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RESEARCH ARTICLE

Association between microsatellite genotypes and body weight at different ages in indigenous chicken ecotypes

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Abstract : The present study was carried out in six indigenous ecotypes of two divisions of Karnataka to assess association of twenty microsatellite genotypes belonging to thirteen chicken autosomes with Body weight at different ages. The general molecular technique protocols suggested by Sambrook *et al*. (1989) were adopted wherever required in PCR, electrophoresis, gel staining and reading. The analysis revealed significant difference ($p<0.05$) among genotypes combined across ecotypes for nineteen microsatellite loci for body weight at sexual maturity. The validity of using thus, identified markers or alleles need further authentication by research in other populations and further proof by expression studies. Considerable numbers of significant associations were identified in later ages (particularly from sixth week) except for first week in earlier ages across all the microsatellite regions explored except MCW007. There was no significant difference among genotypes of any microsatellite regions for traits like day old, second, fourth, fifth and sixth week body weights suggesting absence of definite trend in the influence of microsatellite regions on body weights at different ages in the indigenous chicken ecotypes.

Key words : Microsatellite, Association, Significance, Genotypes

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INTRODUCTION

Substantial advances have been made in the improvement of some important economic traits in livestock and chicken by artificial selection. However, most of such selections were initiated decades ago on the basis of observable phenotypes without knowledge of genetic architecture of the selected characteristics (Dekkers and Hospital, 2002) and thereby the improvement in the traits of interest was limited. The development of molecular biology techniques for uncovering variation at the DNA level has opened new avenues to identify genes affecting quantitative traits (Beckman and Soller, 1983; Haley and Knott, 1992 and Lander and Kruglyak, 1995), in which molecular maker-assisted selection (MAS) acts as a promising tool to improve the trait through conventional means.

It is generally necessary to complement molecular study with population data, using a field survey to describe the main socio-economic features of the population as well as phenotypes (Besbes *et al.*, 2007). The regions of chromosome that affect a trait, but not necessarily a single locus are termed as QTL (Falconer and Mackay, 1996). A significant association between ADL0351 and the dominant effect of body weight at 6, 8, 10 weeks was observed (Poompramun *et al*., 2015).

Several methods are available for QTL analysis and consist of scanning the genome through molecular markers with the objective of determining markers linked to QTL. The earliest method for QTL analysis included single-marker analysis (Gupta, 2002), which can be based on regression, t-test or analysis of variance. These approaches are easily implemented employing available statistical packages. Also, this method is appropriate to investigate associations between microsatellite marker genotypes and quantitative traits; does not require the previous construction of linkage maps and may be used to find and eliminate non-informative markers (Liu *et al.*, 1999).

Microsatellite markers were originally utilized for genetic mapping (Tuiskula-Haavisto *et al.*, 2002 and Ambady *et al.*, 2002) and were extensively used for linkage analyses in the association with disease susceptibility genes (Mcelroy *et al.*, 2005 and Wardecka *et al.*, 2004).Microsatellite markers have been used for quantitative trait locus (QTL) detection in several programs involving chicken, turkeys, ducks and quails in various parts of the world (Hocking, 2005). Estimation of association between microsatellites and economic traits was undertaken (Pandey *et al*., 2005; Chatterjee *et al*., 2008 and 2010 and Rajkumar *et al*., 2008).

Most of the earlier association studies were based on the divergent parent populations and consequent Hybrid generations to identify stable QTL regions. The current study conducted on single generation of six indigenous chicken ecotypes is significant for understanding the molecular inheritance details of economic traits, which may lead to faster development of efficient low input chicken variety.

RESEARCH METHODOLOGY

Research sample and identification :

The indigenous chicken belonging to Ramanagara, Bangalore rural and Chikkaballapura districts of Bangalore division and Chamarajnagara, Mysore and Mandya districts of Mysore division of Karnataka state maintained at AICRP on Poultry for meat, Veterinary College, Hebbal formed the material for the present study. Thirty five randomly chosen adult birds from each of the above districts formed the research sample for the present study.

The birds belonging to the six districts were wing banded at hatch from randomly collected fertile eggs in respective districts. The birds chosen in each district belonged to three hatches and were badged while allocating to breeding pens after growing phase. The wing bands and serial numbers were noted down at the time of blood collection from each bird. The serial numbers for each district were maintained on the 2ml eppendorf tubes and subsequent tubes till the successful amplification of desired segment in each marker. The numbers were maintained till genotyping and further statistical analysis.

Microsatellite markers :

Fourteen microsatellite markers namely MCW014, MCW183, ADL278, MCW067, MCW104, MCW123, MCW330, MCW103, MCW034, MCW081, MCW284, MCW078, ADL268 and ADL112 recommended by FAO (2011) for have been utilized in this study. The remaining six microsatellite markers, ADL020, ADL023, ADL176, MCW007, MCW041 and MCW165 utilized for Indian chicken breeds for both genetic diversity and association study have been selected for this study (Pirany *et al*., 2007; Rajkumar *et al.*, 2008 and Chatterjee *et al*., 2008 and 2010). The selected twenty microsatellite regions are located on chicken autosomes 1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 14, 17 and 23. The general molecular technique protocols suggested by Sambrook *et al*. (1989) are adopted wherever required in the present study in PCR, electrophpresis, gel staining and reading.

PCR :

PCR reactions were set up on clean benches with autoclaved laboratory disposables like micro tips, Flat/dome

shaped PCR tubes, 1.5- 2.0ml eppendorf tubes and autoclaved milliq water ensuring proper thawing, mixing of PCR reaction components and cold chain of around 4 ^{0}C through a minicooler and gel pack. The primer pairs were stored at -20 ^{0}C till final dilution and further usage. The primer pairs were taken out from the -20 ^{0}C and thawed for five minutes and spun at 2000 rpm for one minute to dislodge all the molecules. The yield (nm/µl) in forward and reverse primer was noted and 1 x TE buffer was added at the rate of 10 times the yield of each primer to get a final concentration of 100µM. The same procedure was adopted for all the primer pairs utilized in this study.

Electrophoresis and staining :

The genomic DNA and PCR products were run in minigel apparatus (Biorad) with agarose (0.8 % - 4 %) and TAE buffer to assess genomic DNA quality and amplification of PCR products for various microsatellite markers. The PCR products of each primer pair or set of primer pairs after successful amplification were run in 12 per cent non-denaturing PAGE. The silver staining adopted by Halima *et al*. (2006) with little modification was used for staining of polyacrylamide gels in this study.

Genotype recording :

Each of the lanes with clearer bands was compared with the 50bp ladder lane to determine whether the bands fall in the expected region. Then the alleles were identified in base pairs for each lane in the stained gel and the same was noted as genotype for that lane representing the individual sample. The genotypes belonging to twenty microsatellite regions were recorded as and when the electrophoresis for either single or multiplex PCR reaction products was completed into a column of particular locus in base pairs. The individual lanes without any band or less appreciable bands were not recorded. The original genotype data was entered and saved in MS Office Excel 2007.The same data was entered into GenAlex format and saved in GenAlex for further analysis. The genotype data of the entire twenty microsatellite regions in base pairs was converted alphabet format so as to be compatible IBM-SPSS, which was employed to carry out association studies.

Phenotypic performance recording :

The phenotypic performance data generated for the first generation of indigenous chicken ecotypes belonging to six districts of Bangalore and Mysore division formed the material for association studies with the microsatellite markers in this study. The phenotypic performance of the birds used for the microsatellite genotyping in the traits mentioned below was entered against their wing band numbers. The following traits have been considered for this association study.

- Dayold to eighth week body weights (g)
- Twelfth week body weight (g)
- Twentieth week body weight (g)
- Thirty two week body weight (g)
- Forty week body weight (g).

Statistical analysis :

The genotype and economic traits data across six ecotypes was pooled for association studies due to higher genetic identity among the ecotypes as revealed by genetic divergence results. The phenotypic performance of egg production related traits and genotypes in each of the twenty microsatellite markers are posted against individual birds of each ecotype. The data is then subjected to one-way anova by pooling genotype data for all the six ecotypes using the software IBM-SPSS-Statistics to compare the influence of twenty microsatellite regions on four traits mentioned above.

RESULTS AND DISCUSSION

The results for two microsatellite markers are depicted in the Tables 1-2 and one marker in the Fig. 1. The

observed difference among the genotypes of ADL020 was significant ($p \leq 0.05$) only for traits, eighth week body weight and twentieth week body weight. The observed difference among the genotypes of ADL023 was statistically significant only for traits, first week, twentieth week, thirty two and forty week body weights. The observed difference among the genotypes of ADL176 was statistically significant only for traits, first week, eighth week body weight and twentieth week body weights.

The observed difference among the genotypes of MCW007 was statistically non-significant for all body weight related traits. The observed difference among the genotypes of MCW014 was statistically significant only for traits first, seventh, eighth, twelfth and thirty two week body weights. The observed difference among the genotypes of MCW041 was statistically significant only for traits eighth, twentieth, thirty two and forty week body weights. The observed difference among the genotypes of MCW183 was statistically significant only for traits first, seventh, eighth, twelfth, twentieth and thirty two week body weights. The observed difference among the genotypes of ADL278 was statistically significant only for traits first, eighth and twentieth week body weights. The observed difference among the genotypes of MCW067 was statistically significant only for traits twelfth, twentieth, thirty two and forty week body weights. The observed difference among the genotypes of MCW104 was statistically significant only for traits first, seventh, eighth, twelfth, twentieth and thirty two week body weights. The observed difference among the genotypes of MCW123 was statistically significant only for traits first, seventh, eighth, twelfth, twentieth, and thirty two week body weights. The observed difference among the genotypes of MCW330 was statistically

Table 1: Mean squares for body weight related traits for ADL020

Trait	Source	df	Mean square	F
DOBW	Between genotypes	22	17.82	1.16 ^{NS}
	Within genotypes	158	15.33	
FWKBW	Between genotypes	22	135.17	1.54 ^{NS}
	Within genotypes	157	87.65	
SWKBW	Between genotypes	22	156.12	1.34 ^{NS}
	Within genotypes	157	116.06	
TWKBW	Between genotypes	22	439.76	0.80 ^{NS}
	Within genotypes	158	543.51	
FRTHWKBW	Between genotypes	22	1275.20	0.69 ^{NS}
	Within genotypes	158	1844.16	
FFTHWKBW	Between genotype	22	3223.74	0.79 ^{NS}
	Within genotypes	158	4044.37	
SXTHWKBW	Between genotypes	22	7352.17	0.92 ^{NS}
	Within genotypes	158	7956.63	
SEVTHWKBW	Between genotypes	22	16311.14	1.43 ^{NS}
	Within genotypes	157	11400.82	
EGTHWKBW	Between genotypes	22	28661.02	1.67 *
	Within genotypes	157	17114.09	
TWETHWKBW	Between genotypes	22	45064.92	1.58 ^{NS}
	Within genotypes	148	28506.29	
TWENTTHWKBW	Between genotypes	22	101637.54	1.70 *
	Within genotypes	151	59680.23	
TRYTWKBW	Between genotypes	20	195886.02	1.62 ^{NS}
	Within genotypes	109	120822.22	
FRTYWKBW	Between genotype	20	160262.79	1.24 ^{NS}
	Within genotypes	98	128783.67	

Note: * indicate significance of value at $P \leq 0.05$, NS = Non-significance

Table 2 : Mean squares for body weight related traits ADL112

Trait	Source	df	Mean square	F
DOBW	Between genotypes	16	8.78	0.53 ^{NS}
	Within genotypes	164	16.30	
FWKBW	Between genotypes	16	163.86	1.89 *
	Within genotypes	163	86.58	
SWKBW	Between genotypes	16	109.67	0.89 ^{NS}
	Within genotypes	163	122.09	
TWKBW	Between genotypes	16	352.28	0.64 ^{NS}
	Within genotypes	164	548.25	
FRTHWKBW	Between genotypes	16	1141.94	0.62 ^{NS}
	Within genotypes	164	1836.34	
FFTHWKBW	Between genotypes	16	4717.37	1.21 ^{NS}
	Within genotypes	164	3868.63	
SXTHWKBW	Between genotypes	16	8514.36	1.08 ^{NS}
	Within genotypes	164	7821.14	
SEVTHWKBW	Between genotypes	16	10880.85	0.89 ^{NS}
	Within genotypes	163	12114.60	
EGTHWKBW	Between genotypes	16	15709.39	0.83 ^{NS}
	Within genotypes	163	18810.45	
TWETHWKBW	Between genotypes	15	24872.068	0.79 ^{NS}
	Within genotypes	155	31208.24	
TWENTTHWKBW	Between genotypes	16	91401.74	1.46 ^{NS}
	Within genotypes	157	62326.83	
TRYTWKBW	Between genotypes	14	141192.52	1.07 ^{NS}
	Within genotypes	115	131396.93	
FRTYWKBW	Between genotypes	14	114451.68	0.83 ^{NS}
	Within genotypes	104	136766.65	

Note: * indicate significance of value at P≤0.05, NS- Non significance

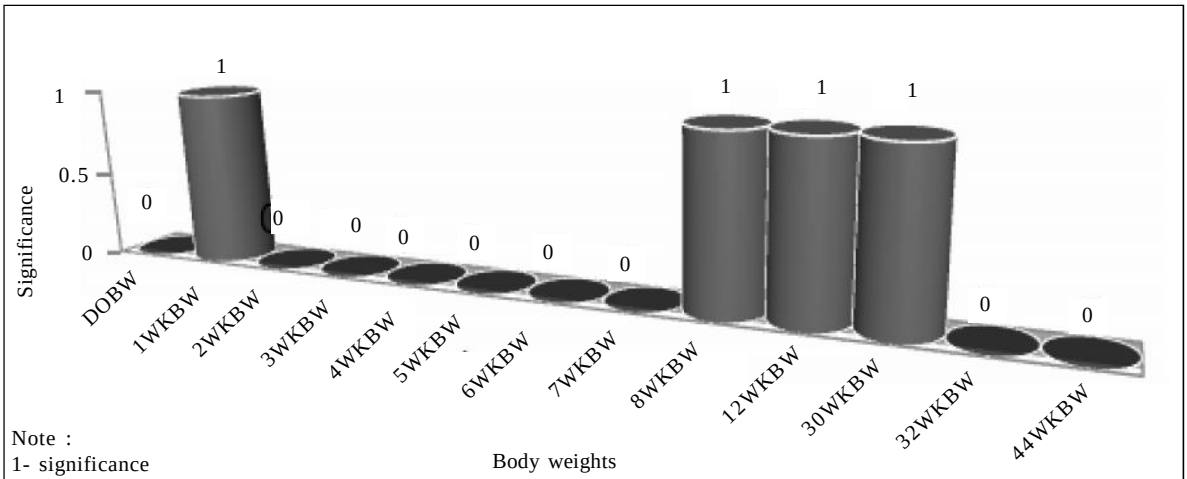

Fig. 1 : ANOVA results for microsatellite marker ADL 268

significant only for traits first, third, seventh, eighth, twelfth, twentieth, and thirty two week body weights. The observed difference among the genotypes of MCW165 was statistically significant only for traits first, eighth, twelfth, twentieth, thirty second and forty week body weights. The observed difference among the genotypes of MCW103 was statistically significant only for traits seventh, eighth, twelfth and twentieth week body weights. The observed difference among the genotypes of MCW034 was statistically significant only for traits first, seventh, eighth, twelfth, twentieth and thirty two week body weights. The observed difference among the genotypes of MCW081 was statistically significant only for trait first week body weight. The observed difference among the genotypes of MCW284 was statistically significant only for trait first week body weight. The observed difference among the genotypes of MCW078 was statistically significant only for traits first, seventh, eighth, twelfth and twentieth week body weights. The observed difference among the genotypes of ADL268 was statistically significant only for traits first, eighth, twelfth and twentieth week body weights. The observed difference among the genotypes of ADL112 was statistically significant only for trait first week body weight.

The body weight at different ages has been an important economic trait in commercial broilers, layers as well as indigenous chicken because of direct influence on monetary returns and other economic traits. Physiologically healthy poultry birds will produce and reproduce better. It is a fact that body weight influences ASM leading to earlier onset of egg production and consequent higher egg production. Quantitative trait loci (QTL) can be defined as the marker interval that co-segregates with variation in the traits of interest. The markers including such intervals can be used in marker assisted selection to introduce or retain beneficial QTL allele. However, markers have to be very closely linked to the causative mutation in the trait gene if they are to remain associated with specific QTL alleles through several generations of selection and, therefore, be useful in practical breeding programmes.

The present study made on the indigenous chicken ecotypes of Karnataka employing twenty microsatellite markers located on thirteen chicken autosomes revealed significant ($P<0.05$) differences among genotypes of all microsatellite regions for body weights at one or the other ages except MCW007, which did not show any significant difference among it's genotypes for body weight at any age. Considerable numbers of significant associations were identified in later ages (particularly from sixth week) except for first week in earlier ages across all the microsatellite regions explored except MCW007. There was no significant difference among genotypes of any microsatellite regions for traits like day old, second, fourth, fifth and sixth week body weights suggesting absence of definite trend in the influence of microsatellite regions on body weights at different ages in the indigenous chicken ecotypes. The association studies in chicken by earlier researchers for body weight related traits (Zhang *et al.*, 2008; Boschiero *et al*., 2009 and Nassar *et al.*, 2012) have revealed significant associations till the microsatellite region only, while Chatterjee *et al.* (2010) reported significant associations beyond microsatellite region, till allele level in each of the microsatellite region they investigated. One of the reason for latter's revelation could be omission of genotypes with less than one bird per genotype in each of the microsatellite region.

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