*Research paper*

# Performance analysis and prolactin gene variability of Kuttanad ducks of Kerala

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## ABSTRACT

The Kuttanad ducks of Kerala are dual purpose ducks known for their adaptability in different agro-climatic zones of the country. The performance evaluation of Kuttanad ducks in the influenza and flood affected regions of their breeding tract revealed the mean body weight at hatch, at 20, 40, 52 and 72 weeks of age to be 46.34±1.03 g, 1.43 ±1.13 kg, 1.58 ±1.36 kg, 1.65 ±1.54 kg and 1.99 ±1.63 kg respectively. The mean age at first egg, body weight at first egg, egg weight at 40, 52 weeks of age and annual egg production at 72 weeks of age were 139.2 ±2.34 days, 1.43 ±1.13 kg, 60.6 ±2.33 g, 67.5 ±2.12 g and 192.6 eggs respectively. PCR-SSCP and sequence analysis of duck-*PRL* gene (204 bp *exon 5*) influencing growth and egg production revealed the locus to be monomorphic and highly conserved in Kuttanad ducks.

**Key words**: Body Weight, Egg Production, Prolactin gene, Kuttanad ducks

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## INTRODUCTION

The local ducks found in Kerala popularly known as Kuttanad ducks are appreciated for their dual purpose utility and are reared by around 1500 farmers in the breeding tracts of Alappuzha, Kottayam and Pathanamthitta districts of the State (Ravindran*et al*., 1984; Mahanta*et al*., 1998; Senani*et al*., 2005; Peethambaran, *et al*., 2006). Following the Avian Influenza (H5NI) outbreaks in 2014 and 2016, nearly 3.60 lakh breeder ducks were culled and many fertile eggs were destroyed. The devastating floods of 2018 and 2019 also deranged the ecosystem of their breeding tract, drowning several elite duck clusters and aggravating the decline of duck germplasm in the State. In this context, an assessment of the performance of Kuttanad ducks in the post-influenza scenario along with a scientific analysis of the genetic variability of important genes controlling economic traits would facilitate the formulation of future strategies for their conservation, selection and multiplication. Prolactin (*PRL)* gene is known as an important candidate gene for growth (Irma *et al*., 2014; Mazurowski*et al*., 2016), egg production and egg quality (Li *et al*., 2009; Cui *et al*., 2011) in ducks. The gene is of 10 kb size consisting of five exons and four introns, encoding 229 amino acids for prolactin, an anterior pituitary hormone regulating the onset of incubation, brooding behavior and follicular development (Chang *et al*., 2012). Hence, the present study was undertaken to assess the performance of Kuttanad ducks in their breeding tract and to detect the genetic variability of *exon 5* locus of duck-*PRL* gene in these *desi* duck populations.

## MATERIALS AND METHODS

### *Sampling and phenotypic data collection*

A total of 80 Kuttanad ducks belonging to different farming clusters maintained in the breeding tract, hit twice by avian influenza outbreaks and heavy floods were evaluated for their growth and production performance. Body weights at hatch and 20, 40 weeks of age, age at first egg, body weight at first egg, egg weights at 40 and 52 weeks of age and the annual egg production at 72 weeks of age were recorded. Blood samples of 2 ml each were collected from wing vein of ducks in EDTA vacutainers and stored at-20° C.

### *DNA extraction and amplification*

Genomic DNA was extracted from whole blood using the standard phenol-chloroform method (Sambrook and Russell, 2001) and frozen at -20° C until use. Purity of DNA was detected by NanoDrop®2000 spectrophotometer. Genomic DNA was used for the amplification of 204 bp fragment (*exon 5*) of *PRL* gene using primers designed by the Primer 3 software (V.4.0) (http://bioinfo.ut.ee/primer30.4/ ). The PCR primers for *PRLexon 5* were synthesized (Sigma-Aldrich, USA) as follows: *PRL* (*exon 5*) - F: 5′-TTCATTCTGGCGACAGC-3" and *PRL* (*exon 5*) - R: 5′-GAAGCCCAGGAGTACTTAGCCG-3". The PCR was conducted in a 20µL reaction mixture containing 1µl of DNA, 2 µl of 10X PCR buffer, 1 µl of 2mM of $MgCl_2$, 0.4µl of dNTPs, 1µl of 10 pmol of each primer and 0.2 µl of *Taq* DNA polymerase. The PCR protocol involved an initial denaturation at 94° C for 30s followed by 35

cycles of denaturation at 94° C for 15s, annealing at 63° C for 20s, extension at 72° C for 30 s and final extension at 72° C for 5 min (Bio-Rad Thermal cycler, USA). Electrophoresis of PCR products was performed in 2% agarose gel in parallel with 50 bp DNA marker (Fermentas) in 1x TBE buffer at a constant voltage of 80 V for 45 min. After ethidium-bromide staining, products were visualized by ultraviolet transilluminator (Bio-Rad, USA).

*PCR-SSCP analysis*

The PCR product was then subjected to single-strand conformation polymorphism (SSCP) analysis using vertical electrophoresis (Hoefer, USA). Aliquots of 10 µL of PCR products were mixed with a 20 µL of denaturing solution (containing 9.5 ml of deionized formamide, 0.4 ml of 0.5MEDTA, 2.5 mg of xylene-cyanole and 2.5 mg bromophenol blue) centrifuged, denatured by heating at 95° C for 10 min and immediately chilled on ice. Denatured amplicons were loaded on 12% PAGE gel in 1x TBE buffer at a constant voltage of 130 V for 18 h. The gel was stained by silver staining (Sanguinetti and Simpson, 1994) to identify polymorphisms if any, at the locus.

*DNA sequencing analysis*

The PCR product from the single SSCP pattern obtained were sequenced using a commercial service (SciGenom Labs Pvt. Ltd. Cochin) in forward and reverse directions to confirm the variability status at the locus.

*Statistical analysis*

The phenotypic data on body weights and egg production collected from the breeding tract were averaged and standard errors estimated.

## RESULTS AND DISCUSSION

Kuttanad ducks under study were maintained in their home tract under extensive system of management. The drakes of Kuttanad origin were found to be squat in posture and gait. Their head was dull to lustrous greenish black in colour. Neck of drakes was distinct brownish black with full or part white bands. The bill was either orange or yellow with or without black spots. Feet were always orange in both the sexes. The females were found to be brownish black on head, breast, back and tail, squat in posture and erect in gait. The estimates of various growth and production traits in Kuttanad ducks are given in Table 1. The performance of Kuttanad ducks in growth and production traits were comparable with the pre-influenza reports on the Chara and Chembally varieties of Kuttanad ducks under various agro-climatic zones of the country (Mahanta*et al*., 1998; Senani*et al*., 2005; Mahanta*et al*., 2009; Anitha, *et al*., 2012). The PCR-SSCP analysis of the exon 5 fragment (204 bp) of duck-*PRL* generevealed only one distinct banding pattern in the duck population (Fig. 1 and Fig 2). Sequence analysis further confirmed the duck-*PRL* gene in Kuttanad ducks to be devoid of any nucleotide polymorphism for the *exon 5*locusand hence, only a single genotype (PP) was observed in the population (Fig. 3).

**Table 1.** Growth and production performance of Kuttanad ducks (n = 80)

| Traits | Mean ±SE |
|---|---|
| Body weight at hatch (g) | 46.34 ±1.03 (80) |
| Body weight at 20 weeks (kg) | 1.43 ±1.13 (58) |
| Age at first egg (days) | 139.2 ±2.34 (58) |
| Body weight at first egg (kg) | 1.43 ±1.13 (58) |
| Body weight at 40 weeks of age (kg) | 1.58 ±1.36 (55) |
| Egg weight at 40 weeks of age (g) | 60.6 ±2.33 (55) |
| Egg weight at 52 weeks of age (g) | 67.5 ±2.12 (50) |
| Body weight at 52 weeks of age (kg) | 1.65 ±1.54 (50) |
| Body weight at 72 weeks of age (kg) | 1.99 ±1.63 (40) |
| Annual egg production at 72 weeks of age | 192.60 (40) |
| Mortality below 8 weeks of age (%) | 23.30 (60) |

**Figures in parenthesis indicate the number of observations (n)*

The study has pointed out that being monomorphic, the *exon 5* fragment of duck-*PRL* gene in Kuttanad ducks can be considered as a highly conserved locus with low or negligible genetic variation. The various exonic and intronic loci of duck-*PRL* gene are reported to be highly polymorphic in many breeds (Cui *et al*., 2011; Chang *et al*., 2012; Irma *et al*., 2014; Mazurowski*et al*., 2016) and therefore, the monomorphic status of the *exon 5* fragment of duck-*PRL* in the Kuttanad variety is a finding contrary to it's more common polymorphic status in most duck breeds. The reasons commonly attributed towards the loss of genetic variability at important loci governing traits in populations are either a lowered population size and the subsequent genetic drift operating in the populations (Falconer, 1960) or the chances of limited interbreeding of the population on account of some habitat loss or habitat fragmentation of man-made or environmental origin (Christopher, 1981). The present study found that the recent repeated avian influenza outbreaks and the Kerala floods contributed to a sharp decline in the breeding stock and paved way for fragmentation of the habitat of these native ducks. Consequently, the lowered rate of outbreeding among the existing populations could have contributed to the loss of genetic variability at the prolactin *exon 5*fragment, making it a matter of concern regarding the duck biodiversity of the region.

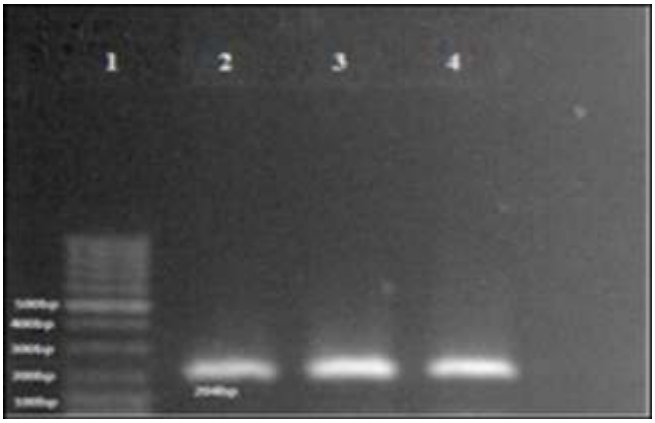


**Figure 1.** PCR amplification of 204 bp fragment of duck-PRL gene (exon 5) in Kuttanad ducks. Lane 1: 100 bp DNA marker, Lane 2 - 4: 204 bp product

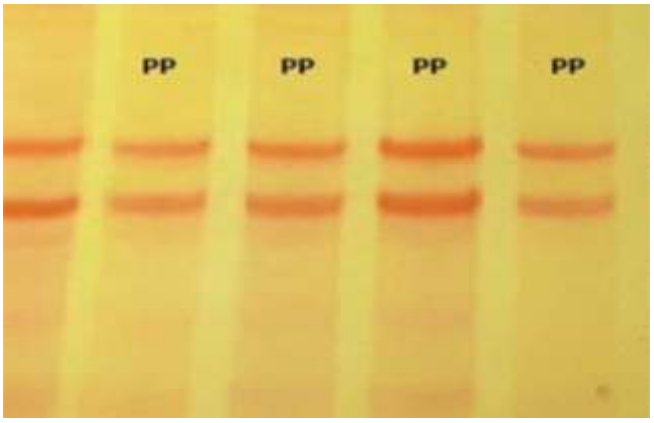


**Figure 2.**SSCP pattern of 204 bp fragment of duck-*PRL* gene(*exon 5*) in Kuttanad ducks

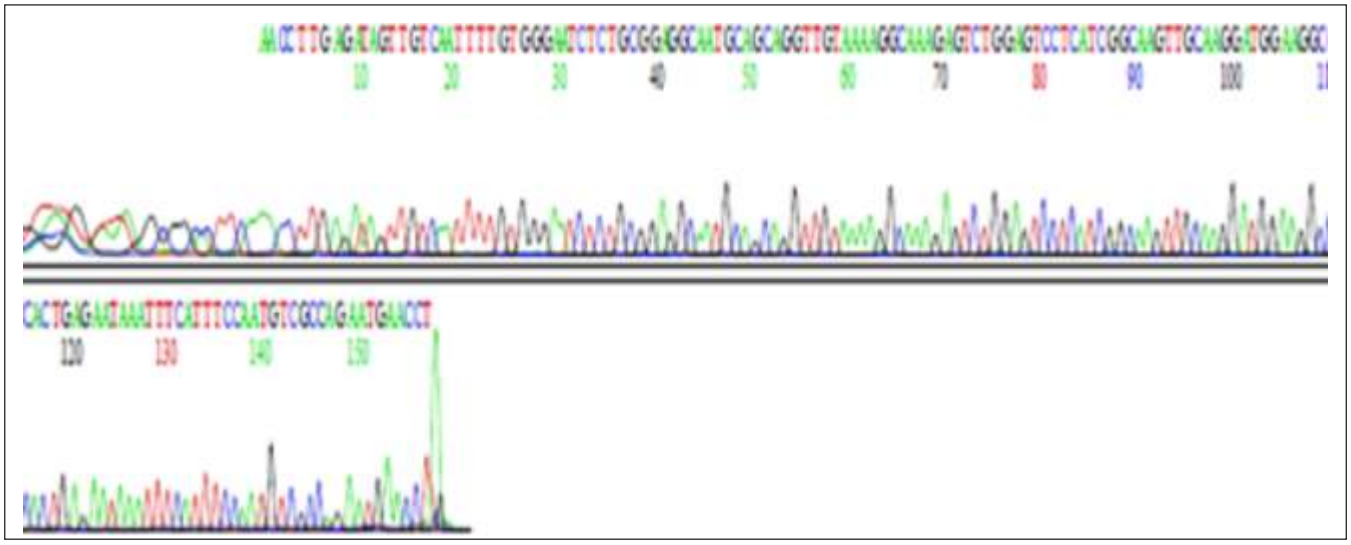

**Figure 3.**Sequence map of PP genotype of duck-PRL gene (exon 5) in Kuttanad ducks

## CONCLUSION

The present study revealed the growth and egg production of Kuttanad ducks in their disaster-hit breeding tract to be conforming to the breed standards and this pointed out the resilience and hardiness of these *desi* duck populations in the post- calamity ecosystem. The *exon 5* locus of duck-*PRL* gene in Kuttanad ducks was found to be monomorphic with a single genotype. It can be inferred from the study that there was considerable loss of genetic variability at the *PRL exon 5* locus and this could probably be attributed firstly, to the loss of alleles and germplasm following the massive slaughter of breeder ducks and destruction of fertile eggs during the avian influenza outbreaks. Secondly, the limited interbreeding of the ducks under considerable habitat loss following the devastating floods in the region also must have contributed to the loss of genetic variability in the major candidate gene of *duck-prolactin* under study. The investigation throws light on the urgent need of concerted efforts towards the planned conservation of Kuttanad ducks, prevention of the environmental degradation of their breeding tract and the quick adoption of appropriate bio-security measures to prevent avian influenza outbreaks in the region.

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