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Genetic diversity of Prolactin gene in Japanese quail *(Coturnix Coturnix Japonica)*

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Abstract

This study was carried out to investigate the genetic diversity of prolactin gene (PRL gene) in Japanese quails using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) marker. One hundred and thirty nine quails were sampled for PRL loci analysis. DNA was extracted from the samples. Polymerase chain reaction (PCR) and electrophoresis was used to characterize a 24 base pair (bp) insertion/deletion in a 358 bp PCR product. Test for Hardy-Weinberg Equilibrium were carried out. From the results, two alleles A (0.63) and B (0.37) and three genotypes AA, AB and BB were observed from this locus. Results of the study showed that genetic variability exists in the study population of Japanese quails in Nigeria and its segregation could be assessed for reproductive capacity.
Keywords: Prolactin, diversity, segregation, Japanese quail.

1. Introduction

Domestic quails (*Coturnix coturnix japonica*) have been used by man for various purposes, including meat and egg production [1]. In Nigeria, there is the move toward the production of micro livestock which includes quails. Yet no proper breeding strategy is in place. Prolactin (luteotropic hormone or luteotropin), is a single chain hormone secreted from the anterior pituitary. It influences a number of activities in vertebrates [2]. It plays an essential role in reproduction, metabolism, regulation of the immune system and pancreatic development. Prolactin plays role in crop-sac development and regulates brooding behaviour in birds [3]. It enhances luteinizing hormone-receptors in Leydig cells, resulting in testosterone secretion and hence spermatogenesis [4]. High blood prolactin interferes with the function of the ovaries, causing a decrease in estradiol, which may result in infertility. In the testis, it affects the production of testosterone resulting in decreased sperm production. Prolactin within the normal reference ranges can act as a weak gonadotropin but at the same time suppresses GnRH secretion [5]. Prolactin stimulates the production of oligodendrocyte precursor cells, which multiply into oligodendrocytes, the cells responsible for the formation of myelin coatings on axons in the central nervous system [4]. The use of molecular markers for candidate gene studies like that of prolactin, can expand genetic and ecological information. This is important in structuring breeding and selection programme and relating gene flow to traits of economic importance, as well as strengthening decision making or inference on specific aspects of population structure, to enhance environmental adaptation and maintain genetic diversity [6]. These form the basis for market-associated selection (MAS) [7]. Data from this study can be used as basic input for innovative breeding program to select for reproductive traits associated with prolactin like broodiness and nestling behaviour. Previous studies carried out give indications of the usefulness of PRL-RLFP for candidate gene studies [6,8-11]. However, information on the polymorphism of prolactin gene in Japanese quails in Nigeria is scant. This study was aimed at investigating the variability of Prolactin (PRL) gene in Japanese quail in Nigeria.

2. Materials and Methods

2.1 Sample size and blood collection

Blood samples were randomly collected from 139 Japanese quail birds obtained from a research station in

NAPRI for PRL loci analysis. The samples were collected through jugular venepuncture into 2ml vacutainer treated with K3-Ethylenediaminetetra acetic acid (EDTA) and inverted several times to ensure proper mixing in order to prevent coagulation. Ethical permission was obtained prior to the sampling (see ethics statement below).

2.2 DNA Isolation

Genomic DNA was extracted manually from 200 µl of individual blood samples using a commercial kit Thermo Scientific (GeneJET Whole Blood Genomic DNA Purification Mini kit). The DNA yield was assessed and quantified using Nanodrop ND-1000 UV/Vis Spectrophotometer (Nanodrop Technologies, Inc., DE) and gel electrophoresis on 1% agarose. DNA concentration was adjusted to 50ng/µl.

2.3 PCR and Electrophoresis Procedure

Polymerase Chain Reaction (PCR) was carried out, using the Applied Biosystems Veriti™ Gene Amp® thermocycler and the PCR Master Kit (Thermo Scientific). The kit of master mix consisted of 0.5 U/µl of *Taq*DNA polymerase, 2.5 ul of 10X PCR buffer, 2mM of $MgCl_2$ and 2mM dNTPs (each). Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA solution (50 ng/µl), 1 µl of each primer (5 pmol/µl) and 10.5 µl of deionized water to make up to 25 µl in PCR tubes. The tubes were positioned in the interchangeable blocks of 96 wells of 0.2ml. Amplification of PRL gene fragment (130 or 154 bp, containing the 24 bp indel in a 358 bp) using the thermo-cycler ABI9700 was carried out using primers described by Cui *et al*[8] , PRL-F (5´-TTT AAT ATT GGT GGG TGA AGA GAC A-3´) and PRL-R (5´-ATG CCA CTG ATC CTC GAA AAC TC-3´), with an initial incubation and enzyme activation of 94°C for 5 min; followed by 36 cycles of 30 sec at 94°C, 30 sec at 61°C, and 45 sec at 72°C; and a final extension of 5 min at 72°C. The PCR-products of the 24 bp were run on 6% polyacrylamide gel, staining was done using silver nitrate.

2.4 Statistical Analysis

Basic measures of genetic diversity, such as total number of alleles, allele frequencies and polymorphic information content (PIC), PRL loci informativeness in relation to expected heterozygosity were computed using Powermaker version 3.25. POPGENE version 1.31software [12] was used to determine Hardy-Weinberg Equilibrium and heterozygosity was calculated using GENPOP software version 4.13 [13].

3. Results

A total of 278 alleles were observed at the PRL gene loci. PIC for the population was 0.38. The observed heterozygosity, *Ho* was 0.47. Chi Square result indicated that the populations were not in Hardy–Weinberg equilibrium for this region of the PRL gene. Based on present results two alleles, [insertion (A) or deletion (B)] and three genotypes, AA, AB, and BB were observed in the population. The observed frequencies of alleles (A 0.51) and (0.49 B) and genotypes AA 0.27 (insertion insertion), AB 0.48 (insertion deletion) and BB 0.26 (deletion deletion), for the PRL gene are shown in Table 1. Results of heterozygosity values calculated to determine the level of genetic variation in the population are shown in Table 2.

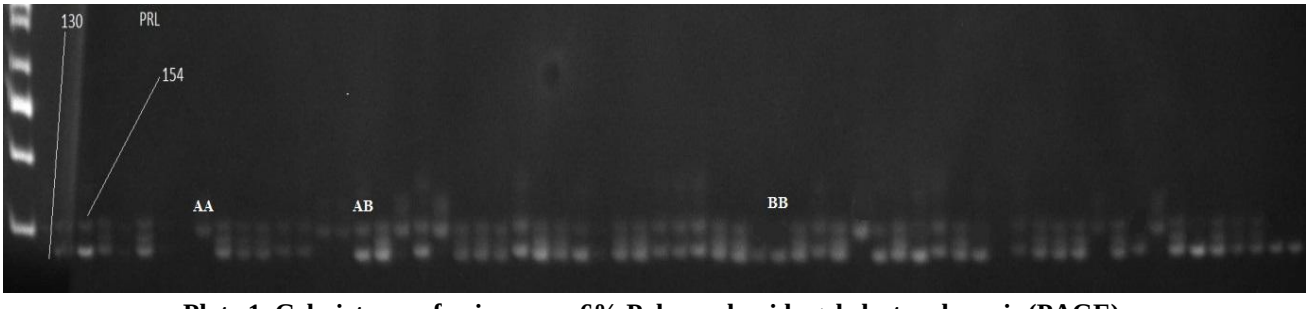

Plate 1. Gel pictures of primers on 6% Polyacrylamide gel electrophoresis (PAGE)

Table 1: Alleles and genotypes frequency in the population

Gene	Allele frequency (%)		Genotype frequency (%)		
	A	B	AA	AB	BB
PRL	0.51	0.49	0.27	0.47	0.26

*A = insertion allele; B = deletion allele; AA = insertion-insertion; AB = insertion-deletion; BB = deletion- deletion.

Table 2: Chi-square test for Hardy-Weinberg equilibrium in the population

Genotypes	Observed (O)	Expected (E)	$(O-E)^2/E$	$\chi 2$
AA	38	35.63	0.16	0.65
AB	65	69.74	0.32	
BB	36	33.63	0.17	

*A = insertion allele; B = deletion allele; AA = insertion-insertion; AB = insertion-deletion; BB = deletion- deletion; χ^2=Chi square

4. Discussion

Prolactin is an important gene for reproduction, it plays roles in crop-sac development, regulates broody behaviour in birds, as well as immune response in various species [14]. Various studies on characterization of the prolactin gene have been reported [8,15-17] .

The efficiency of PRL was determined both as the amount of polymorphism and PIC coefficient. In the study, population PRL marker was reasonably informative at 0.38. However, Kulibaba and Podstreshnyi [1] reported PIC values lower than those obtained in this experiment while Amirinia *et al*[18] recorded PIC values 0.427 in Panda strain and 0.815 in Golden strain in a study using four strains of Japanese quail. The values of PIC reported for chicken vary. Ya-Bo *et al* [19] reported 0.523-0.702 in a study of 19 Chinese native chicken breeds. Yeh *et al*[20] reported values of between 0.560 and 0.641 in a study using 12 indigenous chicken populations in Southern China. Kayang *et al* [21] reported 0.426-0.599 in Turkish native chicken breeds and Davila *et al* [9] reported values ranging from 0.172-0.847 in a study with 13 Spanish chicken breeds population.

4.1 Genotypic and allele frequencies

A total of 278 alleles were identified in the Japanese quail assessed at one Polymerase Chain reaction, Restriction fragment length Polymorphism loci. In this study, two alleles and three genotypes were detected for this locus. Results of the present study are in agreement with the previous report of Yu-Shi *et al* [10] and Rashidi *et al* [11], who reported two alleles (I = 0.52 D = 0.48) in Japanese quail. Gregg *et al* [15] reported two alleles (I=0.76 and D=0.24) in native hens; Alipanah *et al* [16] observed two alleles T and C(0.67 and 0.33) in chickens and Cui *et al* [23] reported in native and commercial chickens respectively. Similar to results of the present study, Ya-Bo *et al* [19] and Lotfi *et al* [24] observed two alleles (I and D) and three genotypes (II, ID and DD) for PRL gene in chicken, duck and Rhine geese respectively. These earlier reports are in line with the results of the present study where two alleles and three genotypes were obtained.

4.2 Hardy-Weinberg Equilibrium (HWE)

Hardy-Weinberg equilibrium test (Chi-square test) for this region of PRL gene was conducted for the populations. The results (Table 2) show that the observed and expected counts were not significantly different from one another. This suggests that for the population the allele and genotype frequencies at the PRL locus are in Herdy-Weinberg equilibrium ($p<0.05$) for this region of PRL gene. This indicates that this particular locus is not changing. The frequency of alleles at just a single locus may change over generations due to evolving population. This change may be as a result of large population size, with less individual migration or presence of more neutral mutations and random mating.

However, this result is not in agreement with previous report by Yu-Shi [10] and Rashidi *et al* [11] on PRL study in Japanese quail. Davila *et al*[9] also reported significant deviations from the Hardy-Weinberg equilibrium on microsatellite study in 64 chicken populations from different continents and some Spanish chicken breeds. Similarly Bao *et al* [22], on microsatellite study of genetic diversity and phylogenetic relationships among red jungle fowls and Chinese domestic fowls reported results different from those obtained in the present study.

5. Conclusion

Results of the present study suggests that polymorphism exist in the population of Japanese quail reared in Nigeria, thus providing a basis for decision making for future conservation, utilization and genetic improvement programmes. This study also provides basic information for future selection programs.

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Competing interests

There are no conflicting interests.

Ethical approval

The author hereby declares that Principles of laboratory animal care were followed, as well as specific national laws where applicable. All experiments were examined and approved by the appropriate ethics committee of the University of Ibadan.

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