

ASSOCIATION OF BONE MORPHOGENETIC PROTEIN 15 (*BMP15*) EXON 1 AND RECEPTOR 1B (*BMPR1B*) INTRON 6 POLYMORPHISMS WITH EGG PRODUCTION AND EGG QUALITY TRAITS IN *DARAG* NATIVE CHICKEN

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ABSTRACT

Bone Morphogenetic Protein (*BMP15*) and Bone Morphogenetic Protein Receptor Type 1B (*BMPR1B*) play key roles in follicular growth and ovulation. *Darag* native chickens were genotyped for polymorphic sites in exon 1 of *BMP15* and intron 6 of *BMPR1B* genes using PCR-RFLP. Gene and genotypic frequencies were calculated and tested for Hardy-Weinberg equilibrium. A general linear model was used to determine the association of the genotype with animal performance. All three genotypes of *BMP15* (CC, TT, CT) and *BMPR1B* (AA, AG, GG) were detected. *BMP15* genotype CT (0.74) and allele C (0.58), and *BMPR1B* genotype AG (0.71) and allele A (0.57) had the highest frequencies. In *BMP15*, shells were heavier and thicker ($P<0.05$) in CC compared to CT. In *BMPR1B*, genotype AA had heavier egg and yolk weights and greater egg length ($P<0.05$) than AG genotype. While polymorphisms in *BMP15* and *BMPR1B* genes were previously reported to be associated with egg production in chickens, the results of the present study provide evidence that egg quality may be impacted as well. Thus, under the conditions of the study, *BMP15* and *BMPR1B* polymorphisms may serve as possible genetic markers to select for certain egg quality traits in *Darag* native chickens.

Keywords: *BMP15*, *BMPR1B*, *Darag*, egg quality

INTRODUCTION

Darag (*Gallus gallus domesticus*) native chicken from Western Visayas is known for its flexibility to a variety of habitats, low resource requirements, and outstanding taste in heritage dishes. However, compared to exotic breeds, their egg production performance falls significantly short (Cocjin *et al.*, 2007), and with the demand for high-quality eggs and meat increasing exponentially, the need to employ genetic improvement is warranted.

By understanding the physiology of egg production, candidate genes that are postulated to be involved in the observed variation of this trait are validated using molecular techniques and statistical analyses. Bone morphogenetic proteins (BMPs), specifically BMP15, plays a pivotal role in oocyte development, folliculogenesis, and regulation of ovulation rates (Paulini and Melo, 2011; Han *et al.*, 2015; Zhang *et al.*, 2025). The action of this growth factor is mediated through receptor BMPR1B, which influence downstream

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signaling pathways in granulosa cells (Bai *et al.*, 2026; Stephens and Johnson, 2016).

A single nucleotide polymorphism (SNP) in exon 1 of *BMP15* was seen to be associated with age at first egg, egg number, and body weight at first egg of various native chicken breeds (Huang *et al.*, 2015; Han *et al.*, 2015). While introns are non-coding regions, it contains polymorphisms that may be associated with phenotypic outcomes. For example, SNP A287G in intron 6 of *BMPR1B* influenced egg production from 47 to 56 weeks (Zhang *et al.*, 2008). The significance of these associations may differ among populations, leading to breed-specific differences. Thus, there is a need to validate the associations of these genetic markers with egg production and egg quality traits of *Darag* native chickens.

The objectives of the study were to determine the gene and genotypic frequencies of *BMP15* and *BMPR1B*, and to evaluate the association between genotypes and egg production and egg quality traits of *Darag* native chickens. These genetic markers may be used to enhance traditional breeding methods by increasing the accuracy of selection, suggesting that this gene is a promising candidate gene for improving egg production traits in *Darag* native chickens.

MATERIALS AND METHODS

Procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños (IACUC protocol number: CAFS-2019-003). A total of 42 *Darag* native chickens raised in the Native Animal Farm of the University of the Philippines Los Baños, Brgy. Putho-Tuntungin, Los Baños, Laguna, were used in the study. These chickens were raised in a semi-intensive housing setup following standardized management procedures from incubation, brooding, and growing. At 18 weeks of age, pullets were transferred to individual laying cages to monitor the total number of eggs produced until 40 weeks (EN40) and 52 weeks of age (EN52). Egg quality at 40 weeks of age, including egg weight, egg length, egg width, shell weight, shell thickness (top, middle, and bottom), albumen height, yolk weight, and yolk color, were also taken. Egg yolk and shell weight were measured using a weighing scale with a readability of 0.01g. Furthermore, a caliper was used to measure egg length, egg width, and shell thickness. Shell thickness was measured at three points, specifically, the top (head), middle, and bottom (butt-end) parts. Albumen height was determined using an albumen height meter, measured 2mm away from the yolk. The mean values for each trait were calculated and recorded.

Blood samples were collected from the brachial wing vein of 14-week-old *Darag* native chickens using a 3 mL sterile syringe. The samples were blotted onto NucleoCard® cards and allowed to dry at room temperature. DNA isolation was performed using the GF-1 Blood Extraction Kit (Vivantis Technologies) following the manufacturer's protocols. The DNA concentrations (ng/μL), absorbance ratios at A260/A280, and at A260/A230 were measured to check for quantity and purity.

Fragments of exon 1 of *BMP15* and exon 6 to 7 region of *BMPR1B*, which includes the target SNP in intron 6, were amplified through Polymerase Chain Reaction (PCR) using primer pairs from Tenzin *et al.* (2020) and Zhang *et al.* (2008), respectively. The final concentrations of the different PCR components were optimized for a total reaction volume of 20 μL. Each PCR reaction for *BMP15* and *BMPR1B* included the following components: 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 μM of each forward and reverse

primer, 0.2 U *Taq* polymerase for *BMP15* and 0.4 U *Taq* polymerase for *BMPR1B*, 2.0 μ L DNA template, and nuclease-free water. Amplification was performed using the Veriti™ 96-Well Fast Thermal Cycler (Applied Biosystems) with the following conditions: initial denaturation (94°C for 2 mins), 30 cycles of denaturation (94°C for 30 sec), annealing (63°C for *BMP15* and 55°C for *BMPR1B*, 30 sec), extension (72°C for 30 sec) and final extension (72°C for 5 mins). Agarose gel electrophoresis (AGE) was performed using Mupid® One Electrophoresis System Complete Apparatus (Eurogentec, Belgium) to verify the size of the amplicons. The PCR products were loaded along with 2 μ L of loading dye with GelRed® in a 1.5% agarose gel at 100V for approximately 30 minutes. A 100 bp DNA ladder was utilized, and visualization was performed through ENDURO™ GDS Gel Documentation System.

PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) was carried out to detect polymorphisms in *BMP15* and *BMPR1B*. The restriction enzymes that were utilized are *Bbv12I* for *BMP15* and *HindIII* for *BMPR1B*. The digested products were loaded in a 3% agarose gel along with 2 μ L of loading dye with GelRed®, and run at 100 V for approximately 50 minutes. A 50 bp ladder was used as reference.

The identified genotypes were scored and calculated for the gene and genotype frequencies. The chi-square goodness of fit was used to evaluate if the population is in Hardy-Weinberg Equilibrium (HWE). Shapiro-Wilk and Bartlett's tests assessed data normality and variance equality, respectively. General Linear Models (GLM) Procedure of SAS® OnDemand for Academics SAS® Studio (SAS Inc, Cary, USA) was employed to investigate the association between the genetic variants in the *BMP15* and *BMPR1B* genes and the egg production and egg quality traits. At $P < 0.05$, genotype effect will be considered significant. Genotype TT (*BMP15*) and GG (*BMPR1B*) were removed due to small sample size. Least Significant Difference was utilized for post-hoc analysis. Data are presented as least squares means (LSM) $\pm$ standard error of the mean (SEM).

RESULTS AND DISCUSSION

Amplified *BMP15* and *BMPR1B* products showed the target fragment sizes of 517bp and 581bp, respectively (Figure 1A and 1B). After digestion, CC (271bp, 170bp and 76bp), TT (347bp and 170bp), and CT (347bp, 271bp, 170bp, and 76bp) genotypes were observed in *BMP15*, while in *BMPR1B*, AA (294bp and 287bp), AG (581bp, 294bp, and 287bp), and GG (581bp) genotypes were detected (Figure 2A and 2B). The product sizes and fragment sizes matched those reported by Tenzin *et al.* (2020) and Zhang *et al.* (2008).

Gene and genotypic frequencies for each gene were computed. For *BMP15*, genotype CT (0.74) was the most observed genotype, followed by CC (0.21) and TT (0.05). The frequency of allele C (0.58) was higher than its allele T (0.42) counterpart. The higher frequencies of allele C and genotype CT were consistent with other indigenous dual-purpose or meat type chickens, the LaiWu Black chickens (Han *et al.*, 2015), and Shaobo (Huang *et al.*, 2015). For *BMPR1B*, genotype AG (0.74) was predominantly higher than genotypes AA (0.21) and GG (0.07). Allele A (0.57) also attained a higher frequency than Allele G (0.43). These observations align with the study of Zhang *et al.* (2008), where allele A and genotype AG are more frequent in the four chicken populations investigated. Genotype AG is also more frequent in the Fayoumi and Rhode Island Red chickens; however, allele

G is more predominant than allele A (Awad and El-Tarabany, 2015). Differences in gene and genotypic frequencies may be attributed to variation in breeding history, population structure, or historical selection pressures across chicken breeds. Meanwhile, the observed frequencies of both *BMP15* and *BMPRII* genotypes in *Darag* native chicken differ from the expected frequencies, suggesting deviation from the Hardy-Weinberg equilibrium. Possibly, one or a combination of forces that change gene and genotypic frequencies are present in the *Darag* native chicken population.

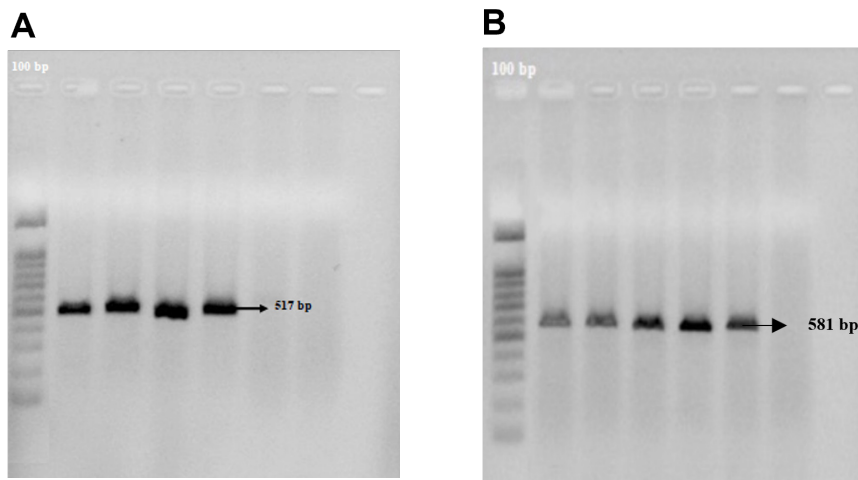


Figure 1. *BMP15* exon 1 (A) and *BMPRII* (B) PCR amplicons visualized in 1.5% agarose gel. Lane 1 shows the 100 bp ladder.

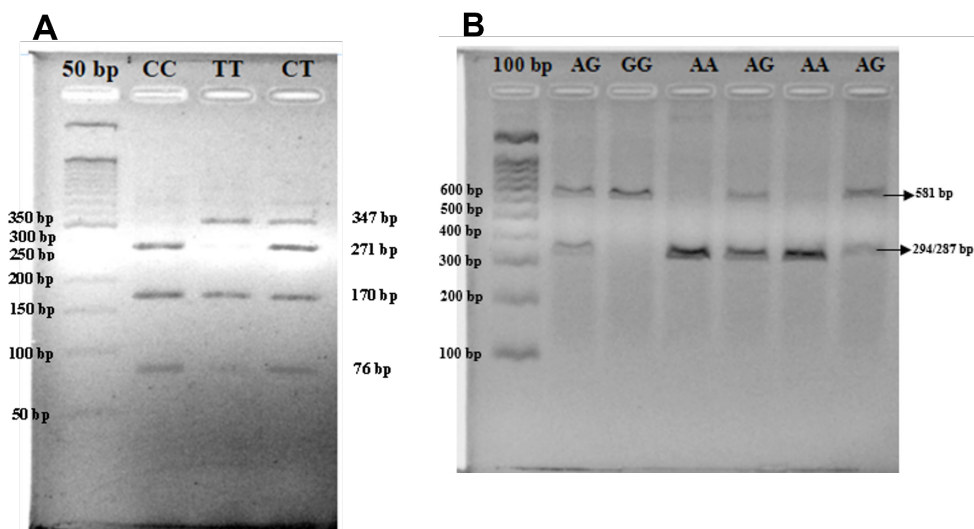


Figure 2. Genotyping by PCR-RFLP of (A) SNP C231T in *BMP15*, where lane 1 contains the 50 bp ladder and lanes 2 – 4 show CC, TT, and CT genotypes; (B) SNP A287G in *BMPRII*, where lane 1 contains the 100 bp ladder, and lanes 2-7 show AA, GG, and AG genotypes.

Egg production was not significantly different between *BMP15* and *BMPR1B* genotypes. This is in contrast to previous reports associating these genotypes with egg number and other reproductive traits (Zhang *et al.*, 2008; Han *et al.*, 2015; Huang *et al.*, 2015).

Notably, *BMP15* and *BMPR1B* genotypes are associated with some egg quality traits. For *BMP15*, the CC genotype had heavier shell weight (P= 0.04) and greater shell thickness (P= 0.002; Table 1) than CT, while for *BMPR1B*, birds with the AA genotype had heavier egg (P=0.02) and yolk (P=0.05) weights and greater egg length (P=0.02) at 40 weeks of age (Table 2). Previous reports showed an association between *BMP15* genotypes and egg weight (EW300) and body weight (BW300) at 300 days (Tenzin *et al.*, 2020) and with age at first egg and total egg production (Han *et al.*, 2015).

BMP15 is preferentially expressed in the oocyte of laying hens (Abadjieva and Grigorova, 2023), which aligns with its function in follicular development. This role may explain *BMP15*’s contribution to the regulation of egg production. The results of the present study suggest an indirect involvement of *BMP15* in egg quality, thereby providing a basis for further investigation of its underlying mechanism through the conduct of functional assays or validation experiments.

Recent transcriptomic studies confirmed the high expression of *BMPR1B* in granulosa cells of hierarchical follicles, where it regulates cellular differentiation and follicular progression (Bai *et al.*, 2026). Its interaction with *BMP15* stimulates pathways that enhance steroidogenesis and FSH receptor sensitivity (Zhang *et al.*, 2025), contributing to yolk precursor accumulation and deposition (Nys and Guyot, 2011). Collectively, this may relate to the observed associations between *BMPR1B* and yolk and egg weights of *Darag* native chickens. In terms of egg length, no direct relationship with *BMPR1B* was seen; thus, further research is needed in this area.

Table 1. Association of *BMP15* on egg production and egg quality traits of *Darag* native chickens.

<i>BMP15</i>			
TRAITS	CC (n=9)	CT (n=31)	p-value
Egg production			
EN40	67.00 ± 7.32	64.83 ± 4.00	0.77
EN52	81.90 ± 10.70	72.07 ± 5.83	0.37
Egg quality			
EggWT40	42.25 ± 1.74	39.91 ± 0.92	0.23
Egg length	51.78 ± 1.05	50.94 ± 0.55	0.47
Egg width	38.77 ± 0.65	38.10 ± 0.34	0.35
Albumen height	4.14 ± 0.18	3.87 ± 0.10	0.19
Yolk weight	13.96 ± 0.60	13.70 ± 0.32	0.71
Yolk color	10.32 ± 0.48	10.24 ± 0.25	0.89
Shell weight	4.76 ± 0.21	4.27 ± 0.11	0.04
Shell thickness	0.32 ± 0.006	0.29 ± 0.003	0.002

Table 2. Association of *BMPR1B* on egg production and egg quality traits of *Darag* native chickens.

<i>BMPR1B</i>			
TRAITS	AA (n=9)	AG (n=31)	p-value
Egg production			
EN40	69.22 ± 6.40	70.30 ± 3.51	0.88
EN52	89.44 ±11.52	87.97 ± 6.31	0.91
Egg quality			
EggWT40	43.43 ± 1.50	39.39 ± 0.81	0.02
Egg length	53.15 ± 0.87	50.80 ± 0.47	0.02
Egg width	39.00 ± 0.59	38.19 ± 0.32	0.23
Albumen height	3.86 ± 0.16	4.01 ± 0.09	0.44
Yolk weight	14.89 ± 0.53	13.63 ± 0.29	0.05
Yolk color	10.86 ± 0.51	10.08 ± 0.28	0.16
Shell weight	4.37± 0.27	4.16 ± 0.15	0.51
Shell thickness	0.29 ± 0.007	0.30 ± 0.003	0.29

CONCLUSION

The findings of the present study provide initial evidence of the association of *BMP15* and *BMPR1B* genotypes with selected egg quality traits, thereby emphasizing their potential for molecular-assisted selection in *Darag* native chickens. The present work was limited to DNA association analysis, which did not include gene expression, biochemical, or ligand-receptor binding assays. It is recommended to expand this work to include a larger sample size of *Darag* or other Philippine native chickens, together with the conduct of RNA- and protein-based assays to strengthen the biological relevance of the growth factor and its receptor on egg quality traits.

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