and *PRLR* Slick1 gene markers as selection tools.

Further validation of the results of the current study would be provided if more cows with milk production records were genotyped and the data analyzed. The *CT* genotype could be tested in other areas of the Philippines that are hotter or more humid than the South of Luzon, where the current study was done. A larger dataset would minimize bias, enable more robust analysis and determine the values of the *PRL* and *PRLR* Slick1 mutations in the local dairy cattle population. This is important because the available dairy cattle genetics are from temperate

breeds and thus the animals may have difficulty adapting to hot, humid conditions. The need for sires with better heat tolerance in tropical countries prompted the development and availability of bulls with thermotolerance/tropical bloodlines. In addition, future plans should include the development of genotyping test protocols using the qPCR.

Conclusions

This study demonstrated that the locally developed PCR-RFLP method for screening for the *PRL* and *PRLR* Slick mutations in dairy cattle was comparable to a commercially available genotyping method. The PCR-RFLP method was an effective and reliable tool for genotyping, providing valuable insights into the genetic makeup of the sampled populations of dairy herds. The proportion of *PRLR* Slick1 carriers in the four local dairy farms was low, underscoring the need for targeted breeding strategies to increase the frequency of the *T* allele of the *PRLR* Slick1 mutation. The *GG* genotype of the *PRL* gene was associated with significantly higher milk yield than other variants, suggesting the *G* allele is favorable for production in this population, especially when combined with the *PRLR* Slick1 gene marker to mitigate heat stress. While the *PRLR* Slick1 mutation did not significantly increase milk yield in hetero-slick cows compared to non-carriers, it provided stable performance. Hetero-slick (*CT*) cows maintained consistent milk production during the hot-humid season of calving, regardless of the *PRL* genotype. The current study was the first to determine, if only preliminarily, the milk production performance of local dairy cows based on genotype for the *PRL* and *PRLR* genes. Due to the small samples size, further studies are recommended using a larger dataset to assure more robust statistical analysis. Furthermore, this was the first time that a genetic testing service was made available locally as a tool for selection, especially since there is still no organized genetic evaluation system on island-born animals as the basis for selection of replacement/breeder animals. By selecting for both the presence of the *PRLR* Slick1 mutation and favorable *PRL* genotype, breeding programs can more effectively enhance economically important traits, leading to improved productivity and animal welfare under heat-stress conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgement

Essential services were provided the Animal Breeding and Genomics Section and the Reproduction and Physiology Section of the Philippine Carabao Center, National Headquarters Science City of Muñoz, Nueva Ecija, the Philippines. Funding support and coordination were provided by DOST-PCAARRD, as this study was a part of a funded project and was instrumental in the successful completion of this study. The dairy farmers and farm staff cooperated and assisted with providing the cattle samples necessary for this research that were crucial in achieving the research objectives.

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