GENETIC POLYMORPHISM IN THE EXON 5 OF THE PROLACTIN GENE IN TWO DUCK BREEDS ADAPTED TO NIGERIA

OH Osaiyuwu[1], IM Egunjobi[2], LO Igbatigbi[3]
[1,2]Animal Breeding and Genetics, Department of Animal Science, University of Ibadan, Ibadan, Nigeria
[3]Department of Zoology, Federal University Lokoja, Kogi state Nigeria.

Corresponding author: LO Igbatigbi, Lydia.igbatigbifulokoja.edu.ng

ABSTRACT

Purpose:
This study aimed at determining genetic variants of prolactin gene in the Muscovy and Mallard ducks in Nigeria, and to evaluate the genetic relatedness or diversity of the duck species at the exon 5 of Prolactin (PRL) gene loci in the two selected Duck breeds, by determining the genetic polymorphisms, genetic parameters and genetic variation in the duck breeds. 80 adult ducks sourced from Lagos, Ogun and Oyo states of Nigeria, were used for this study.

Method:
Blood samples (2ml) collected by jugular venipuncture from the ducks, using sterile needles and syringes, were stored in ethylene diamine tetra acetic acid (EDTA) bottles, to prevent coagulation and preserve the integrity of the samples, were transported in ice packs, to the laboratory for analysis. Genomic DNA was extracted from whole blood samples, amplified using PCR were digested using *HinfI* Restriction Enzyme. The data were analyzed using POPGENE 1.32 software.

Results:
Two alleles with frequencies A (0.1034, 0.2188), B (0.8966, 0.7812), and three genotypes with frequencies AA (0.0000, 1.0000), AB (6.0000, 5.0000) and BB (23.000, 10.0000) were identified at the Exon 5 of of PRL gene in Mallard and Muscovy ducks respectively. Indicating that prolactin gene was polymorphic in both populations. The Chi-square test (0.367, 0.2381) suggests that the populations conform to Hardy-Weinberg equilibrium. Also, noted were observed heterozygosity (0.2069, 0.3125) and expected heterozygosity (0.1887, 0.3528).

Conclusion:
The sampled populations were polymorphic for PRL gene at exon 5, and are also in Hardy–Weinberg equilibrium, based on the observed parameters and statistical analysis. The values of observed heterozygosity and expected heterozygosity, indicates higher genetic variation within the Mallard breed population, compared to the Muscovy population, which can be attributed to inbreeding. This indicates the need for Genetic intervention to prevent this indigenous breed of ducks from extinction.

INTRODUCTION

Ducks are waterfowls that are commonly raised in regions of high rainfalls, deltas, riverine areas and coastal district of the tropics. They belong to the group of livestock comprising of chickens, ducks, turkeys, guinea fowls and pigeons. These species are generally referred to as indigenous or local poultry species. The genetic resources of these local poultry species are mainly represented by, domestic local chicken (*Gallus gallus domesticus*), guinea fowl (*Numida meleagris*), and ducks (C*arina spp.*) (Youssao *et al.,* 2010). Ducks are very common and prominent in Asia, Europe, North America and South America, but they are one of the neglected or underutilized poultry species in Africa and Nigeria in particular, this is regardless of their innate potentials for meat and egg production, as well as their adaptability to different climatic conditions (Oguntunji, 2013). The world's duck population has been placed at 1.24 trillion, with Asia continent possessing the highest number (Ismoyowati and Sumarmono, 2019). Likewise, Nigeria's duck population, has been estimated to be about 9,553,911(NBS, 2012) and they are spread across all the agro-ecological zones of Nigeria (Oguntunji and Ayorinde, 2015).

Duck farming and consumption is not as widespread in Nigeria, in comparison to other poultry species like chicken and turkey. This may be due to many factors such as its unpleasant and unattractive appearance, marketability, taboos and dirtiness associated with its bathing and scavenging habits (Ola, 2000). The main duck species used for production of duck meat are the Mallard duck (*Anas platyrhynchos*) also known as the common duck, the Muscovy duck (*Cairina Moschata*) and the hybrids from the cross breeding of Muscovy drakes with Mallard ducks called Mulards or more commonly Mules (Baeza, 2006). However, due to their good adaptation to rearing conditions, Mallards are most times reared in place of Muscovy ducks in very popular farms in Europe. As a result of genetic improvement in Mallard ducks, they have exhibited better performance than broilers, in terms of their weight gain and feed efficiency (Adzitey and Adzitey, 2011), this has been achieved through the application of modern methods of molecular genetics, by identifying candidate genes associated with quantitative traits, to improve productive traits and enhance breeding programs (Basumatary *et al.,* 2019). Genetic improvement programs for meat-type ducks have successfully enhanced the productive performance of these ducks.

The Prolactin gene (PRL) is a single-chain polypeptide which belongs to the family of growth hormone genes, and it is synthesized by the anterior pituitary gland of poultry (Wang *et al.,* 2011). The expressed product of this gene is the prolactin hormone, which is a multifunctional hormone involved in the control of lots of physiological processes in vertebrates. Prolactin hormone combines with its receptor (prolactin receptor, (PRLR)) to act on target cells. Both PRL and PRLR are mainly associated with reproductive performance. For instance, in poultry species, prolactin is a very crucial hormone in induction and maintenance of incubation behaviour, as well as the regulation of the follicular development (Wang *et al.*, 2011).

Alaraji, F (2024) also observed that the prolactin hormone is involved in egg formation. However, this 'maternity hormone' has diverse functions not only in reproductive traits of birds but also growth and development of mammals to include osmoregulation, reproduction, immune responses, water, and electrolyte balance, promoting the development of breast gland, inducing lactation, maintenance of pregnancy, impelling the development of embryos and cellular proliferation in vertebrates and mammals (Li-Yuan, 2002; Byrnes and Bridges, 2005).

Studies have shown that, The *PRL* gene is associated with the reproductive traits of ducks, it is a very important gene important in egg production, including egg shell strength, and egg weight, all influenced by the exon 2, 4, and 5, respectively (Wang *et al*., 2011). As an agriculture poultry species, duck egg is fast becoming a major source of protein in human diet, but the egg performance of some native duck breeds needs to be improved. Identification of the genetic forms or polymorphisms of PRL gene can be employed as genetic markers, for the selection of genetically superior ducks to increase productivity. (Oyebanjo *et al.,* 2023). Therefore, this study is aimed at determining genetic variants of the prolactin gene in the Muscovy, and Mallard ducks in Nigeria and to evaluate the genetic relatedness or diversity of the duck species at the prolactin gene loci.

MATERIALS AND METHODS

Experimental birds

80 adult ducks (40 Mallard and 40 Muscovy breeds) were used for this experiment. Samples were collected only from location where these duck breeds were reared. These ducks were sourced from Lagos, Ijebu-Ode, and Ibadan all in South-West Nigeria. 2ml of blood samples were collected by jugular venipuncture from each of the 80 ducks with the assistance of a state-certified veterinary doctor, with the use of sterile needles and syringes for each bird. The blood samples were immediately stored in ethylene diamine tetra acetic acid (EDTA) bottles, to prevent coagulation and to preserve the integrity of the blood samples until DNA extraction. The samples were transported in icepacks from the field to the lab where DNA extraction was carried out.

DNA extraction

Genomic DNA was extracted using the JENA Bioscience Blood DNA Preparation Column Kit following the manufacturer's protocol. Samples were prepared by mixing 200µl of the blood samples with 1ml of the Blood Lysis buffer in an Eppendorf microtube, and the mixture was incubated on ice for 10min making sure that the tube was vortexed intermittently two to three times during incubation. The mixture was then centrifuged at 10,000g for 10min to pellet the white blood cells, and the supernatant was disposed after centrifuging. 300µl of the Lysis buffer and 2µl of the Rnase A were added to the white blood cell pellets and the tube was vortexed vigorously. Incubation was done for 5min at 60°C and 8µl Proteinase K was added to the tube and mixed by pipetting. Another incubation was done for 10 min at 60°C, then tube content was allowed to cool to room temperature. 25 300µl of Binding Buffer was added to the tube, the contents were mixed by inversion, then the tube was placed on ice to cool. Afterwards, the tube was centrifuged for 5min at 10,00g and the supernatant was transferred into a new Eppendorf tube. 500µl Ethanol was added to the tube. The contents were mixed by continuous pipetting. A spin column was inserted into a 2ml Collection Tube, 100µl activation buffer was added to the spin column and was centrifuged alongside the collection tube at 10,000g for 30 seconds. The flow-through was discarded and 600µl of the supernatant from the DNA Binding process was pipetted directly into the spin column. It was centrifuged for another 1 min at 10,000g and the resulting flow-through was discarded. 500µl wash buffer was added into the spin column and spined for 30sec at 10,000g and the resulting flow-through was again discarded. Residual wash buffer was removed by centrifuging the spin column for 2 minutes at 10,000g and the 2ml collection tube was discarded. The spin column was placed into a new eppendorf tube. Elution of DNA was done by adding 5µl of the elution buffer into the center of the spin column and incubating the spin column at room temperature for 1 min. The spin column was again centrifuged at 10,000g for 1 min and the DNA extract in the Eppendorf tube was stored at -20°C until further analysis.

Polymerase chain reaction (PCR)

The primer sequence used to amplify Exon 5 of PRL Gene Primer sequence were;
(5' → 3') PRL
F- TGCAAACCATAAAAGAAAAGA
R- CAATGAAAAGTGGCAAAGCAA
DNA isolated was amplified using Jena Bioscience Ruby Hot Start mastermix (2x) and each PCR reaction mixture contained 25µl of mastermix (2x), 2µl (10 pmol) of each PRL primers, 2µl of DNA extract, and 19µl sterile nuclease-free water, to make up a total reaction volume of 50µl. The PCR was then performed using an Applied Biosystem 2720 Thermocycler. The reaction mixture was subjected to: Initial denaturation at 94°C for 5 min, Denaturation involving 35 cycles at 94°C for 60sec, Annealing for 30 sec at 54°C, Extension for 2 mins at 72°C; and Final extension for 7 min at 72°C. The reaction mixture containing 10µl of PCR product, 3µl of 10x buffer was digested using 1µl *HinfI* Restriction Enzyme (Jena Bioscience, Germany) and 12µl nuclease free water. The mixture was incubated at 37°C for 10mins and inactivated at 80°C for 20mins.

Visualization of PCR products

The PCR products were visualized on a 2% agarose gel dissolved in 0.5x Tris-borate buffer, stained with maestrosafe, under Blue Light Transilluminator (New England BioGroup, USA). The gel was loaded with a midrange DNA ladder marker (1000bp; Jena Bioscience, Germany) to assess the size of the amplified product.

Statistical analysis

After visualization, the data were analyzed using POPGENE 1.32 software package (Yeh *et al.*, 1999) to estimate the genetic differences. The fragment bands in each sample were determined from the gel pictures as seen on the gel documentation system. The allele and genotype frequencies, observed heterozygosity, Nei- expected heterozygosity and chi square test were determined using POPGENE 1.32 software package (Yeh *et al.,* 1999).

RESULTS AND DISCUSSION

The amplification of the isolated DNA fragments resulted in the generation of 414bp, also two alleles (A and B), and three genotypes (AA, AB and BB) were identified in prolactin gene at the exon 5, in both breeds of duck. This is similar to a study carried out by Sabry *et al*. (2020) who discovered a DNA fragment of 417bp in the exon 5 of the prolactin gene across 5 Egyptian duck breeds. Also, Artur *et al*. (2016), reported a DNA fragment with 400bp in the exon 5 across the Pekin and Mullard breeds of ducks. The variations in these research results may be attributed to differences in the duck breeds studied.

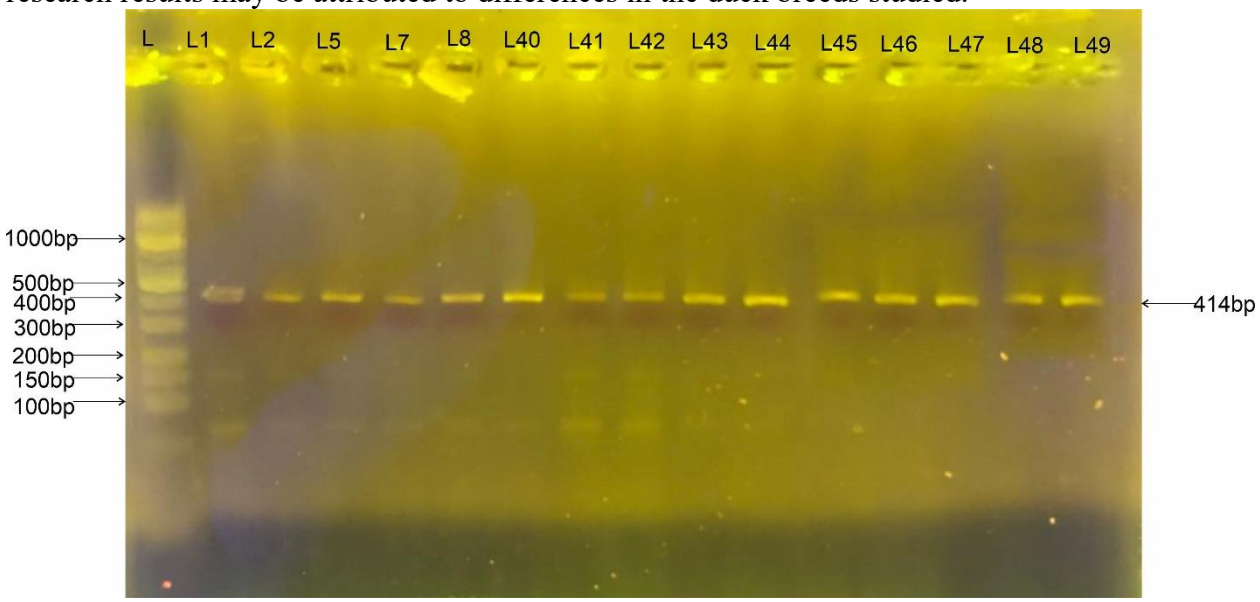

PLATE 1 Mallard PCR product on Gel Electrophoresis

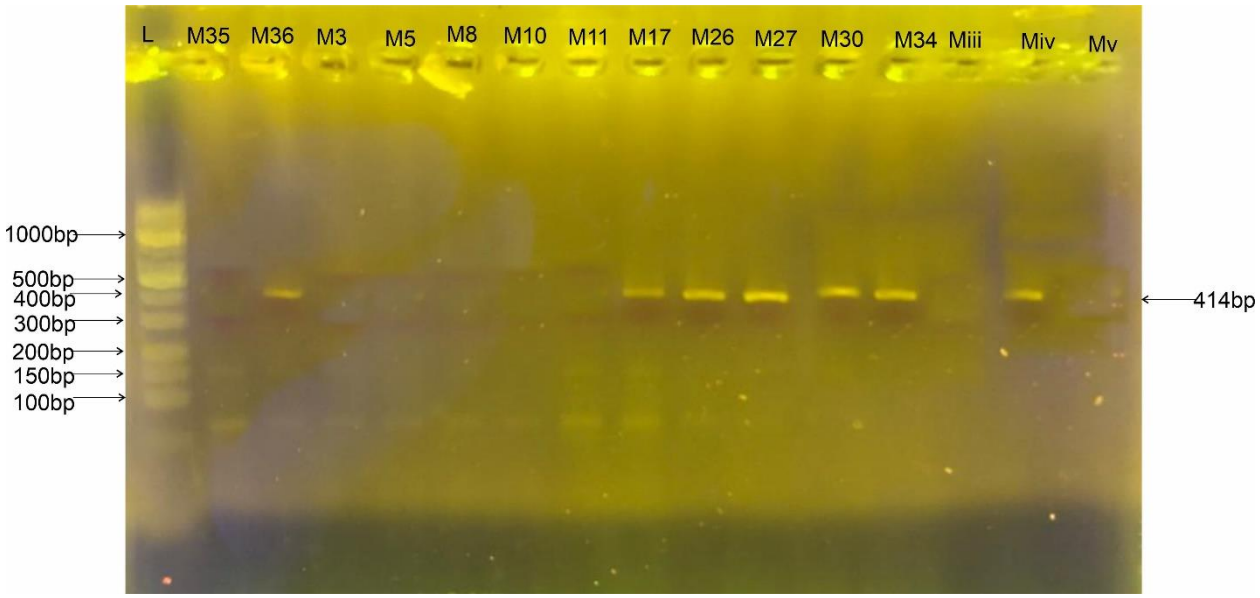

PLATE 2 Muscovy PCR product on Gel Electrophoresis

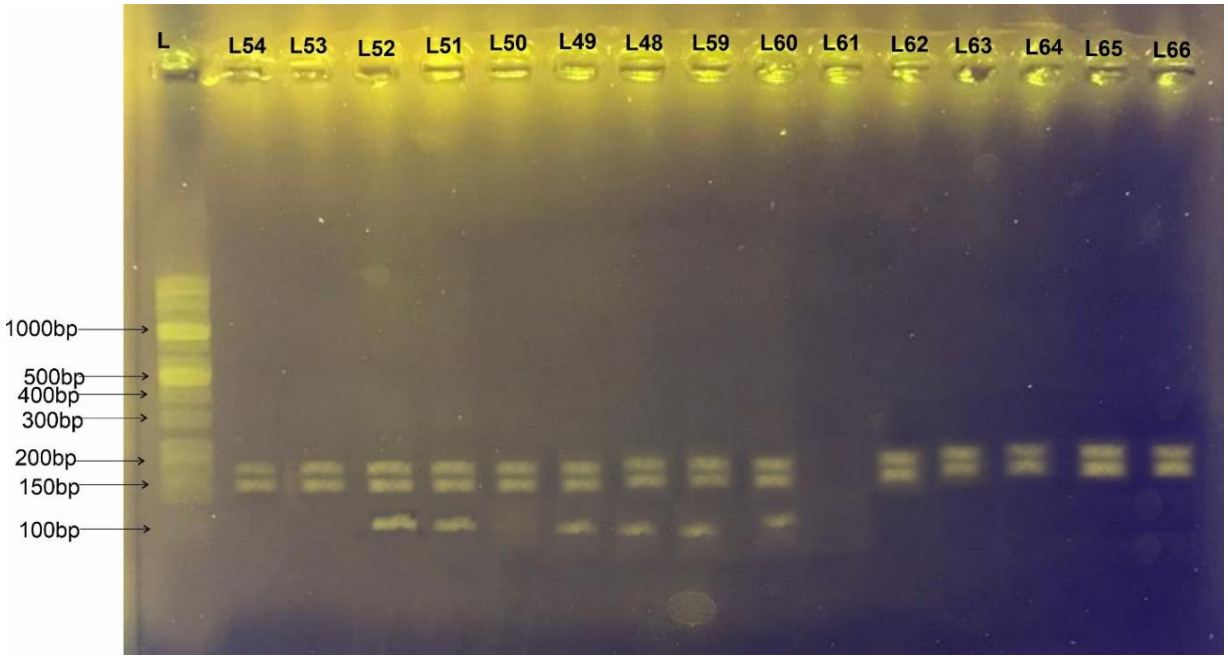

Plate 3 DNA fragment bands of PRL gene of Mallard Duck Breeds obtained by digestion using *Hinf1***.** 1 Band: genotype AA; 2 Bands: genotype BB; 3 Bands: genotype AB.

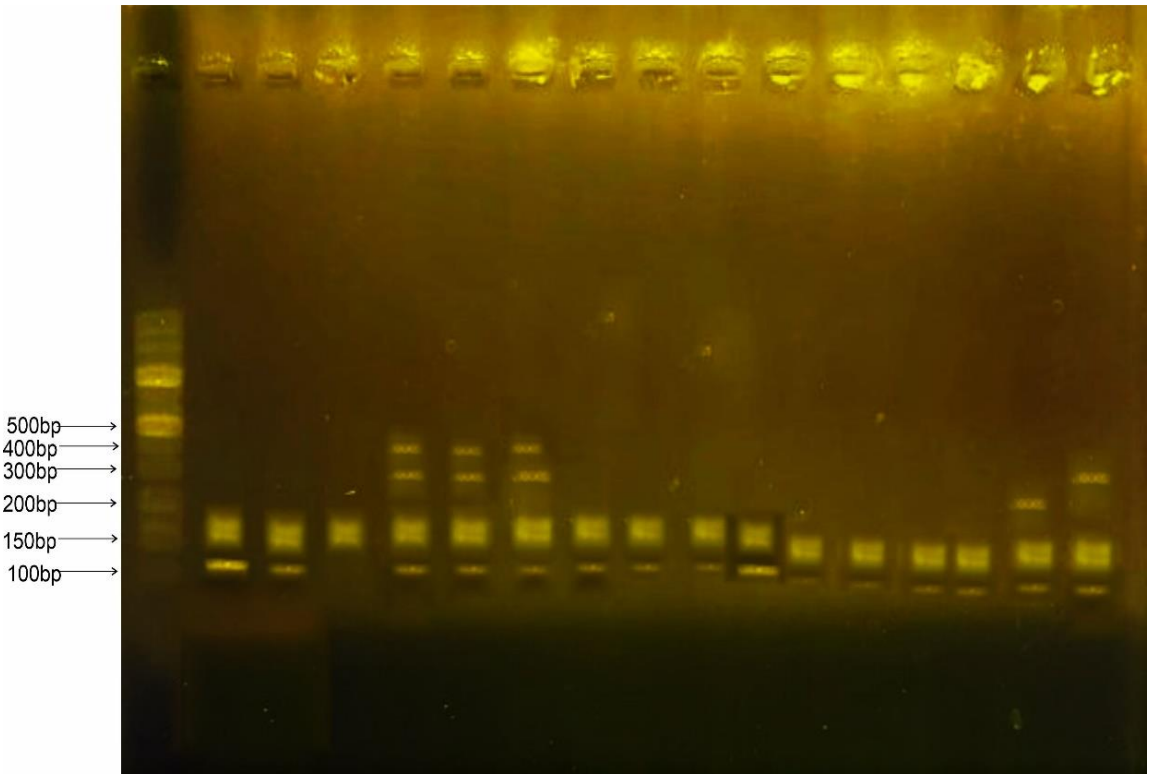

Plate 4 DNA fragment bands of PRL gene of Muscovy Duck Breeds obtained by digestion using *Hinf1***.** 1 Band: genotype AA; 2 Bands: genotype BB; 3 Bands: genotype AB.

The estimation of the prolactin gene's allele frequency, genotype frequency and heterozygosity revealed a higher frequency for allele B (0.8966, 0.7812) than allele A (0.1034, 0.2188), in Mallard and Muscovy ducks respectively. And the most prevalent genotypes were AB (6.0000, 5.0000) and BB (23.000, 10.0000) in Mallard and Muscovy ducks respectively, indicating that prolactin gene was polymorphic in both populations. According to Hartl and Clark (2000), if an allele has a frequency of 0.99 or below, then it can be referred to as polymorphic. The observed number of alleles which is the actual number of alleles found in the population for the Mallard and Muscovy populations were both 2.0000, while the effective number of alleles which is the number of equally frequent alleles that it would take to achieve the same expected heterozygosity as in the studied population for the Mallard and Muscovy populations were 1.2277 and

1.5193 respectively. A chi-squared test ($\chi 2$) was carried out to see if the selected duck breed populations were in the Hardy–Weinberg equilibrium. The chi square value for Hardy Weinberg equilibrium implies that the PRL gene locus in the selected populations did not deviate from the Hardy-Weinberg hypothesized population and is said to be at equilibrium.

The values of observed heterozygosity and expected heterozygosity, showed that the observed heterozygosity in the mallard population was higher than the expected heterozygosity, implying that there is high genetic variation, within the population which may be due to the mixing of two previously isolated populations (Jemmali, 2018). However, in the Muscovy population, the value of observed heterozygosity was lower than the value of expected heterozygosity, indicating low genetic variability, and this can be attributed to inbreeding (Jemmali, 2018). Hoffmann *et al.* (2021), also confirmed that when expected heterozygosity is higher than observed heterozygosity, it depicts the presence of inbreeding. This observed inbreeding is an important parameter that indicates a need for genetic intervention in threatened species (Ralls *et al.,* 2018).

Table 1.0. Allele/Genotype Frequency of *PRL* Gene in Selected Duck Breeds

Duck breed	Allelic frequency		Genotypic frequency		
	A	B	AA	AB	BB
Mallard	0.1034	0.8966	0.0000	6.0000	23.0000
Muscovy	0.2188	0.7812	1.0000	5.0000	10.0000

Table 2 Heterozygosity statistics of *PRL* gene in Muscovy and Mallard ducks

Breed	Obs_Hom	Exp_Hom	Obs_Het	Exp_Het	Ave_Het	Nei	x^2
Mallard	0.7931	0.8113	0.2069	0.1887	0.2636	0.1855	0.3167
Muscovy	0.6875	0.6472	0.3125	0.3528	0.2636	0.3418	0.2381

CONCLUSION

The results of this study showed that the sampled populations were polymorphic for PRL gene at exon 5, as indicated by the two alleles of the gene found in this region, and the populations are also in Hardy–Weinberg equilibrium. According to observed parameters and statistical analysis, the percentage of polymorphic loci for both populations was found to be 100.00 % which indicates that the PRL in both selected populations is highly polymorphic. With the values of observed heterozygosity and expected heterozygosity, there is high genetic variation within the Mallard breed population, while low genetic variation was observed in the Muscovy population, which may be attributed to forces such as inbreeding. This inbreeding can be an indication to the importance of genetic interventions to protect this indigenous breed of ducks from extinction.

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