B-5315
[1-7]

RESEARCH ARTICLE Indian Journal of Animal Research

Polymorphisms in the Growth Hormone Gene and Their Association with Pre-pullet Body Weight in Kuttanad Ducks of Kerala, India

V.N. Sahana[1], Bindya Liz Abraham[1]

10.18805/IJAR.B-5315

ABSTRACT

Background: Kuttanad ducks are dual purpose native ducks found in Kerala state of India. Identification of single nucleotide variations in candidate genes like growth hormone gene and exploring their association with body weight at grower and pre-pullet stages is essential for the marker assisted selection of breeder ducks for genetic improvement.

Methods: Genomic DNA was extracted from blood samples collected from 101 Kuttanad ducks. PCR-amplification of two duck growth hormone gene *(dGH)* loci *viz*, exon 3 and partial intron 3 (227 bp), exon 5 and partial intron 4 (366 bp) were undertaken using custom-synthesised primers. The 366 bp region was genotyped using Single Strand Conformation Polymorphism. The 227 bp region was genotyped using DNA pooling assay to yield a 139 bp product for subsequent High-Resolution Melting analysis. The association of *dGH* genotypic variants for these loci with fortnightly body weight at pre-pullet stages up to 16 weeks of age was analysed.

Result: In the 366 bp fragment, GG and GA genotypes were revealed with one novel SNP (g 74G>A). The GG homozygotes had significantly higher body weight at all stages from two to 14 weeks of age ($p \leq 0.01$). A novel SNP (g 71A>G) was revealed at the 139 bp locus within the 227 bp region with three genotypes *viz*., AA, AG and GG. The GG homozygotes had significantly higher body weight at six, 14 and 16 weeks of age ($p \leq 0.05$). The study revealed that *dGH* polymorphic loci have jointly contributed to the variation in growth and hence can be used as markers for the primary selection of growers at sixth week of age followed by their secondary selection at 14-16 weeks of age during the multi-stage selection programmes for breeder ducks.

Key words: Ducks, Growth hormone gene, Polymorphism.

INTRODUCTION

Duck production is a traditional ancillary occupation and sustainable livelihood option for the rural farmers and women folk of the wetlands of the Indian subcontinent, offering high returns under low input in a short period of time (Veeramani *et al.,* 2016; Purabi *et al.,* 2021; Veeramani *et al.,* 2023; Kamal *et al.,* 2023). Kerala is one of the leading duck producing States in India with 1.77 million duck population amounting to 5.97% of total poultry population of the country (Anonymous, 2019). The native ducks of Kerala known as Kuttanad ducks are well known for their dual-purpose utility. Their adult body weight is 1.6 Kg and the annual egg production in the breeding tract ranges from 150-200 (Bindya, 2021). The Chara and Chemballi ecotypes of Kuttanad ducks have also earned pan-India popularity through their adoption as a model duck for the genetic improvement of non-descript local ducks in other Union Territories and States of India such as the Andaman-Nicobar Islands and Assam (Senani *et al.,* 2005; Mahanta *et al.,* 2009; Deka *et al.,* 2019).

Growth traits like body weight determine the economic value of any meat-type poultry and it is crucial in selecting a breeding stock (Cyriac *et al.,* 2020). Selection of breeding stock with superior growth attributes by employing polymorphisms found in major candidate genes forms the basis of marker-assisted selection (MAS) programmes. The studies on markers of such candidate genes influencing growth, production and disease resistance in Indian ducks is however limited (Keleson *et al.,* 2019; Bindya and Priya, 2020; Bhavana *et al.,* 2022). Among these, growth hormone (GH) gene is a promising candidate gene, influencing growth, carcass quality, egg production and immune response. The duck GH gene (*dGH*), which is 5.2 kb with five exons and four introns is located on chromosome 28 and expressed in the anterior pituitary (Kansaku *et al.,* 2008). The polymorphisms of *GH* loci have also been reported to be associated with body weight,

[1]Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad-673 576, Kerala, India.

Corresponding Author: Bindya Liz Abraham, Department of Animal Genetics and Breeding, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad-673 576, Kerala, India.
Email: bindya@kvasu.ac.in

How to cite this article: Sahana, V.N. and Abraham, B.L. (2024). Polymorphisms in the Growth Hormone Gene and Their Association with Pre-pullet Body Weight in Kuttanad Ducks of Kerala, India. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5315.

Submitted: 24-01-2024 **Accepted:** 02-07-2024 **Online:** 07-08-2024

biometrics, dressing weight and breast weight in various duck populations of the world (Wu *et al.,* 2012; Yurnalis *et al.,* 2017; Asmaa *et al.,* 2020). Studies on GH gene variations exist for exotic duck breeds (Wu *et al.,* 2012; Wu *et al.,* 2014; Mazurowski *et al.*, 2015; El-Araby *et al.,* 2020) while such research on native Indian duck varieties is scanty. Hence the present study was undertaken to identify single nucleotide polymorphisms (SNPs) in the GH gene of Indian native Kuttanad ducks and explore their association with body weight at pre-pullet stages of growth.

MATERIALS AND METHODS

The experimental population comprised of 101 Kuttanad ducks maintained at the University Poultry and Duck Farm, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala from January to May 2022 under standard management conditions. Day-old female ducklings were wing banded, fed with starter feed (CP: 22-23%, ME: 2800 Kcal/kg) from 0 to 11 weeks of age and grower feed (CP: 18-19%, ME: 2600 Kcal/kg) from 12 to 16 weeks of age. They were vaccinated against Pasteurella and duck plague at 4, 8 weeks of age and at 12, 16 weeks of age respectively. Data on body weight was recorded individually at fortnightly intervals from hatch to 16 weeks of age.

Isolation of DNA and PCR amplification

One milliliter of blood was collected from the wing vein of each bird and transferred into vacutainer tubes containing ethylenediamine tetra acetic acid (EDTA) as anticoagulant. Genomic DNA extraction was carried out using the Quaigen DNeasy Blood and Tissue Kit (cat. Nos. 69504 and 69506), adhering to the manufacturer's protocol with necessary modifications. The concentration, purity and quality of the extracted DNA were evaluated using a NanoDrop spectrophotometer (ThermoScientific, USA) and the resulting DNA was stored at -20°C until further use.

Oligonucleotide primers were designed from the *Anas platyrhynchos* gene sequence (>NC_051799.1:c6089943-6085452 *Anas platyrhynchos* isolate Z2 breed Pekin duck chromosome 28, ZJU1.0, whole genome shotgun sequence) using Primer 3 (V.0.4.0) software (http://bioinfo.ut.ee/primer30.4/). The primers custom synthesized (Sigma-Aldrich) and selected for 366 bp fragment (exon 5 partial intron 4 and intron 3) were Forward: 5'CTCCTC CTGTCCAGTGGCTA3' and Reverse: 5'CGATTATCGTG AGAGAGAAC3 and those for 227 bp fragment (exon 3 and partial intron 3) were Forward: 5'CGCTATTC TCCC AGTG CACG3' and Reverse: 5'TGGAAACCCGCTG TTCATTG3'.

PCR amplification was carried out in a BIORAD thermal cycler using a reaction mixture composed of 1.5 µl of DNA template (50 ng/µl), 12.5 µl of 2X emerald green master mix, 1.5 µl of forward primer (10 pM/µl), 1.5 µl of reverse primer (10 pM/µl) and 8 µl of nuclease-free water resulting in a final volume of 25 µl. The PCR reaction conditions included an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30s, annealing temperature of 61°C for 45 sec (exon 3 and partial intron 3) and 66.4°C for 1 min (exon 5 and partial intron 4), extension at 72°C for 1 min and a final extension at 72°C for 5 min. Subsequently, electrophoresis of PCR products was conducted in a 2% agarose gel along with a 100 bp ladder (Fermentas) using 1xTBE buffer at a constant voltage of 80 V for 50 min. The gels were stained with ethidium bromide and visualized under UV light. Gel documentation was performed with a Bio Rad gel documentation system (USA).

Genotyping of the *dGH* exon 5 and partial intron 4 (366 bp) locus

The exon 5 and partial intron 4 locus was genotyped by Single-Strand Conformation Polymorphism (SSCP) analysis. The PCR products of exon 5 and partial intron 4 (366 bp) locus were subjected to single-strand conformation polymorphism (SSCP) analysis. Eight microlitres of PCR products were mixed with 10 µl of denaturing dye (9.5 ml of deionized formamide, 0.4 ml of 0.5M EDTA, 2.5 mg of xylene-cyanole and 2.5 mg bromophenol blue) centrifuged, denatured at 95°C for 10 min and snap chilled immediately on ice for 10 min before conducting the PAGE. The SSCP analysis was done using vertical electrophoresis (Hoefer, USA). Denatured amplicons were loaded on 12% PAGE gel (6 ml of 30% Acrylamide / Bis-acrylamide (29:1), 10X TBE (1.5 ml), N, N, N2 , N2 , Tetra Methyl Ethylene Diamine (0.015 ml) and 10% Ammonium per sulphate (0.0832 ml) in nuclease-free water of 7.32 ml) in 1xTBE buffer with voltages of 9, 11 and 15 at respective running times of 90, 120 and 45 minutes for various PCR products. The gel was stained by a silver staining method (Sanguinetti and Simpson, 1994). The PCR products from different SSCP patterns were sequenced in forward and reverse directions using a commercial service (SciGenom Labs Pvt. Ltd. Cochin). Nucleotide sequence alignments and comparisons were carried out using reference sequence (NC_051799. 1:c6089943-6085452) in GenBank using BLASTn and EMBOSS merger. The NCBI (National Centre for Biotechnology Information) Blast algorithm was used to search the NCBI GenBank database (http:/www.ncbi.nlm.nih.gov/) for homologous sequences.

Genotyping of the *dGH* exon 3 and partial intron 3 (227 bp) locus

The exon 3 and partial intron 3 region was genotyped using DNA pooling sequencing and High-Resolution Melt curve (HRM) analysis. For this, the amplicons of exon 3 and partial intron 3 (227 bp) were screened for identifying the SNPs using DNA pooling sequencing assay (Bansal *et al.,*2002). A DNA pool containing PCR amplicons of four microliter of each sample was prepared and sequenced using the respective forward and reverse primers to detect the variations, if any, at nucleotide level by an automated sequencer at Agrigenome Labs Pvt. Ltd. Cochin using Sanger's dideoxy chain termination method. The obtained sequences were aligned with other sequences in GenBank

using Sequence Manipulation Suit (SMS), BLASTn (http://www.ncbi.nlm.nih.gov/blast), Clustal Omega and EMBOSS merger (http://emboss. bioinformatics.nl/cgi-bin/emboss/merger). The presence of SNP was confirmed only after comparing with the reference sequence using BLASTn (GenBankNC_051799.1:c6089943-6085452). The genotyping of the detected SNP was carried out using high resolution melt curve analysis (HRM) in Eco Real-Time PCR system (Illumina). The primers (HRME3F and HRME3R) were designed to amplify the regions flanking the SNP (139 bp product). The reaction consisted of 10 µL total reaction volume with 0.5 µL of genomic DNA (50 ng/µl), 5 µL Sso Fast EVA green supermix, 0.5 µL of forward primer (10 pM/µl), 0.5 µL of reverse primer (10 pM/µl) and nuclease free water added to make up a final volume of 10µl. Thermal profile of reactions included 95°C for 10 min, 95°C for 30s, 65.4°C for 30s, 72°C for 30s followed by the melt curve analysis. The results were confirmed by sequencing the representative samples from each genotype after their detection through melting temperature (Tm) shift and the melt curve patterns.

Statistical analysis

Allelic and genotypic frequencies for each locus were calculated by the standard procedure (Falconer and Mackay, 1996). The observed (H0) and expected (He) heterozygosity at the loci and chi-square test for Hardy-Weinberg equilibrium were estimated using Pop Gene 3.1 software. The following General Linear Model for fixed effects was used to analyze the association of *GH* variants with body weight at fortnightly intervals using SPSS (V.21). Duncan multiple range test (Kramer, 1957) was used to compare the means. The model used was:

$$y_{ij} = \mu + g_i + e_{ij}$$

Where,

y_{ij}= body weight measured on ijth bird at different stages;

ì= Overall mean.

gi= Fixed effect associated with ith genotype (i = 1, 2,3);

eij= Random error.

Least squares means and their standard errors were computed for all the genotype effects using SPSS V.21.

RESULTS AND DISCUSSION

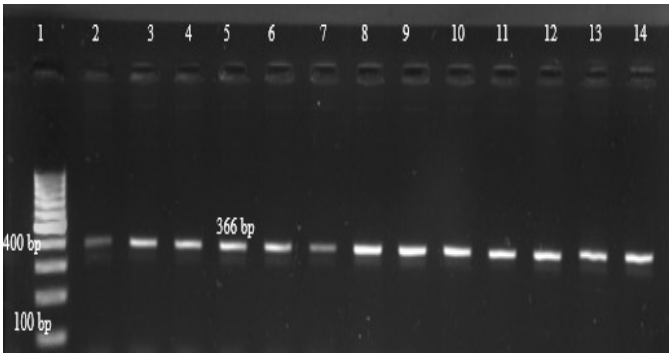
PCR-SSCP analysis of *dGH* exon 5 and partial intron 4 (366 bp) locus

The PCR- SSCP analysis of exon 5 and partial intron 4 (366 bp) of duck GH gene exhibited two typical banding patterns suggesting the Kuttanad population to be polymorphic for the locus (Fig 1 to 3). Representative PCR products from each type were sequenced to confirm the presence of a novel SNP at the 74th position (G>A) of the 366 bp *dGH* exon 5 and partial intron 4 (Fig 6). Two genotypes *viz*., GG and GA were recognized in the population with alleles, G and A. The AA genotype was not found in the population and this could be due to genetic drift operating in small populations. The polymorphic pattern detected at the locus was in accordance with the reports in Chinese Tsaiya ducks (Chang *et al.,* 2012) and Indonesian Bayang ducks (Yurnalis *et al.,* 2019).

On estimation of population indices for the locus, it was found that GG genotype (0.61) was more predominant than GA genotype (0.39). The frequencies of G and A alleles were 0.81 and 0.19, respectively. The observed and expected heterozygosity were 0.38 and 0.31, respectively. Chi-square analysis revealed that there was no significant difference between the observed and expected heterozygosity ($p \geq 0.05$). Hence, the Kuttanad population was found to be in Hardy-Weinberg equilibrium for the 366 bp (exon 5 and partial intron 4) polymorphic locus of *GH* gene.

Sequence analysis of *dGH* exon 3 and partial intron 3 (227 bp) locus

Sanger sequencing of pooled amplicons of 227 bp fragment (exon 3 and partial intron 3) of *dGH* revealed a novel SNP at the 190th position (A>G) (Fig 4). Primers were designed to produce a 139 bp amplicon from the 227 fragment such that the identified SNP, g.190A>G was at the 71st position on the 139 bp amplicon, making it suitable for an HRM



Lane 1: 100 bp DNA marker;Lane 2-14: 366 bp product

Fig 1: PCR amplification of *dGH* exon 5 and partial intron 4 (366 bp).

analysis. The genotyping of the novel SNP by HRM technique revealed three distinct melt curves corresponding to three genotypes *viz*., AA, AG and GG at the 227 bp locus in the native ducks under study (Fig 5). The results were confirmed by sequencing of the representative samples of each genotype (Fig 6). The polymorphic nature of the locus was in accordance with the reports in Chinese Tsaiya ducks (Chang *et al.,* 2012), Muscovy ducks (Ismoyowati *et al.,* 2017) and Indonesian Bayang ducks (Yurnalis *et al.,* 2019).

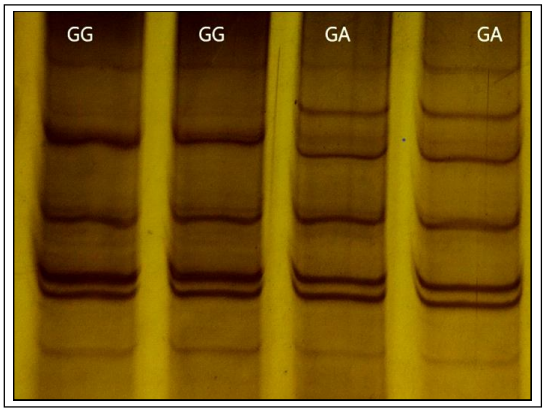


Fig 2: SSCP pattern of *dGH* exon 5 and partial intron 4 (366 bp).

The population indices revealed that the AG genotype was more predominant (0.37) than the other genotypes AA (0.27) and GG (0.36). The allelic frequencies of A and G were 0.46 and 0.54 respectively indicating G to be more predominant than A. The observed and expected heterozygosity were 0.38 and 0.50 respectively. Chi-square test revealed that there was no significant difference between the observed and expected heterozygosity ($p \geq 0.05$) and hence the population was in Hardy-Weinberg equilibrium for the exon 3 and partial intron 3 locus.

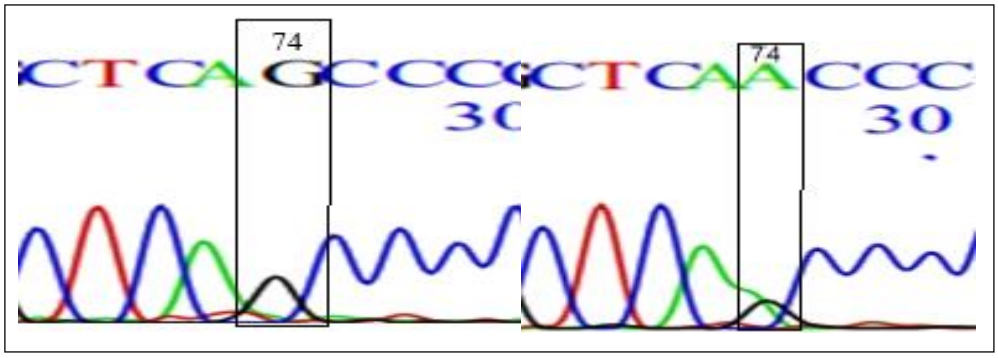


Fig 3: Chromatogram of GG and GA genotype of *dGH* exon 5 and partial intron 4 (366 bp).

Table 1: Least squares means with standard error of body weight (g) for *GH* exon 5 and partial intron 4 (366 bp) genotypes.

	BW0	BW2	BW4	BW6	BW8	BW10	BW12	BW14	BW16
GG(62)	36.84^{a}±	247.31^{a}±	526.63^{a}±	849.48^{a}±	1137.15^{a}±	1328.15^{a}±	1469.31^{a}±	1562.02^{a}±	1638.23^{a}±
	0.30	5.05	10.87	21.87	18.69	20.73	22.44	20.67	18.46
GA(39)	36.06^{a}±	214.92^{b}±	387.13b±	602.02b±	897.38b±	1157.41b±	1349.64b±	1478.21b±	1595.64^{a}±
	0.47	8.01	15.35	28.20	33.71	27.80	24.80	27.84	24.84

Means with different superscripts within each column differ significantly ($p \leq 0.01$).
Figures in parenthesis represent the number of observations.

Table 2: Least squares means with standard error for body weight (g) at different stages for *dGH* exon 3 and partial intron 3 (139 bp) genotypes.

	BW0	BW2	BW4	BW6	BW8	BW10	BW12	BW14	BW16
GG(36)	37.16^{a}±	237.14^{a}±	480.14^{a}±	851.91^{a}±	1070.94^{a}±	1282.14^{a}±	1471.11^{a}±	1584.44^{a}±	1683.61^{a}±
	0.51	6.29	19.03	37.28	36.89	30.71	29.17	26.09	20.19
AA(27)	36.98^{a} ±	226.56^{a}±	456.70^{a}±	678.15b±	1026.81^{a}±	1249.15^{a}±	1390.37^{a}±	1527.41^{a}b±	1625.19^{a}±
	0.49	9.04	26.23	41.15	42.73	29.85	22.05	24.04	23.56
AG(38)	35.64^{a}±	238.45^{a}±	477.18^{a}±	717.61b±	1033.29^{a}±	1252.63^{a}±	1400.87^{a}±	1479.34b±	1560.79b±
	0.50	8.71	14.91	31.30	31.04	34.06	33.88	32.26	27.51

Means with different superscripts within each column differ significantly ($p \leq 0.01$).
Figures in parenthesis represent the number of observations.

Association of *dGH* exon 5 and partial intron 4 (366 bp) genotypes with body weight

The two variants identified in *GH* 366 bp fragment (exon 5 and partial intron 4) *viz*., GG and GA, differed significantly ($p<0.01$) for fortnightly body weight at all stages upto 14 weeks of age, except at hatch (Table 1). The GG homozygotes were significantly heavier than GA homozygotes ($p\leq0.01$). The results indicated that the SNP, g.74G>A identified in the 366 bp GH exon 5 and partial intron 4 locus contributed to the variation in all except hatch weight indicating the locus to exert a marked influence on body weight at all stages in Kuttanad ducks. The results are contrary to the report of monomorphic nature of *GH* intron 4 in Pekin ducks (Eris and Elmaci, 2022).

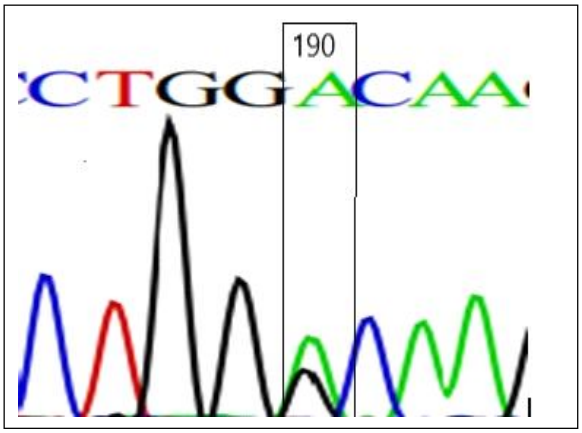


Fig 4: Sequence map of pooled amplicons of *dGH* exon 3 and partial intron 3 (227 bp).

Association of *dGH* exon 3 and partial intron 3 (139 bp) genotypes with body weight

The three 139 bp *dGH* exon 3 and partial intron 3 variants identified *viz*., GG, AA and AG differed significantly ($p<0.01$) for body weight at six (BW6), 14 (BW14) and 16 (BW16) weeks of age in the duck population under study (Table 2). The GG homozygotes were found to be heavier at these stages than AA and AG genotypes ($p<0.01$). The results indicated that the SNP g.71A/G in the GH exon 3 and partial intron 3 locus and its significant association with early (BW6) as well as pre-pullet or finisher body weights (BW14, BW16) has contributed to the variation in early and finisher growth at 6, 14 and 16 weeks of age in Kuttanad ducks and hence exerts a marked influence on these economic traits. The finding is in agreement with the polymorphisms reported in *dGH* promoter, exon 3 and intron 1 regions to be associated with body weight from 4 to 8 weeks of age in Pitalah -

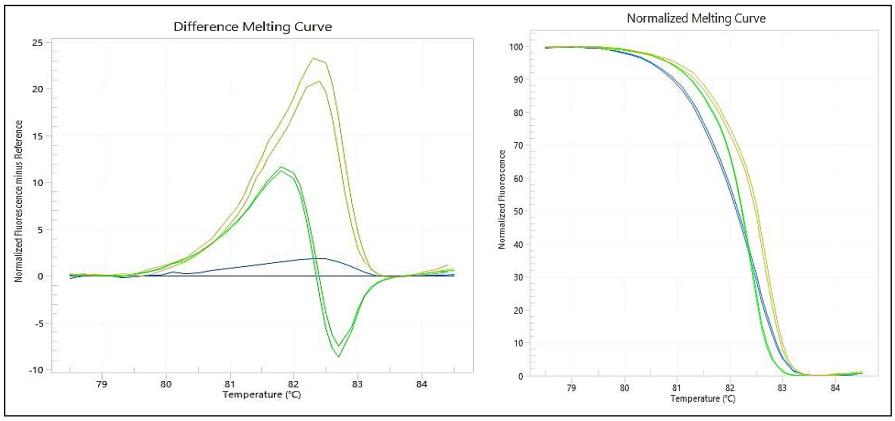


Fig 5: Difference melting curve (left); Normalized melting curve (right) for 139 bp locus with AA (yellow); GG (blue) and AG (green) genotypes.

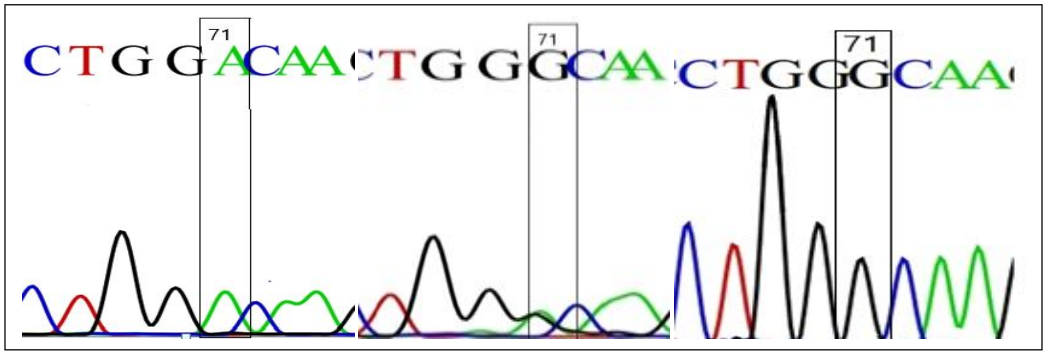


Fig 6: Chromatogram of *dGH* exon 3 and partial intron 3 (139 bp) AA, AG and GG genotypes.

Kumbang Janti ducks (Yurnalis *et al.,* 2017) as well as the Bayang ducks (Yurnalis *et al.,* 2019).

CONCLUSION

The study in overall revealed that the 227 bp and 366 bp loci of duck growth hormone gene were polymorphic in Kuttanad ducks. The *dGH* exon 5 and partial intron 4 (366 bp) also revealed one novel SNP *viz*., g.74G>A with GG and GA genotypes in the population. The 139 bp HRM fragment of *dGH* exon 3 and partial intron 3 (227 bp) revealed one novel SNP g.71A>G, yielding AA, AG and GG genotypes. The GG homozygotes at both the loci were found to have significantly higher body weight than all other genotypes at crucial stages of growth ($p \leq 0.01$). The two exonic loci and their partial introns being polymorphic appeared to have jointly contributed to the variation in growth at the early and finisher stages of six, 14 and 16 weeks of age in the population. The SNPs identified *viz*., g.71A>G and g.74G>A, on account of their marked influence on the growth of ducklings at these grower and pre-pullet stages can be used as potential candidate markers for the primary selection of grower ducks at six weeks of age and later for the secondary selection of pullets at 14-16 weeks of age in the multi-stage duck selection programmes.

Conflict of interest

The authors declare no potential conflicts of interest concerning the research, authorship and/or publication of this article.

REFERENCES

Anonymous (2019). 20th Livestock Census-2019. Department of animal husbandry, dairying and fisheries. Ministry of Agriculture and Farmers Welfare. Government of India. New Delhi. dahd.nic.in/sites/default/filess/20thLivestock Census2019AllIndiaReport.pdf.

Asmaa, W.Z., Fardos, A.M.H., Elshimaa, M.R. and Amir, H.A. (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: 41-46.

Bansal, A., van den Boom, D., Kammerer, S., Honisch, C., Adam, G., Cantor, C.R., Kleyn, P. and Braun, A. (2002). Association Testing by DNA Pooling: An Effective Initial Screen. In: Proceedings of the National Academy of Sciences of USA. 99: 16871-16874.

Bhavana, K., Foote, D.J., Srikanth, K., Christopher, N.B., Vandana, R.P., Sankaralingam, S., Hijam, S.S., Gopalakrishnan, A. and Nagarajan, M. (2022). Comparative transcriptome analysis of Indian domestic ducks reveals candidate genes associated with egg production. Science Reports. 12: 943- 949.

Bindya, L.A. (2021). Duck Production and Management in India. In: Poultry in India (Year book 2021). Mahi Goswami, White Wing Media, Ghaziabad. pp. 940.

Bindya, L.A. and Priya, M. (2020). Genetic variability analysis of duck-PRL exon 5 in Kuttanad ducks of environmentally challenged breeding tracts. Environment and Ecology. 39: 309-313.

Chang, M.T., Cheng, Y.S. and Huang, M.C. (2012). The SNP genotypes of growth hormone gene associated with reproductive traits in Tsaiya ducks. Reproduction in Domestic Animals. 47: 568-573.

Cyriac, S., Joseph L., Anitha P. and Girbin G.T. (2020). Effect of individual selection on growth and carcass characteristics in Kuttanad ducks (*Anas platyrhynchos domesticus*). Indian Journal of Animal Research. 54: 1578-1583. doi: 10.18805/ijar.B-3910.

Deka, R.J., Zakir, A.M.M. and Kayastha, R.B. (2019). Improvement of rural livelihood through rearing of Chara-Chemballi ducks in Assam. World's Poultry Science Journal 70: 397-404.

El-Araby, I.E., Awad, A.S., Saleh, A.A., Abd El-Fattah, A.H. and Tahoun, M.M. (2020). Growth hormone gene polymorphisms in mature healthy male Muscovy ducks in Egypt. Zagazig Veterinary Journal. 48: 79-87.

Eris, C. and Elmaci, C. (2022). Growth hormone gene polymorphism in Pekin ducks reared in Turkey. Journal of Tekirdag Agricultural Faculty 19: 798-806.

Falconer, D.S. and Mackay, T.F.C. (1996). Introduction to Quantitative Genetics, (4th Edn.) Longmans Green, Essex, England. pp 464.

Ismoyowati, Tugiyanti, E., Mufti, M. and Purwantini, D. (2017). Sexual dimorphism and identification of single nucleotide polymorphism of growth hormone gene in Muscovy duck. Journal of the Indonesian Tropical Animal Agriculture. 42: 167-174.

Kamal, R., Chandran, P.C., Dey, A., Sarma, K., Padhi, M.K., Giri, S.C., Bhatt, B.P. (2023). Status of indigenous duck and duck production system in India- A review. Tropical Animal Health and Production 55: 15.

Kansaku, N., Hiyama, G., Sasanami, T. and Zadworny, D. (2008). Prolactin and growth hormone in birds: Protein structure, gene structure and genetic variation. Journal of Poultry Science. 45: 1-6.

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

Kramer, C.Y. (1957). Extension of multiple range tests to group corrected adjusted means. Biometrics. 13: 13-18.

Mahanta, J.D., Raj, J., Deka, Sapcota, D. and Jalaludeen, A. (2009). Certain Performance Traits of Chara-Chemballi Ducks of Kerala under Range Conditions in Assam. In: Proceedings of IV World Waterfowl Conference. 11-13. Thrissur, Kerala. pp. 112-114.

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

Purabi, K., Jyanashree, S., Kalita, K., Deka, R. and Sahariya, J. (2021). Genetic characterization of indigenous ducks of North-eastern region using microsatellite markers. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-4673.

Sanguinetti, C.J., Dias, E.N. and Simpson, A.J. (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques 17: 914-921.

Senani, S., Jalaludeen, A., Rai, B.R., Jaisunder, Kumar, S.J., Chatterjee, R.N., Kundu, A. and Bhagat, D. (2005). Performance evaluation of Chara-Chemballi ducks of Kerala in the Andaman Islands. Livestock international. 8: 21-23.

Veeramani, P., Prabakaran, R., Sivaselvam, S.N., Sivakumar, T., Selvan, S.T. and Karthikeyan, S.M.K. (2016). Phylogenetic analysis of six duck populations in India. Indian Journal of Animal Research. 50: 626-628. doi: 10.18805/ijar.9301.

Veeramani, P., Prabakaran, R., Sivaselvam, S.N., Sivakumar, T., Selvan, S.T. and Karthikeyan, S.M.K. (2023). Genetic diversity of six duck populations in India. Indian Journal of Animal Research. 57: 1113-1119. doi: 10.18805/IJAR.B-4379.

Wu, X., Yan, M.J., Lian, S.Y., Liu, X.T. 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, A.L., Pi, J.S., Pu, Y.J., Du, J.P., Liang, Z.H. 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: 8027-8033.

Yurnalis, Arnim, Putra, D.E., Kamsa, Z. and Afriani, T. (2019). Identification of *GH* gene polymorphisms and their association with body weight in Bayang duck, local duck from West Sumatra, Indonesia. In: Proceedings of 6th International Conference on Sustainable Agriculture, Food and Energy (SAFE2018); IOP conference series: Earth and Environmental Sciences. 347(1): 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: 203-208.