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Original Research

Polymorphism of Prolactin (PRL) gene in Native Chicken, 'ZO-AR' of Mizoram, India

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Abstract

Broodiness is a behavioural trait which is observed in most common breeds of domestic and indigenous fowl. The onset and regulation of incubation behaviour is caused by this trait and due to this fundamental role in avian production, it has been of great interest to scientists, producers and breeders. The chicken Prolactin (PRL) gene has been accepted to have crucial effect in egg production as its increase in secretion causes the onset of incubation (broodiness). Therefore, the present study has been aimed to investigate the Single Nucleotide Polymorphisms (SNPs) in the PRL gene of native chicken 'Zo-ar' of Mizoram. DNA extraction followed by PCR-RFLP has been done in 50 blood samples collected from different parts of the state. Two alleles C and T have been detected for the PRL gene in the population. The T allele was found to be predominant in contrast to the C allele with a frequency of 0.66 and 0.34 respectively. The genotype frequencies of CC, CT and TT were found to be 0.06, 0.56 and 0.38 respectively in the studied population. The population conforming to the Hardy-Weinberg equilibrium indicates that no selection pressure was applied for any economic traits to the population in the past.

Keywords: Allele, Broodiness, Incubation Behaviour, Genotype Frequencies, Prolactin, Native Chicken, Single Nucleotide Polymorphism

Introduction

The local chickens of Mizoram are mainly game birds, locally called 'Zo-ar' by the natives and are scavenging in nature. They are reared for both table and game purpose by the local people as the chicken are large in size. The body weight gain and feed conversion ratio of this chicken were found to be higher than Nicobari, Frizzle fowls and Naked neck breeds (Haunshi and Doley, 2011) which are three well known breeds of chicken in India. The egg quality traits, egg shell thickness, shape index and specific gravity of eggs of this chicken has also been found to be superior as compared to Nicobari fowls and some improved varieties of chicken such as Vanaraja and Gramapriya (Haunshi and Doley, 2011). The Hen Day Egg Production % on the other hand was low for the Mizo-local chicken which may be attributable to their persistent broodiness and incubation behaviour due to which the average annual egg production becomes less. The chicken prolactin (PRL) hormone, secreted in the anterior pituitary gland has been proved to be playing crucial role in reproduction and egg production in chicken and other poultry species as has been found to be directly associated with the onset of broodiness, maintenance of broody behaviour, regulation of gonadal functions (Kansaku *et al.*, 2008), production of crop milk, regression of the ovary and cessation of egg production (Sharp 1997). The chicken PRL gene has been therefore extensively studied for genetic variability in order to make selection against broodiness and improve the egg production of domestic fowls. Due to the different biological activities of the PRL gene it can be used as a potent candidate gene in molecular poultry breeding programme for increasing egg production.

Therefore, this study has been aimed to study the different polymorphisms in the PRL gene in the local chicken of Mizoram to find out the diversity that exist within the population and possibility of making genetic improvement thereafter.

Materials and Methods

Animals and Blood Samples

The present investigation was conducted on a total of 50 unrelated '*Zo-ar*' chicken of Mizoram, India. These chickens were randomly selected from backyard reared flocks in three districts viz. Aizawl, Mamit and Kolasib districts of Mizoram. The blood samples were collected aseptically via the wing vein from the randomly chosen chickens. About 1 ml of blood was collected in vacutainer containing EDTA. The samples were kept in ice immediately and cold chain was maintained during the transit of the sample from farm to laboratory after collection and was stored at -20˚C until further use.

Genomic DNA Isolation

Genomic DNA was extracted using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) according to the instruction manual of the kit. The quantity and quality of DNA extracted from the samples were checked using a NanoDropMultiscanGo Spectrophotometer (Thermo Scientific, USA). The primers and restriction enzyme used for PCR-RFLP analysis are given in Table 1.

Table 1: Gene, size of PCR amplified product, primer sequence, annealing temperature for PCR and restriction enzyme used for RFLP analysis

Name of Primers		Primer sequence (5´ - 3´)	RE	Product size (bp)	T^A (°C)	Reference
PRL	F	F: AGAGGCAGCCCAGGCATTTTAC	*Alu*I	439	61	Bagheri *et al.*, 2006
	R	R: CCTGGGTCTGGTTTGGAAATTG				

PCR and RFLP

The PCR amplification was carried in a 25 µl reaction mixture of 10X PCR buffer, 2mM of $Mgcl_2$, 200 µM of each dNTPs, 5 pM of each primers, 2U Taq DNA polymerase and 60 ng genomic DNA. The following PCR cycles were applied: Initial Denaturation of 95ºC for 5 min, followed by 35 cycles of – 95ºC for 45 sec, 61ºC for 45 sec, 72ºC for 45 sec and final synthesis at 72ºC for 5 min. The amplified DNA of 439 bp was digested using *Alu*I enzyme by incubating at 37ºC overnight. The digested products were separated in 3% agarose gel in 0.5 X TAE containing 1.0

µM ethidium bromide and visualized under UV trans-illuminator and after which photographs were taken using Gel Doc system for manual interpretation of the genotypes available.

Statistical Analysis

The allele and genotypic frequencies along with observed and expected homozygosity and heterozygosity were calculated using the POPGENE 32 software.

Results and Discussion

The prolactin (PRL) gene is found in Chromosome 2 in birds (Miao *et al*., 1999; Alpinah *et al.*, 2011) and consists of five exons and four introns (Li *et al*., 2009; Yousefi *et al*., 2012). The polymorphism in the 5'-flanking promoter regions of the PRL gene was detected using the PCR-RFLP method in the local chicken 'Zo-ar' of Mizoram. The digestion of PRL gene PCR amplified fragment (439 bp) with the restriction endonuclease enzyme *Alu*I showed polymorphism yielding three different genotypes *i.e*., CC (304, 81 and 54 bp) with three fragments, CT (439, 304,81 and 54 bp) with four fragments and the TT (439 bp) genotype resulted in an uncut fragment. All the three genotypes CC, CT and TT are shown in Fig. 1.

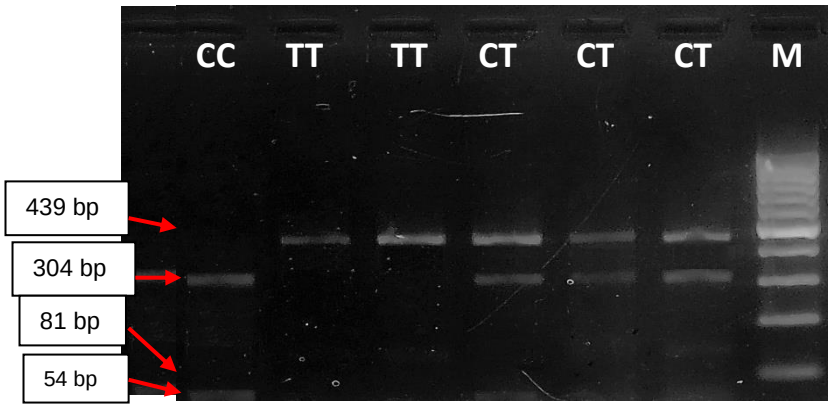

Figure 1: Genotype of PRL gene digested with RE *Alu*I in 3% agarose gel: CC, TT and CT - Genotypes, M - 100 bp Ladder (SM0244 Gene Ruler^{TM} 100bp DNA ladder)

The genotypic frequency distribution along with the observed and expected heterozygosity values for the PRL locus in native 'Zo-ar' chicken of Mizoram has been presented in the Table 2.

Table 2: Genotypic frequency distribution in native chicken 'Zo-ar' of Mizoram

Genotypes (n=50)	Genotypic frequency
CC (3)	0.06
CT (28)	0.56
TT (19)	0.38
Observed Heterozygosity	0.56
Expected Heterozygosity	0.45
χ^2 value	2.84^{NS}

n = Number of animals; NS = Not significant

Among the three genotypes present in the population, the heterozygote genotype, CT was found to be predominantly distributed among the native chicken in Mizoram as it showed a genotype frequency of 0.56, while the CC was the least found genotype with a frequency of 0.06 as only 3 of the samples had this genotype. On the other hand, TT genotype was moderately found with a frequency of 0.38. The observed heterozygosity value (0.56) was also found to be higher than the expected value. The native chicken population of Mizoram was seen to be conforming to the Hardy-Weinberg equilibrium with respect to the PRL locus. In the present study, the allele T has been seen to be predominantly prevalent with a frequency of 0.66 in the local chicken population of Mizoram under the present study (Table 3). On the other hand, it has been recorded that the C allele (0.34) was rarely distributed in the population. Similarly, the T allele of PRL locus was found to have a higher frequency in most of the native Chinese breeds (Nguyen *et al*., 2018), like Lien Minh chicken (0.81), Taihe Silkies 1 (0.70), Taihe Silkies 2 (0.87), Nongdahe

(0.58), Yangshan (0.95) and White rock (0.65).

Table 3: Allele frequency of PRL gene in native chicken 'Zo-ar' of Mizoram

Locus	Allele	Allelic frequency
PRL	C	0.34
	T	0.66

On the other hand, in the same study it was found that the T allele was completely absent in commercial egg laying breeds like White leg horn where the C allele had a frequency of 1.00, giving rise to the idea that the C allele has a correlation with egg production traits.

Similarly, in Polttavaskaya Glinistaya chicken breeds also reports of PRL gene polymorphisms were put forward by Kulibaba (2015). All the three genotypes were found to be present in the breed with a frequency of TT (0.37), CT (0.52) and CC (0.11). The T allele (0.63) was seen to be having major distribution in this chicken population too. In Noi chicken breeds of Vietnam (Vu and Ngu, 2016), results similar to our findings have been reported where the T allele was predominantly present and had a frequency of 0.83. On the other hand, contrary to our findings, Abdi *et al*. (2014) reported the predominance of C allele (0.78) in West Azerbaijan native poultry lines. Rashidi *et al.* (2012) reported genetic correlation between the PRL variants and different production traits. It was found that the T allele was completely absent in commercial layers. Moreover, traits like age at sexual maturity, body weight at hatch and total egg production were seen to be affected by the PRL gene variation in these chickens. The chicken having the CC genotype for PRL gene were found to be performing better for various reproductive traits like age at puberty, body weight on 12^{th} week of age, mean egg weight and mean egg production as reported by Abdi *et al*. (2014). Some traits like egg production at 12^{th} week, 40^{th} week and egg weight at 30^{th} week were found to be significantly higher in CC genotypes as compared to the TT genotype by Kulibaba (2015). Similar results were also obtained by Alpinah *et al*. (2011), in Zaboli breed of chicken. Thus, PRL variants can be considered in playing important role in egg production as well as broodiness of chicken.

The observed heterozygosity in the native chicken population was calculated to be 0.56 for the PRL locus which can be considered as an intermediate level of heterozygosity.

Conclusion

The presence of genetic variability and the favourable alleles for most of the production traits in the population suggests the possibility of increasing production performance with the help of proper selection methods and breeding systems. The population conforming to the Hardy-Weinberg equilibrium for all the loci indicates that no selection pressure was applied for any economic traits to the population in the past.

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Conflict of Interests

There is no conflict of interest.

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