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Full Length Research Paper

Association of LEI0258 microsatellite alleles with antibody response against newcastle disease virus vaccine and body weight in two Tanzania chicken ecotypes

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A study was carried out to evaluate the prospects of using marker assisted selection (MAS) in improving primary antibody response against Newcastle disease virus (NDV) vaccine and body weight in two Tanzania chicken ecotypes, namely Kuchi and Tanzania Medium (*Medium*). The study involved evaluation of the association between LEI0258 microsatellite alleles (a microsatellite located within the chicken Major Histocompatibility Complex) and primary antibody response and body weight. Results indicated that the allele 205 bp was significantly (P<0.001) positively associated with the elevated primary antibody responses against NDV vaccine, while the allele 307 bp was significantly (P<0.05) negatively associated with this trait. The allele 307 bp was also significantly (P<0.05) positively associated with body weight. However, based on the magnitude of R^2 (which were less than 0.10), it was envisaged that incorporation of these alleles into breeding programs would results into marginal response and hence their use in resource poor countries may sometimes not be justified. Therefore use of cheaper methods for the chicken MHC typing was recommended.

Key words: Microsatellites, PCR, Newcastle-disease, body-weight, local-chicken.

INTRODUCTION

Low genetic potential and high prevalence of diseases are among of the major factors limiting productivity of the local chickens in the tropics (Yongolo, 1996; Alexander, 2001; Otim, 2005). Among many diseases of poultry endemic in Tanzania and other developing countries, Newcastle disease (ND) has been reported to be the most important (Rahman et al., 2002; Illango et al., 2005; Otim, 2005). When Newcastle disease strikes, 60 to 100% of chickens in a household can be lost (Yongolo, 1996; Alexander, 2001; Acamovic et al., 2005). Although the strategy of mass vaccination has largely been an effective control of the Newcastle disease, however, combining vaccination programs and development of genetically resistant stocks will further maximize protection of the chickens from the disease. Improving disease resistance in poultry by direct selection or by selection for immune response may hardly be feasible due to quantitative nature of these traits, their low to moderate heritability, and the difficulties associated with obtaining reliable measurements (Yonash et al., 2000). In this situation, marker assisted selection (MAS) is expected to be a more effective breeding approach. Genes located within the Major Histocompatibility Complex (MHC) B region on chicken Micro-chromosome 16 has been demonstrated by many workers to be associated with immune response and disease resistance as well as

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productivity (Heller et al., 1991; Dunnington et al., 1992; Caron et al., 1997; Liu et al., 2002; Juul-Madsen et al., 2002; Parmentier et al., 2004; Taylor, 2004; Joiner et al., 2005; Boonyanuwat et al., 2006; Fulton et al., 2006). Results from these studies further showed Restriction Fragment Length Polymorphism (RFLP), and micro-satellites linked to this region (LEI0258 in chickens) to be promising DNA markers in characterizing MHC B genes. Identifying marker alleles (bands) associated with superiority is important before MAS can be used. Using MHC-RFLP, results from some of MHC studies revealed that marker alleles associated with superiority vary depending on population under consideration i.e. background genome (Dunnington et al., 1992). Therefore there is a need for identifying bands associated with superiority in a population of interest before MAS can be used to improve immunocompetence of that population.

Past research on local chicken MHC in Tanzania (Lawrence, 1998) and other countries such as Bolivia and India (Baelmans et al., 2005) has only tested the presence of different MHC haplotypes using specific alloantisera. Similar work has been done on Brazilian local chickens using LEI0258 microsatellite (Lima-Rosa et al., 2005). However, no study has been carried out to identify MHC alleles/haplotypes responsible for either high or low immune responses (a pre-requisite for MAS) in the local chicken populations of Tanzania. Therefore this study was carried out to identify LEI0258 micro-satellite alleles associated with antibody response against NDV vaccine and body weight in two Tanzania chicken ecotypes viz. Kuchi and Tanzania Medium (*Medium*). Previous studies (Lawrence, 1998; Msoffe et al., 2001) have shown these ecotypes to be relatively superior to other Tanzania chicken ecotypes in terms of body weight and egg production.

MATERIALS AND METHODS

Study site and experimental materials

This study was carried out at Sokoine University of Agriculture Poultry Research Unit, Morogoro, Tanzania. The place is located at an altitude of about 525 m above sea level. The relative humidity at the location is about 81%, while the monthly mean and maximum temperatures are 18.7 and 30.1°C, respectively. The area has a mean annual rainfall of 846 mm. Experimental chicks were derived from two parent stocks, one representing *Kuchi* ecotype obtained from drier parts of north west Tanzania, and another representing Tanzania Medium (*Medium*) ecotype obtained from central part of the country. A total of 85 and 88 chicks for *Kuchi* and *Medium* ecotypes, respectively randomly sampled from five batches were involved in this study.

Management of experimental birds

Birds were fed a starter ration (20% CP and 2800 Kcal ME/kg) from day old to 8th week of age, growers ration (16% CP and 2750 Kcal ME/kg) from 9th to 16th week of age, and layers ration (17% CP and 2700 Kcal ME/kg) from 17th week of the age to the rest of the period. Water was supplied on *ad libitum* basis. Furthermore, birds were vaccinated against Gumboro disease when they were 10 to 14 days of age, and also due to the experimental set-up they were first vaccinated against Newcastle disease when they were 4 weeks of age, and vaccinations were repeated 3 weeks post vaccination, and later on after every 3 months.

DNA isolation and MHC haplotyping

For each chicken, DNA was isolated from 200 µl packed cells from EDTA-stabilized blood using a salt protocol, as described by Juul-Madsen et al. (1993). The MHC haplotypes were determined by PCR-based genotyping of the LEI0258 microsatellite locus (Dalgaard et al., 2005; Lima-Rosa et al., 2005; Fulton et al., 2006; Schou et al., 2006). The PCR amplification from genomic DNA was carried out in 25 µl reaction volumes using standard buffer (Amersham) containing 0.05 µM of each primer (the forward primer having been labelled with fluorescein), 0.4 mM of each dNTP, 1.5 mM $MgCl_2$ and 1unit of *Taq* DNA polymerase (Amersham). After an initial 5 min of denaturation at 94°C, the amplification went through 25 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 2 min. The amplification was completed with a final extension for 10 min at 72°C. The amplicons were determined by electrophoresis on a denaturing polyacrylamide gel in an ALF DNA sequencer (Amersham) to detect allelic polymorphism. A mixture of 10 fluorescein-labelled fragments of 50-500 bp (Amersham) was used as a size marker. Furthermore, DNA samples from three well-characterised lines of White Leghorn homozygous for the MHC haplotypes B13, B19 and B21 (Miller et al., 2004) were included on a gel as control samples. The different MHC haplotypes were finally classified according to the repeat motif of the LEI0258 microsatellite (Fulton et al., 2006).

Traits studied

The traits considered were primary antibody response against NDV vaccination, and body weight at 16 weeks of age. Egg production and related traits were not included in the association analysis as there were very few observations per allele for statistical analyses in nearly all the alleles considered.

Assessment of antibody response against NDV vaccine

The chicks were vaccinated with Newcastle disease virus vaccine (La Sota) according to manufacturer's instructions at the age of 4 weeks and antibody levels were assessed just prior and 2 weeks post vaccination. Blood from each chick was collected from wing vein using syringes. Samples were titrated for Newcastle disease virus (NDV) specific antibodies by the microtitre method of the haemagglutination inhibition (HI) test (Allan and Gough, 1974) using NDV antigen. Four haemagglutination (HA) units were used and twofold serial dilutions of sera added with a starting dilution of 1:2. The titres were expressed in $\log_2 10$ form of the highest dilution causing HI. Since antibody titre prior to vaccination were almost zero in nearly all chicks, then only antibody titre 2 weeks post vaccination (primary antibody response) was considered in subsequent analyses.

Statistical analyses

Since the distribution of different alleles were similar in the two ecotypes, the data for the two ecotypes were pooled together, and six most frequent alleles in both ecotype (i.e. 205, 215, 234, 307, 321, and 345 bp size alleles) were chosen for the association study. Choosing the most frequent alleles was based on the concept that

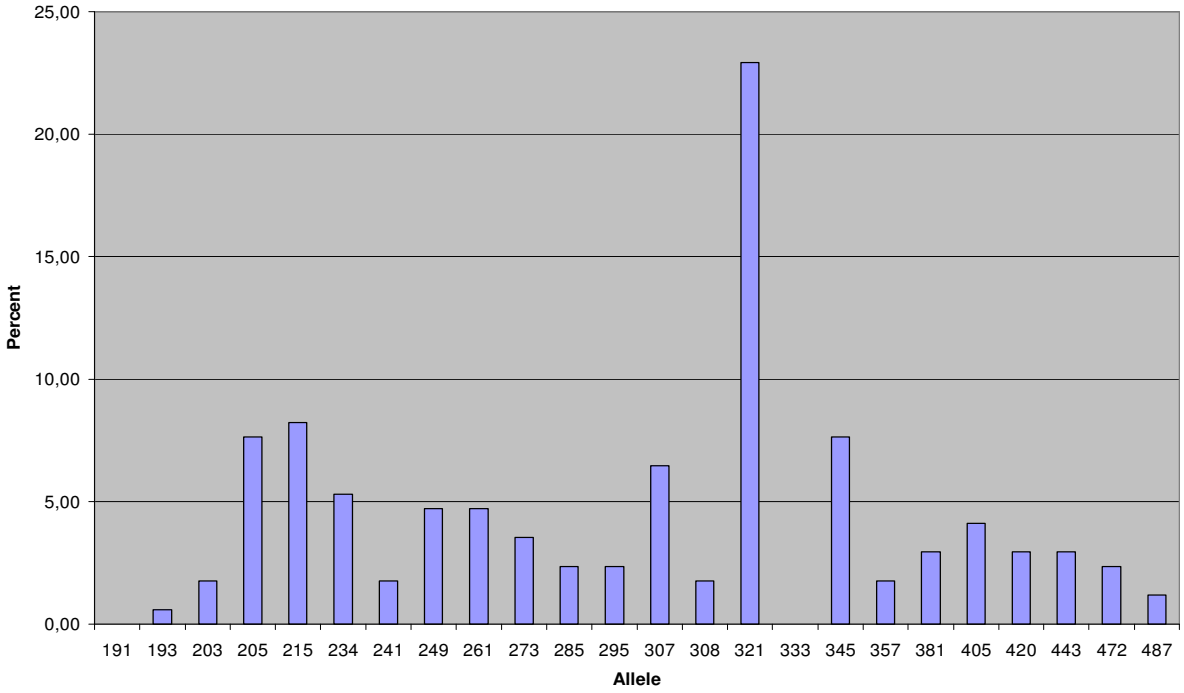

Figure 1. Allele frequencies in *Kuchi* ecotype.

for a random mating population, at a particular locus, frequency of a certain allele in a population is expected to be increased by natural selection if it plays a significant role in the survival of the individuals in the environment (Jeffery and Bangham, 2000; Sabeti et al., 2002; Saunders et al., 2002; Verrelli et al., 2002). For each band (allele), all individuals were scored as a carrier (1) or non carrier (0) of the allele. Then single band analysis was carried out to determine the association of each band with the traits considered by regression analysis using REG procedures of SAS (2000). In this analysis it was assumed that the mode of gene action for these alleles is complete dominance (Schou et al., 2006). Furthermore, before analysis data were adjusted for the fixed effects of ecotype, sex and hatch using GLM procedures of SAS (2000).

RESULTS AND DISCUSSION

LEI0258 microsatellite allele frequencies in the two Tanzania chicken ecotypes

Results from the current study revealed that 22 and 23 alleles of LEI0258 were identified in *Kuchi* and *Medium* ecotypes, respectively (Figures 1 and 2). In a study by Schou et al. (2006) in local chickens of Vietnam and Lima-Rosa et al. (2005) in local chickens of Brazil, a total of 19 and 15 alleles were identified in their populations, respectively, using the same microsatellite, which are somewhat lower than the number obtained in the current study. However, the number of alleles in these studies including the current work are much higher than those reported for commercial breeds such as Lohman Silver Line (3 alleles) (Fink et al., 2005), and in Lohaman brown line (5 alleles) (Schou et al., 2006). In most cases local chicken are kept under free range conditions in which a variety of diseases are prevalent compared to intensive management in which commercial chickens are kept (Pinard-van der Laan, 2002). The observed high number of alleles in free range local chickens in the present study and the study by Lima-Rosa et al. (2005) and Schou et al. (2006) are therefore not surprising. Increased polymorphism at MHC increases their ability to respond to various disease antigens and hence high chance of surviving in their environments. Furthermore, apart from rearing environment, reduced polymorphism at MHC in commercial chickens is also likely being contributed by selection for productivity, as opposed to the out-bred populations.

Results from current study also revealed high level of heterozygosity in the studied populations, in which the proportion of heterozygous individuals in *Kuchi* and *Medium* ecotypes were 88.2 and 86.4%, respectively, and the difference between the two ecotypes was not significant ($P>0.05$) (Table 1). These values are in close agreement with that of 91% reported by Schou et al. (2006) in one population of Vietinamese local chickens, but higher than those of 50% and 75% reported by Lima-Rosa et al. (2005) in two populations of Brazilian local chickens, typed using the same microsatellite. As reviewed by Wegner et al. (2004), MHC heterozygosity seems to be advantageous in MHC mediated disease resistance due to increased diversity of antigens capable of being presented to T cells. Therefore the frequency of heterozygosity at the MHC is expected to be higher in out-bred populations exposed to all kinds of infectious agents as

Table 1. Frequency of homozygous and heterozygous individuals summarized by ecotype.

Status	*Kuchi* (n = 85)		*Medium* (n = 88)	
	Frequency	%	Frequency	%
Homozygous	10	11.8	12	13.6
Heterozygous	75	88.2	76	86.4

X^2 (Chi-square) value = 0.136, P > 0.05.

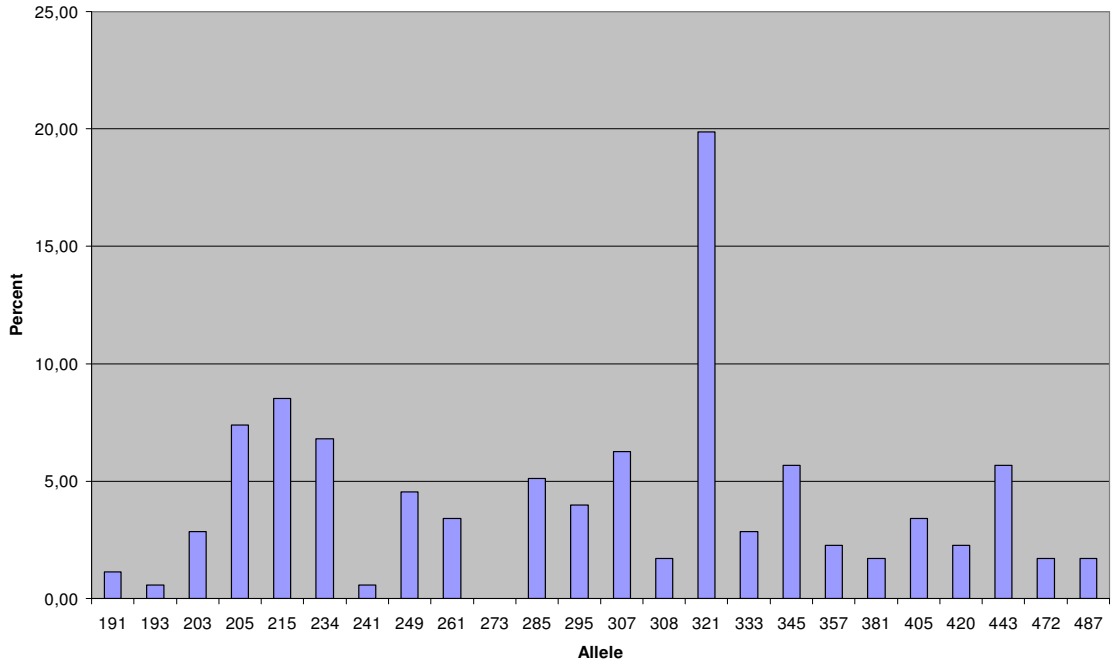

Figure 2. Allele frequencies in *Medium* ecotype**.**

observed in the current study. The low degree of heterozygosity reported for Brazilian local chickens by Lima-Rosa et al. (2005) compared to the results of the current study could probably be attributed to relatively low antigenic diversity prevailing in the environments in which their chickens have evolved compared to those of populations in the current study (*Kuchi* and *Medium*). This is also reflected in the degree of polymorphism (number of alleles), in which lower number of alleles (15) were reported in these Brazilian chickens compared to 22 and 23 alleles found in the present study for *Kuchi* and *Medium* ecotypes, respectively.

Association of LEI0258 microsatellite alleles with primary antibody response and body weight

Association between LEI0258 and the performance was also evaluated in the current study. As started earlier, since distribution of the different alleles among the two studied ecotypes were very similar (Figures 1 and 2), the data for the two ecotypes were pooled together and six most frequent alleles in both ecotypes (205, 215, 234, 307, 321, and 345 bp size alleles) were chosen for the association analysis.

Results from Table 2 indicate that alleles 205 bp and 307 bp were of special interest. The allele 205 bp was significantly (P<0.001) positively associated with the elevated primary antibody responses against NDV vaccine, while the allele 307 bp was significantly (P<0.05) negatively associated with this trait. Significant influence of LEI0258 microsatellite alleles on fitness parameters in chickens were also demonstrated in a study by Schou et al. (2006) in which allele 276 bp which is not found in the populations under current study was found to be associated to resistance to some species of worms in Vietnamese local chickens. Results from Table 2 further show that body weight was only influenced by allele 307 bp in which its presence was associated with increased body weight at 16 weeks of age. Association of some alleles of microsatellites located within MHC region with performance were also reported in other livestock species such as sheep (Bot et al., 2004).

Despite the presence of significant association between

Table 2. Association between LEI0258 microsatellite alleles and body weight at 16 weeks of age and primary antibody response against NDV vaccine.

Trait	Allele (bp)	β	S.E	Sig.	R^2
Ab	205	1.34	0.26	0.000	0.082
	215	0.03	0.27	0.900	
	234	-0.10	0.29	0.730	
	307	-0.92	0.29	0.001	
	321	0.08	0.18	0.652	0.028
	345	-0.16	0.28	0.557	
Bwt16	205	21.53	46.19	0.641	0.002
	215	-65.32	46.94	0.165	
	234	-18.80	50.07	0.708	
	307	135.33	49.51	0.007	
	321	-12.40	31.17	0.691	
	345	-7.41	48.01	0.877	

some of the alleles and the performance in the present study, results for R^2 in Table 2 indicate that the proportion of total phenotypic variance explained by these alleles is too low (less than 0.10). Using RFLP, studies by Yonash et al. (1999, 2000) have also reported low proportion of total phenotypic variation that is explained by single MHC allele/band with regard to primary antibody response to *Escherichia Coli*, Sheep Red Blood Cells (SRBC), and NDV vaccination in broilers. Low R^2 could be due to the fact that antibody response (humoral immune response) and body weight are controlled by many loci, and some of these loci map outside the MHC region (Yonash et al*.,* 2000, 2001; Zhou and Lamont, 2003).

Due to low total phenotypic variation explained by significant alleles, incorporating these markers in breeding programs would result into marginal additional response. The method used for microsatellite typing in the current study (automated microsatellite typing) involved use of expensive equipments (ALF DNA Sequencer®, Amersham), which might be difficult to obtain in developing countries like Tanzania. Hence, additional response by use of these markers and costs involved in typing could sometimes not be justified, unless cheap methods of MHC typing which does not require expensive equipments (serological method and manual microsatellite typing) are used. The problem of serological method for MHC typing in local chickens is that currently available alloantisera have been derived from inbred lines of commercial chickens, that is, White Leghorn (Baelmans et al., 2005; Fulton et al., 2006). Inbred lines contain a limited combination of BG, BF and BL genes. In contrast, in out-bred populations (local chickens), novel alleles and combinations of alleles are likely to exist. Therefore, available alloantisera might not be able to identify all the existing haplotypes in local chickens (some chickens may not show reaction to any of the available haplotype specific antisera) as it has been demonstrated in studies by Lawrence (1998) and Baelmans et al. (2005). Therefore this method requires development of alloantisera which can adequately type our populations of local chickens. To start with, haplotypes/groups obtained from LEI0258 microsatellite typing can be used in initial development of serological reagents using procedures described by Juul-Madsen et al. (2006). However, since novel alleles and combination of alleles are likely to exist in these out-bred populations (local chickens), this process may result into large panel of alloantisera which could be difficult to manage. The situation can further be complicated by the problem cross-reactivity (Lawrence, 1998; Fulton et al., 2006). In this regard, therefore, manual microsatellite typing currently remains as the method of choice (Msoffe et al., 2005).

Conclusion

Significant associations existed between some LEI0258 microsatellite alleles and antibody response against NDV vaccine and body weight, hence prospects for using MAS in improving these traits in breeding programs. However, considering high costs of automated typing, and marginal additional response (based on R^2) expected from these alleles when incorporated into breeding programs, their use in developing countries could sometimes not be justified, and hence use of cheaper methods for MHC typing is required. Finally, it can be recommended that further studies on association between MHC haplotypes and phenotype should be carried out to explore other components of immune system, which are also involved in immune response to Newcastle disease. Furthermore, other diseases which are also a problem to local chickens should be considered. These studies should go further by challenging prospective MHC haplotypes/groups with the diseases to further investigate the nature of resistance.

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