MHC genes in Tellicherry chicken breed of Kerala, India: an analysis on pleotropic action

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ABSTRACT

The present study was conducted on indigenous chicken breed Tellicherry inhabiting both Western Ghats and coastal Kerala. These birds are known to be resistant to a variety of infections and at the same time produce good quality meat. It was proposed to analyze association of an immune related gene in this breed with important layer traits. Allele specific PCR was carried out in the selected Tellicherry chicken population and the relationship with layer production traits was analyzed. From the present study, it was observed that MHC haplotype B_2 was associated with higher body weight on 1st day and 16th week as well as with high egg number. At the same time, B_{15} haplotype was associated with high egg number and low age at first egg. Because of significant association, this marker could be used for selection of indigenous chicken.

Keywords: Indigenous poultry; Tellicherry chicken; AS-PCR; production traits; association

INTRODUCTION

The only indigenous chicken breed of Kerala state of India is Tellicherry. Its body conformation shows some similarities with red jungle fowl but its plumage is black with shining bluish tinge on hackle, back and tail feathers. These small birds inhabit in Western Ghats as well as coastal Kerala, as free-range birds without cages and rest at night on tree branches.

In recent years, these native chickens are gaining importance due to the increase in demand for the quality of their dark hue meat. The alleged traditional medicinal quality of their meat further adds to their demand (Vij et al 2008). They have undergone population decline and range fragmentation. After a long search for native breeding tract of these birds, a population was spotted in the hilly terrain of Kasaragod district, Kerala. These birds are locally known to be resistant to a variety of diseases.

Since the major histocompatibility complex (MHC) of vertebrates is a prime molecule responsible for disease resistance, it was proposed to study the association of this gene in this native breed for the first time. In the past, work on association of this gene with disease resistance was carried out in other breeds. In white leghorn population, MHC genotypes showed significant effect on antibody titre against Newcastle disease at 21st day. Genotype $B_{15}B_{19}$ showed highest antibody titer followed by B_2B_{19}, B_2B_{15} and B_2B_2 (Shanaz et al 2005). Leghorn and Ancona birds originating from B_2, B_8, B_{12} and B_{19} and B_2/B_{12} haplotypes were studied for susceptibility to infectious bronchitis virus (IBV) infection. The B_2/B_2 and B_5/B_5 haplotypes and B_2/B_{12} birds were found to be resistant to IBV Gray strain infection compared to chicks with MHC homozygous B_{12} and B_{19} haplotypes (Banat et al 2013).

Though multitude of studies on the disease resistance aspect has been turned up, role in production potential of this gene remained hiddened for a long time. Later on, in Iranian indigenous chicken (Khorasan) significant association of LEI0258 microsatellite with body weight, egg weight, egg laying

intensity and weight at sexual maturity was reported. Allele 194 was negatively associated with body weight at 8 and 12 weeks. Allele 310 had negative effect on egg production at 28 and 84 weeks as well as on weight at sexual maturity. Allele 194 was associated with weight of first egg laid and allele 313 with high egg laying intensity (Nikbakht and Esmailnejad 2015).

Molee et al (2016) reported the significant association of MHC B-Lβ2 gene (exon 2) polymorphism with Newcastle disease virus titer and body weight and suggested pleiotropic effect of this region on immunity and growth performance. Study was conducted in indigenous population of Thai chicken Leung Hang Khao by PCR amplification and sequencing. Significant association was observed between alleles of the LEI0258 marker and body weight in the Ghagus breed. As compared to the reference (309 bp) allele, the 194 bp allele had a significant positive influence on body weight recorded at 4 and 8 weeks of age. The 194 bp allele also had a significant positive influence on shank length at 8 weeks of age, egg production and survivability up to 72 weeks of age. However, the 445 bp allele had a significant negative effect on body weight recorded at 40 weeks of age.

Therefore, in order to prove the pleiotropic action of this gene family, allele specific PCR analysis was performed and association with important production traits was analyzed.

MATERIAL and METHODS

Thirty Tellicherry chicks were randomly selected from the native breeding tract in Kasaragod district, Kerala. Data were collected on body weight on first day (BWt 1), body weight at 6 weeks (BWt 6), body weight at 16 weeks (BWt 16), weight of first egg (EWt 1), egg number (EN: number of eggs laid in one month during 20 to 30 weeks of age), age at first egg (AFE) and average weight of eggs (EWt A 30: average weight of 30 consecutive eggs laid during 20 to 30 weeks of age). From the selected sample population, from each bird, 0.5 ml of whole blood was collected by brachial venipuncture into 2 ml syringe. Individual blood samples were transferred into ethylene diamine tetraacetic acid (EDTA) coated vials (1 mg/ml of blood), transported in ice pack and stored at –20°C till further processing. Phenol-chloroform extraction procedure (Sambrook and Russel 2001), which removes protein and other cellular components from nucleic acids, with some modification was applied to isolate genomic DNA from whole blood. The relatively pure DNA prepared was checked for quality (horizontal submarine agarose gel electrophoresis), purity (UV-Spectrophotometry) and concentration (UV-Spectrophotometry) and qualified samples were used for further analysis.

The individual samples were amplified with a pair of primers specific to MHC B-Lβ2 family as per Zheng et al (1999) from relatively conserved segments of exon 2 of the B-Lβ2 II family genes and synthesized at Merck Biosciences, Bangalore, Karnataka. The PCR mixture consisted of 1 X PCR buffer, 4 mM $MgCl_2$, 200 µM dNTPs, 20 p mole of each primer, 1U Taq DNA polymerase and 100 ng of template DNA. The reaction programme involved an initial denaturation at 94°C for 10 minutes followed by 35 cycles of 94°C for 30 sec, 57°C for 30 sec and an extension at 72°C for 30 sec.

The products of this initial reaction were diluted 1:100 times and 1 µl of dilution was subjected to allele specific PCR. Primers specific to five important MHC alleles of broilers (B_2, B_{13}, B_{15}, B_{19} and B_{21}) were chosen for the present experiment (Zheng et al 1999).

Diluted PCR products were subjected to amplification with five sets of allele specific reactions, separately targeting these alleles. Individual reaction mixture contained 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM each of dNTP, 20 p mol of each primer and 1 U Taq DNA polymerase. Annealing temperatures of 55, 60, 50, 55 and 55°C for B_2, B_{13}, B_{15}, B_{19} and B_{21} alleles respectively were used for allele specific reaction. For rest of the conditions, the values of initial amplification were followed.

The results of all the reactions were tabulated and MHC genotypes of entire birds were found. Association of various MHC genotypes with layer production traits was analyzed by software programme Popgene (Version 1.32). Birds with rare alleles were excluded from data analysis.

RESULTS

Mean values of each production parameters and corresponding standard deviations were calculated in each genotypic group (Table 1). Analysis of variation was performed between these groups and wherever

F-value was significant, post hoc analysis was done. It was performed with Tukey HSD to classify means of genotypic groups into homogeneous subsets (a, b). Among genotypic groups, highest body weight on first day was obtained in B_2B_{13} (27.75 g) and lowest in $B_{13}B_{15}$ (26.32 g). On analysis of variance for body weight on first day, significant F-value was obtained (7.43). Post hoc analysis showed that B_2B_{13} and B_2B_{15} groups exhibited high value for BWt 1, whereas, $B_{13}B_{15}$ showed a lower value.

Highest body weight at 6 weeks was obtained for B_2B_{15} (188.62 g) following the same trend as in body weight on first day, whereas, lowest value was obtained for $B_{13}B_{13}$ (171.53 g). For this trait also significant F-value was obtained (11.45) on analysis. Since the genotypes $B_{13}B_{13}$, $B_{13}B_{15}$ and B_2B_{13} exhibited lower value for BWt 6, it was understood that B_{13} allele was associated with lower BWt 6. When mean values of body weight at 16 weeks were calculated for each genotypic group, highest value was obtained for B_2B_{15} and lowest for $B_{13}B_{13}$. Among the F-values calculated, highest was obtained for BWt 16 (37.70). Three sub-sets were obtained for mean value viz a, b and c. The genotypes $B_{13}B_{13}$ and $B_{13}B_{15}$ presented lower BWt 16, whereas, B_2B_{15} a higher value. At the same time, B_2B_{13} presented a medium value BWt 16. From this result, it is inferred that B_2 allele was associated with higher value and B_{13} with a lower value of BWt 16.

As far as weight of first egg is concerned, there was no significant variation between genotypic groups as F-value was low. The B_2B_{15} genotypic group (15.83 ± 0.75) exhibited highest egg number, whereas, $B_{13}B_{13}$ group (13.33 ± 1.15) exhibited lowest. There were significant differences among mean values of egg number among the genotypic groups analyzed with an F-value of 4.81 ($p \leq 0.05$). Since $B_{13}B_{13}$ group exhibited lowest sub-set for egg number and $B_{13}B_{15}$ and B_2B_{13} belonged to both the classes, the B_{13} allele was inferred to be associated with lower number of eggs. At the same time B_2B_{15} belonged to higher sub-set for egg number. So the alleles B_{15} and B_2 were realized to be associated with high egg number.

Age at first egg was found to be associated with MHC genotypes significantly ($F= 28.23$, $p \leq 0.05$). The $B_{13}B_{15}$ and B_2B_{15} genotypes were found to take minimum days for laying first egg, whereas, the $B_{13}B_{13}$ and B_2B_{13} were found to take more days. So the B_{15} was identified to be a quick laying allele. Since the F-value (1.84, $p \leq 0.05$) in case of EWtA 30, was not significant, the MHC haplotypes identified by the

Table 1. Mean values and ANOVA of production characteristics of MHC genotypes in Tellicherry chickens

Layer trait	Genotype				F-value
	$B_{13}B_{13}$	$B_{13}B_{15}$	B_2B_{13}	B_2B_{15}	
BWt 1	26.73 ± 1.08^{ab}	26.32 ± 0.68^{a}	27.75 ± 0.31^{b}	27.58 ± 0.60^{b}	7.43*
BWt 6	171.53 ± 4.31^{a}	174.82 ± 6.51^{a}	178.93 ± 1.87^{a}	188.62 ± 2.90^{b}	11.45*
BWt 16	629.4 ± 8.75^{a}	633.55 ± 5.16^{a}	644.60 ± 5.57^{b}	663.27 ± 6.92^{c}	37.70*
EWt 1	38.30 ± 0.17	39.44 ± 1.18	41.00 ± 2.14	39.95 ± 2.50	1.64^{NS}
EN	13.33 ± 1.15^{a}	13.69 ± 1.49^{ab}	14.50 ± 0.58^{ab}	15.83 ± 0.75^{b}	4.81*
AFE	165 ± 4.36^{b}	149.23 ± 4.11^{a}	166.25 ± 4.27^{b}	148.5 ± 3.94^{a}	28.23*
EWtA 30	43.60 ± 0.92	44.75 ± 1.10	45.85 ± 0.81	45.27 ± 2.04	1.84^{NS}

*Calculated value significant at $p \leq 0.05$, ^{NS}Calculated value non-significant

Table 2. Correlation between production parameters in Tellicherry chickens

Parameter	BWt 1	EWt 1	EN	BWt 16	AFE
BWt 1	1	0.067^{NS}	0.12^{NS}	-	-
EWt 1	0.067^{NS}	1	-	-	-
EN	0.12^{NS}	-	1	0.47*	$-.06^{NS}$
BWt 16	-	-	0.47*	1	$-.184^{NS}$
AFE	-	-	$-.06^{NS}$	–.184	1

*Calculated value significant at $p \leq 0.05$, ^{NS}Calculated value non-significant

present study were proved to be not associated with EWtA 30.

Meaningful correlation between various layer production parameters were analysed for the population of Tellicherry chicken under study. Significant positive relationship could be derived between BWt 16 and EN (0.47, $p \leq 0.05$). All other calculations for correlation coefficients in Tellicherry population resulted in insignificant values (Table 2).

DISCUSSION

Different chicken breeds varying in various production parameters were evolved, meanwhile, the frequency of linked MHC alleles changed. This process lead to variation in frequency of MHC haplotypes in different breeds of chicken. Chicken MHC haplotypes were commonly identified with polyclonal antisera produced by immunization between birds having different haplotypes. Since the serological typing has several limitations, molecular markers were developed.

Nikbakht and Esmailnejad (2015) used microsatellite (LEI0258) study to analyze the association of MHC haplotypes with layer production traits in Iranian indigenous chicken. Associations were obtained with body weight at 8 and 12 weeks, weight of first egg and egg weight at 28 and 84 days as well as weight at sexual maturity. From the present study, it was interpreted that MHC B_2 haplotype was associated with higher body weight on first day and body weight on 16th day. At the same time B_{13} allele was associated with lower body weight at 6 and 16 weeks.

According to Haunshi et al (2020), in Ghagus breed of poultry, MHC was associated with body weight at 4, 8 and 40 weeks and egg production and survivability up to 72 weeks of age. The present study revealed that alleles B_{15} and B_2 were associated with high egg number and the B_{15} was identified to be a quick laying allele. In Nicobari breed LEI0258, alleles 261 (B_{15} haplotype) and 295 (B_5 haplotype) were significantly associated positively with antibody titres to NDV vaccine at 20 weeks of age when compared to reference allele 343 bp. This relationship indicated the role of MHC gene in mounting immune response against an antigen.

A study by Ewald et al (2007) suggested a significant role of MHC haplotypes in survivability trait of chickens (early and late mortality in commercial broiler line). Lwelamira et al (2008) reported that body weight at 16 weeks is positively associated with allele 307 bp (B_{72} haplotype) in Tanzanian chicken. In white leghorn breed, MHC had significant negative influence on body weight recorded at 16 weeks of age. The 552 bp ($B_{19.1}$ haplotype) alleles were significantly negatively associated with egg production up to 72 weeks of age. As per the current correlation analysis, birds with high body weight at 16 weeks are supposed to produce more eggs compared to low weight birds.

This study in Tellicherry chicken revealed that B_2 as well as B_{15} were highly productive haplotypes. So, this molecular marker could be applied to the selection of indigenous layer population in India. For many of the important poultry diseases, immunoprophylatic measures are not effective and epidemics incur huge losses to the poultry farmers. To change this scenario, up-gradation of native breeds having strong immunity as well as good production potential is essential.

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