Research Article

Morphometric characterization and growth hormone gene polymorphism (intron 2) in Kuttanad ducks of Kerala

Sahana V.N.[1], Bindya Liz Abraham[1]*, Marykutty Thomas[1], Aravindakshan T.V.[1] and Binoj Chacko[2]

[1]*Department of Animal Breeding and Genetics,*
[2]*University Poultry and Duck Farm,*
College of Veterinary and Animal Sciences, Mannuthy, Thrissur-680651, Kerala, India

ABSTRACT

Utilization of variations in candidate genes is a viable approach for the selection of superior breeding stock of all avian species including ducks. The present study aimed at assessing the morphometry of native Kuttanad ducks of Kerala and the identification of single nucleotide polymorphism (SNP) in the intron 2 region (752 bp) of their growth hormone (*GH*) gene. The mean body weight (BW) at hatch, 4, 8, 12 and 16 weeks of age were found to be 37.82 ±0.56g, 464.32 ±16.71g, 1045.12 ±25.74, 1421.46 ±90.58 g and 1531.93 ±71.54g respectively. The mean chest girth, shank length and neck length at 12 weeks of age were found to be 28.82 ±0.25cm, 7.14 ±0.05cm and 24.83 ±0.21cm respectively. PCR amplification and direct sequencing of the intron 2 region (752 bp) of duck *GH* gene revealed eight novel SNPs *viz*., 72G>A, 90A>G, 175A>G, 176 T>G, 267C>T, 275A>G, 286A>G and 403A>C. The polymorphisms are likely to serve as appropriate markers for selection of ducks for body size and body measurements on further genotyping and association studies with growth traits.

Key words: Duck, Growth hormone gene, Polymorphism

***Corresponding author:** bindya@kvasu.ac.in

INTRODUCTION

Duck is an important poultry genetic resource reared for meat and eggs and has economic, social and ecological value. Duck farming serves as a traditional livelihood option for the rural landless farmers and womenfolk in coastal areas giving high returns under a low input system of short duration (Jalaludeen *et al*., 2022). *Desi* ducks of Kerala known as Kuttanad ducks are spread across the wetlands of Kottayam, Pathanamthitta, Alappuzha and Ernakulam districts of the State. They are dual purpose with an annual egg production of 192.60 at 72 weeks of age (Bindya and Priya, 2020). Their excellent adaptability to the varied agro-climatic zones outside home State also help them enjoy the privilege of having been adopted by the Government of Assam as the model duck for the genetic improvement of *Pati* ducks under free-range farming conditions (Mahanta *et al*., 2009).

Several candidate genes like *IFNγ*, *GH*, *FGF 7*, *TGIF1*, *Prolactin receptor* and *Prolactin* have been studied to assess the possibility of marker assisted selection for growth, production and disease resistance in Indian duck varieties (Sankhyan and Kumar, 2009; Keleson *et al*., 2019; Bindya and Priya, 2020; Bhavana *et al.,* 2022). Growth hormone (*GH*) gene is known as one of the most promising candidate genes for growth performance in poultry (Asmaa *et al*., 2020).The duck growth hormone gene (*dGH*) is 5.25 Kb in size and located on chromosome 28. It consists of five exons and four introns. Growth hormone is the expressed product of the *GH* gene with primary function of growth and several secondary functions of egg production and reproduction in ducks (Kansaku *et al*., 2003). The mature growth hormone protein of duck consists of 190 amino acids (Buggiotti *et al*., 2006). Many polymorphisms have been reported in intronic fragments of the duck *GH* gene like intron 2 and intron 3 regions in Chinese ducks (Xu *et al*., 2007) and intron 2 region in Pekin and Mulard ducks (Mazurowski *et al*., 2015). Identification of intronic mutations in the candidate genes is known to assist the selection for body size and plumage colour in ducks (Zhou *et al*., 2018). Therefore, this study aimed at the detection of single nucleotide polymorphism (SNP) in intron 2 region (752 bp) of *GH* gene in Kuttanad ducks of Kerala.

MATERIALS AND METHODS

A total of 100 Kuttanad ducks reared under standard management conditions at the University Poultry and Duck farm, College of Veterinary and Animal Sciences, Mannuthy from January 2022 to May 2022 formed the material for the study. The birds were fed with starter feed (CP: 22-23 per cent and ME: 2800 Kcal/kg) from 0 to 12 weeks of age and grower feed (CP: 18-19 per cent and ME: 2600 Kcal/kg) from 12 to 16 weeks of age. Ducklings were wing banded for identification and vaccinated against *Pasteurella* and duck plague virus. Standard deworming was also carried out. Data were recorded on body weight at hatch (BW0) and at different stages of growth (BW), length of trunk with neck (LTN), length of neck (LN), length of breast bone (LBB), chest girth (CG) and length of shank (LS) at fortnightly intervals up to 16 weeks of age. The phenotypic data were subjected to descriptive statistics for morphometric characterization.

Genomic DNA extraction and amplification

One ml of blood was collected from the wing vein of ducks in EDTA (1mg/ml of blood) vacutainers and transferred to laboratory, stored at -40°c for further analysis. Genomic DNA was extracted from the collected blood samples using Qiagen DNeasy Blood and Tissue Kit (Cat. No. 69504) following the manufacturer's protocol. The concentration and purity of DNA was assessed by NanoDrop@2000 spectrophotometer and stored at -20 °c until further use. Genomic DNA was used for the amplification of 752 bp fragment (intron 2) of *GH* gene using primers designed by Primer 3 software (V.4.0) (http://bioinfo.ut.ee/primer30.4/). The PCR primers for *GH* intron 2 were custom synthesized (Sigma-Aldrich, USA) as follows: *GH* (intron 2)-F: 5'-CCGCTGAGACGTACAAAGAG-3" and *GH* (intron 2) - R: 5'-TCTGCTCCTCCGGAATATA GC-3". The PCR was conducted in a reaction mixture containing 1.5 µl of DNA template (50 ng/µl), 12.5µl of 2X emerald green master mix, 1.5µl of forward primer (10 pM/µl), 1.5µl of reverse primer (10 pM/µl) and 8µl nuclease free water, added to make up a final volume of 25 µl. The protocol involved an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30s, annealing at 62.4°C for 2 min 45s, extension at 72°C for 1 min and a final extension at 72°C for 5 min (Bio-Rad Thermal cycler, USA). Electrophoresis of PCR products was performed in 2% agarose gel along with a 100 bp ladder (Fermentas) using 1x TBE buffer at a constant voltage of 80 V for 50 min. After ethidium-bromide staining, the products were visualized under a UV transilluminator (Bio-Rad, USA).

DNA Sequencing

Sequencing was undertaken with a 40 µl pooled sample containing 0.5 µl each of PCR product from 80 samples through a commercial service (SciGenom Labs Pvt. Ltd. Cochin) in forward and reverse directions to confirm the variability status at the locus. Nucleotide sequence alignments and comparisons were carried out using GenBank reference sequence NC_051799.1. The SNPs were detected on sequence alignments using BLASTn (http://www. ncbi.nlm.nih.gov/blast) and EMBOSS: merger (http://emboss.bioinformatics.nl /cgibin/ emboss/merger) applications.

RESULTS AND DISCUSSION

Morphology and morphometric characterization of a population of 125 Kuttanad ducks maintained at University Poultry and Duck farm, College of Veterinary and Animal Sciences, Mannuthy from day old to sixteen weeks of age was undertaken in the study. Two variants *viz., Chara* and *Chemballi* were found in the population. Chara drakes had lustrous greenish black head with yellowish black bills and they were found to be squat in posture and gait. In females, the head was light brown with yellowish bills and the plumage colour was brownish black at neck, breast and tail. Feet were light orange in colour in both sexes. Chemballi males had dark brown head. In females, the head was light brown and the plumage pattern was light brown to brownish black at neck, breast and tail. Beak color varied from light yellow to light orange in both the sexes.

The mean body weight and body measurements at different stages of growth from hatch to 16 weeks of age in Kuttanad ducks are given in Table 1. Hatch weight of Kuttanad ducks in the present study was found to be lower than earlier reports from their breeding tract (Bindya and Priya, 2020). Body weight at four weeks of age was also found to be lower than that of earlier reports (Mahanta *et al*., 2009). The body weight at 12 weeks of age was in accordance with the earlier reports (Renchi *et al*., 1979; Mahanta *et al*., 2009) and slightly higher than that reported for other Indian duck varieties like Pati, Tripura and Nageshwari ducks of North Eastern India (Arundhati *et al*., 2018). The chest girth of Kuttanad ducks was found to be lower than that of Pati ducks of Assam (Purabi *et al.*, 2021) while their shanks and necks were found to be longer than that of indigenous ducks of Tamil Nadu (Veeramani *et al*., 2014), *desi* ducks of Odisha (Kamal *et al*., 2019) and Pati ducks of Assam (Purabi *et al*., 2021).The shank length of Kuttanad ducks was also found to be higher than that of Nageshwari ducks of Assam (Sharma *et al.,* 2003), *desi* ducks of West Bengal (Vij *et al*., 2010) and Pati, Tripura, Manipuri, and Nageshwari ducks of North Eastern India (Arundhati *et al*., 2018). The shank length of Kuttanad ducks in the present study also depicted a significant increase after its first report more than 40 years ago (Renchi *et al*., 1979) which could be of some evolutionary significance in the present context of flood-hit home tract of Kuttanad ducks. Duggan *et al.,* (2015) has reported that change in leg morphology and morphometry of ducks occurs as a result of adaptation to swimming with limitation to terrestrial locomotion. This could be particularly true of the Kuttanad ducks in the background of the yearly episodes of Kerala Floods ever since 2018 wherein several clusters of ducks and ducklings were washed away in the strong water currents. The increase in shank length over several generations could be an indication of an increased adaptiveness of these native ducks to swimming in turbulent flood waters.

Table 1: Fortnightly body weight and body measurements in Kuttanad ducks

Weeks of age	**Body weight (g)**			**Body measurements (cm)**				
	Male	**Female**	**Overall**	**LTN**	**LN**	**LBB**	**CG**	**LS**
Hatch (125)	39.82 ±0.48	36.86 ±0.21	37.82 ±0.56	-	-	-	-	-
2 (110)	222.30 ±6.55	215.54 ±8.20	219.32 ±22.15	18.21 ±0.25	9.97 ±0.13	3.49 ±0.05	10.42 ±0.14	4.21 ±0.08
4 (106)	472.73 ±11.92	426.56 ±10.72	464.32 ±16.71	26.37 ±0.34	13.38 ±0.20	5..26 ±0.08	16.26 ±0.24	5.96 ±0.05
6 (103)	766.57 ±17.45	737.90 ±18.73	751.18 ±21.12	33.49 ±0.35	14.12 ±0.26	6.83 ±0.30	21.70 ±0.26	6.63 ±0.05
8(102)	1119.57 ±22.66	958.10 ± 23.57	1045.12 ±25.74	37.0 ±0.39	15.05 ±0.33	8.79 ±0.20	23.24 ±0.32	6.93 ±0.06
10 (100)	1298.34 ± 25.78	1256.23 ±28.65	1268.36 ±28.41	38.78 ±0.44	22.52 ±0.51	11.88 ±1.68	27.53 ±0.31	7.12 ±0.09
12 (99)	1443.32 ±32.47	1405.92 ±35.36	1421.46 ±90.58	40.78 ±0.36	24.83 ±0.23	12.08 ±0.21	28.82 ±0.25	7.14 ±0.05
14 (97)	1513.42 ±46.69	1489.74 ±67.34	1494.90 ±31.08	41.06 ±0.69	25.52 ±0.32	12.51 ±0.32	30.36 ±0.29	7.29 ±0.09
16 (94)	1549.52 ±54.72	1523.69 ±63.28	1531.93 ±71.54	41.90 ±0.36	26.06 ±0.32	12.86 ±0.11	31.45 ±0.24	7.32 ±0.06

*Figures in parenthesis indicate the number of observations; LTN- length of trunk with neck; LN-length of neck; LBB-length of breast bone; CG-chest girth; LS- length of shank.

PCR amplification of 752 bp region of *dGH* gene (intron 2) in Kuttanad ducks is depicted in Fig. 1. Direct sequencing and further sequence alignments with the reference sequence revealed eight novel SNPs in intron 2 region of GH gene in Kuttanad ducks. There were six transitions of 72G>A, 90A>G, 175A>G, 275A>G, 286A>G, 267 C>T and two transversions of 176T>G and 403A>C in the *dGH* intron 2 (752 bp) under study (Fig. 2). The results revealed that intron 2 (752 bp) of *GH* gene of Kuttanad ducks is highly polymorphic. Several polymorphisms of similar nature have been reported in duck GH gene in ducks of exotic origin at the introns 2 and 3 of Cherry Valley, Muscovy and Jingjiang (Wu *et al.,* 2012), intron 3 of Muscovy (Wu *et al*., 2014), intron 2 of Pekin and Mulard (Mazurowski *et al*., 2015), intron 1 of Pitalah and Kumbang Janti (Yurnalis *et al.,* 2017) and intron 4 of Bayang ducks (Yurnalis *et al*., 2018). All the eight SNPs detected in the study were found to be novel and reported for the first time in any Indian or exotic duck variety. These SNPs may provide appropriate markers for selection of superior ducks on subsequent genotyping of the birds and their possible association with the levels of *GH* mRNA expression.

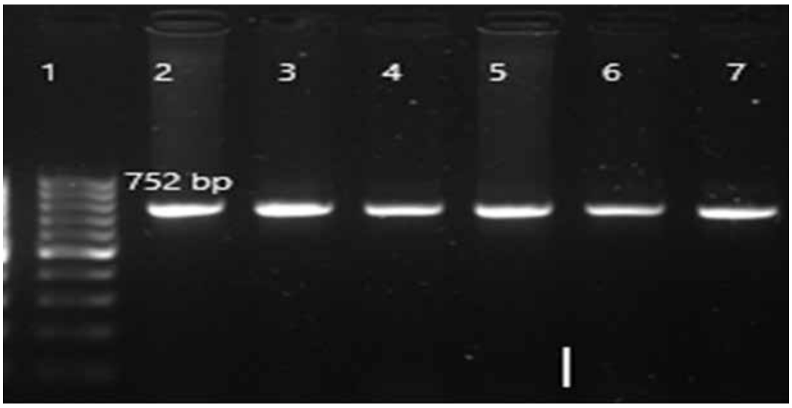

Fig. 1: *PCR amplification of 752 bp region of dGH gene (intron 2) in Kuttanad ducks*

Lane 1: 100 bp ladder; Lane 2-7: 752 bp

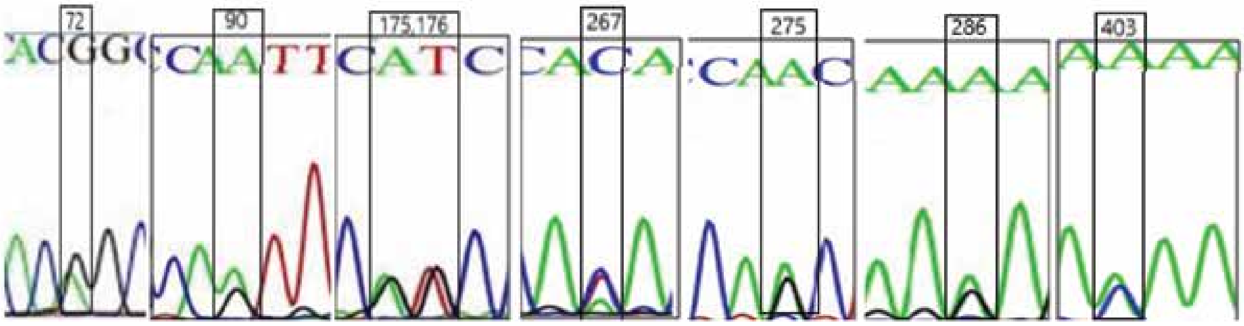

Fig. 2*: Sequence map of intron 2 (752 bp) of dGH gene in Kuttanad ducks*

GH gene variation in introns have been found to be associated with growth and carcass traits in Cherry Valley, Muscovy and Jingjiang ducks (Wu *et al*., 2012), egg-laying performance in Muscovy ducks (Wu *et al.,* 2014), 12th week body weight of Mulard ducks (Mazurowski *et al*., 2015) and 5 to 8 week body weight of Bayang ducks (Yurnalis *et al.*, 2018). Though introns do not encode protein products, they are integral to the regulation of gene expression and are involved in virtually every step of mRNA processing (Kuhnlein *et al*., 1997). Intronic mutations may affect splice or branch sites when situated in regulatory regions of the genes and may influence the quantity of transcripts produced as well as the status of translation (Mrinal and Nagaraju, 2008). The "functional nuclear introns" can accommodate sequences that are crucial for the expression of the gene on which the intron is located. They play key role in the process of alternative splicing, which results in the production of multiple type of messenger RNA from a single gene (Saikhom *et al.,* 2017).

CONCLUSION

The present study revealed the morphometry and growth performance of Kuttanad ducks from the day of hatch to 16 weeks of age and the single nucleotide variations in the intron 2 region (752 bp) of their growth hormone gene. Kuttanad ducks had longer neck and shank lengths when compared to most of the indigenous ducks of India, which can make them excellent swimmers in turbulent waters, an acquired trait of adaptability to the flood situations in their breeding tract. The intron 2 of duck-*GH* gene in Kuttanad ducks was found to be highly polymorphic with eight novel SNPs. The SNPs detected were 72G>A, 90A>G, 175A>G, 176 T>G, 267C>T, 275A>G, 286A>G and 403A>C. These SNPs may provide appropriate markers for selection of superior ducks on subsequent genotyping of the birds and their association with the levels of *GH* mRNA expression in future studies.

ACKNOWLEDGEMENT

The authors duly acknowledge the facilities provided by Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Mannuthy for the research.

REFERENCES

Arundhati P, Bula D, Arpana D, Islam R, Sharma M, Krishna B, and Keleson B. 2018. Morphology, morphometry and certain egg quality traits of indigenous ducks of North Eastern region of India. *International Journal of Chemical Studies,* 6 (2):3131-3136.

Asmaa WZ, Fardos AMH, Elshimaa MR, and Amir HA. 2020. Polymorphisms in intron 2 of growth hormone gene and their associations with economic traits in Muscovy, Pekin and Mallard ducks. *Scholars Journal of Agriculture and Veterinary Sciences,* 7 (2):41-46.

Bhavana K, Foote DJ, Srikanth K, Christopher NB, Vandana RP, Sankaralingam S, Hijam SS, Gopalakrishnan A, and Nagarajan M. 2022. Comparative transcriptome analysis of Indian domestic ducks reveals candidate genes associated with egg production. *Scientific Reports,* 12:943-949.

Bindya LA, and Priya M. 2020. Performance analysis and prolactin gene variability of Kuttanad ducks of Kerala. *Journal of Livestock Biodiversity,* 10 (1): 28-31.

Buggiotti L, Hellstrom MA, and Primmer CR. 2006. Characterization of the first growth hormone gene sequence for a passerine bird the pied flycatcher. *DNA Sequence*, 17:401-406.

Duggan BM, Paul MH, Tobias S, and Dylan NC. 2015. Differences in hind limb morphology of ducks and chickens: effects of domestication and selection. *Genetics Selection Evolution,* 47:88.

Jalaludeen A, and Churchil RR. 2022. Duck Production: An Overview. In: Duck Production and Management Strategies, 1st edition, pp.1-55. *Springer Verlag, Singapore*, Pp. 657.

Kamal R, Dey A, Chandran P, Mohanta R, Giri S, Mohanty S, Kumar G, and Samir B. 2019. Phenotypic and morphometric characterization of desi duck of Odisha. *Indian Journal of Animal Sciences*, 89(3):334-336.

Kansaku N, Nakada A, Okabayashi H, Guémené D, Kuhnlein U, Zadworny D, and Shimada K. 2003. DNA polymorphism in the chicken growth hormone gene: Association with egg production. *Animal Science Journal,* 74:243-244.

Keleson B, Bula D, Probodh B, Luit B, Krishna B and Shantanu T. 2019. Polymorhism of prolactin receptor gene in indigenous ducks of Assam. *Journal of Entomology and Zoology Studies,* 7 (1):922-925.

Kuhnlein U, Ni L, Zadworny D, Weigend S, Gavora JS, and Fairfull W. 1997. DNA Polymorphisms in the chicken growth gene: response to selection for disease resistance and association with egg production. *Animal Genetics, 28* (2):116-123.

Mahanta JD, Raj J, Deka, Sapcota D, and Jalaludeen A. 2009. Certain performance traits of Chara-Chemballi ducks of Kerala under range condition in Assam. *Proceedings of the IV World Waterfowl Conference*, 11-13 November, Thrissur, India. pp.136-138.

Mazurowski A, Frieske A, Kokoszynski D, Mroczkowski S, Bernacki Z, and Wilkanowska A. 2015. Examination of growth hormone gene polymorphism and its association with body weight and selected body dimensions in ducks. *Folia Biological (Kraków),* 63(1):43-50.

Mrinal N, and Nagaraju J. 2008. Intron loss is associated with gain of function in the evolution of the gloverin family of antibacterial genes in *Bombyx mori*. *Journal of Biological Chemistry*, 283:23376–23387.

Purabi K, Jnyanashree S, Kabitabala K, and Rajjyoti D. 2021. Morphology and morphometry characteristics of Pati duck. *International Journal of Chemical Studies*, 9:351-354.

Renchi PG, Nair GR, Nair RS, Nair BRK, and Unni AKK. 1979. Relationship between shank length and body weight in *desi* ducks. *Indian Veterinary Journal*, 56:937-939.

Saikhom R, Sahoo AK, Taraphder S, Pan S, Sarkar U, Ghosh PR, Bhattacharya D, and Baidya S. 2017. Polymorphisms of growth hormone gene in Haringhatta black chicken. *Exploratory Animal and Medical Research*, 7(1):42-47.

CV, and Kumar S. 2009. Nucleotide Polymorphism in Interferon Gamma gene of Indian Runner Native duck using PCR-RFLP. *Proceedings of the IV World Waterfowl Conference*, 11-13 November, Thrissur, India. pp.83-84.

Sharma SS, Zaman G, Goswami RN, and Mahanta JD. 2003. Certain performance traits of Nageswari ducks of Assam. *Indian Journal of Animal Sciences*, 73:831–832.

Veeramani P, Prabakaran R, Selvan ST, Sivaselvam SN, and Sivakumar T. 2014. Morphology and Morphometry of Indigenous Ducks of Tamil Nadu. *Global Journal of Medical Research: Veterinary Science and Veterinary Medicine*, 14(3):17-20.

Vij PK, Tantia MS, Pan S, and Vijh RK. 2010. Morphometric and egg characteristics of indigenous ducks. *Journal of Livestock Biodiversity,* 2(2):77–80.

Wu X, Yan MJ, Lian SY, Liu XT, and Li A. 2014. *GH* gene polymorphisms and expression associated with egg laying in Muscovy ducks (*Cairina moschata). Heriditas*, 151:14-19.

Wu Y, Pan AL, Pi JS, Pu YJ, Du JP, Liang ZH, and Shen J. 2012. One novel SNP of growth hormone gene and its associations with growth and carcass traits in ducks. *Molecular Biology Reports*, 39(8):8027-8033.

Xu SH, Bao WB, Huang J, Cheng JH, Shu JT, and Chen GH. 2007. Polymorphism analysis of intron 2 and 3 of growth hormone gene in duck. *Heriditas*, 29(4):438-442.

Yurnalis, Arnim, Putra DE., Kamsa Z, and Afriani T. 2018. Identification of *GH* gene polymorphisms and their association with body weight in Bayang duck, local duck from West Sumatra, Indonesia. *Proceedings of 6th International Conference on Sustainable Agriculture, Food and Energy* (SAFE 2018); 19th to 21st October, I'M Hotel, Makati, Manila, Philippines. Abstract No.012032.

Yurnalis, Husmaini, and Sabrina. 2017. Polymorphisms of growth hormone gene exon 1 and their associations with body weight in Pitalah and Kumbang Janti ducks. *International Journal of Poultry Science*, 16(5):203-208

Zhou M, Du Y, Nie Q, Liang Y, Luo C, Zeng H, and Zhang X. 2010. Associations between polymorphisms in the chicken VIP gene, egg production and broody traits. *British Poultry Science*, 51:195-203.