GENETIC EVALUATION OF CROSSBREEDING PARAMETERS FOR IMMUNOCOMPETENCE TRAITS IN BROILERS

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INTRODUCTION

Selection experiments in different animal species indicated that it is possible to exploit the genetic differences in resistance to disease (Mallard *et al.*, 1998 ; Morris, 1998). Similar to production traits, the disease-resistance traits are lowly to moderately heritable (Sacco *et al.*, 1994 ; Morris, 1998). A number of immunocompetence traits have been reported in literature for studying the general immunity status in animals. The immune response to non-specific, natural, multi-determinant complex antigen like sheep red blood cells may provide good indicator of natural immunity status of an individual because of its broad immune characteristics, resistance for multiple pathogens, T-cell dependent response and association of the response with MHC haplotypes (Dix and Taylor, 1996 ; Dunnington *et al*., 1996). Complement is the primary humoral mediator of antigen-antibody reactions. It detects the pathogenic organisms and gets rid of them through various activities like opsonisation, phagocytosis, lysis of foreign cells, regulation of inflammatory and immune response and hence it is reported as an immunocompetence tool in literature (Chanh *et al.*, 1976 ; Skeeles *et al.*, 1980 ; Shen *et al.*, 1984). Lysozyme is considered as a non-specific bactericidal substance and its role as immunostimulant in monogastric animal and fish has been reported (Sohn *et al.*, 2000). The T-cell mediated cytotoxicity as a result of *in vivo* proliferative response of T-lymphocytes to mitogen like concanvalin-A has been used as an immunoresponse tool by many workers (Cheng and Lamont, 1988 ; Benda *et al.*, 1990). Therefore, to identify the nature of gene action for general disease resistance status in broilers, the present paper evaluates the crossbreeding parameters of four important immunocompetence traits *viz*. response to sheep red blood cells (SRBC), serum haemolytic complement level (HC), serum lysozyme level (LSZ) and *in vivo* concanavalin-A (CON) response, using a complete diallel experiment.

MATERIALS AND METHODS

Experimental design. A complete 4×4 diallel experiment, involving four synthetic broiler lines, namely Coloured Synthetic Male Line (CSML), White Synthetic Male Line (WSML), Coloured Synthetic Female Line (CSFL) and Naked Neck Line (NNL), maintained at the Experimental Broiler Farm of the Institute, was planned in the present study. Each synthetic parent line was generated independently by mass selection in each generation on the basis of six weeks body weight. All parental lines completed more than five generations of selection. In the present experiment, each purebred and crossbred genetic group was produced using 6 sires and 42 dams selected randomly from the respective parental lines. Chicks were hatched in a single hatch and uniform management practices were given. Body weight of birds was measured at six weeks of age (BW6W).

Collection of sera and injection of concanavalin-A. All experimental birds were bled twice by wing vein puncture method with fine needle. The sera collected at eight weeks of age (day zero) were used for evaluating SRBC, HC, LSZ and sera collected on day 7 were used for evaluating response for post-SRBC immunization. The injection of concanavalin-A intradermally in foot was done on day 14.

Assay of immunocompetence traits. The humoral immune response to SRBC was assayed by the method of Siegel and Gross (1980). The response was determined by the difference between the haemagglutination (HA) titre (the reciprocal of the highest dilution showing clear agglutination) on day zero and day 7 post-injection. The data were generated in term of $\log_2(n+1)$ values, where 'n' is the HA titre. The haemolytic complement activity in sera was measured according to Demey *et al.* (1993) with some modifications. The reciprocal of the highest dilution showing 50 % haemolysis was taken as the titre of the complement of the sera samples. The transformed data ($\log_2(n+1)$) could be considered as the titre that lysed 50 % of the erythrocytes (CH50 U/ml). The lysozyme assay was carried out according to the method described by Sato and Watanabe (1976). The concentrations of lysozyme in test sera samples were determined by the standard curve prepared from different concentration of egg white lysozyme in diphosphate buffer suspended with lyophilised *Micrococcus lysodeikticus*. The *in vivo* cell-mediated immune response to Concanavalin-A (Con-A) was evaluated by the method of Cheng and Lamont (1988). In this assay, the skin test was carried out by injecting Con-A intradermally in foot and measuring the increase in thickness in the skin due to delayed type hypersensitivity at the interval of 24 hours.

Statistical analyses. The data on 476 purebred and crossbred progenies of 16 genetic groups were analysed using model 3 of Harvey (1990) which included the genetic group and sex as fixed effects while sire within each paternal line as random effect. After appropriate correction of data from the previous model, the estimation of different crossbreeding parameters for immunocompetence trait was undertaken by using the diallel model given by Eisen *et al.* (1983). The model included the effects of direct genetic effect (l_i) ; maternal genetic effect (m_j), overall heterosis (h) ; line direct heterosis (h_i) ; specific combining ability (s_{ij}) and residual reciprocal effect (r_{ij}). Simple correlation coefficients between immunocompetence traits and six weeks body weight, were also calculated.

RESULTS AND DISCUSSION

The least-squares analysis of variance indicated that the genetic group had highly significant effect ($P < 0.01$) on all immunocompetence traits. The effects of sex, interaction effect of sex and genetic group and of sire within genetic group were non-significant for all traits.

From data on least-squares means of purebred and crossbred progenies, it was observed that a single purebred or commercial genetic group could not be considered as the best with respect to all immunocompetence traits studied in the present investigation. Since the genetic group effect was found significant for all immunocompetence traits, the diallel analysis of the data using complete diallel model (Eisen *et al.,* 1983) was considered for further analysis. It was observed that the effects of various crossbreeding parameters were highly significant on all immunocompetence traits. The direct genetic, maternal genetict, average heterosis, line direct heterosis and residual reciprocal effects contributed significantly to general immune responses.

Table 1. Least-squares estimates of immunocompetence traits and six weeks body weight in different purebred and crossbred genetic groups of broilers [A]

Gen.Grp.	Obs	SRBC ($log_2(n+1)$)	HC (U/ml)	LSZ (µg/ml)	CON (mm)	BW6W (g)
Pure						
1×1	31	9.25 ± 0.54^{efgh}	4.58 ± 0.18^{a}	5.38 ± 0.17^{g}	2.83 ± 0.15^{cd}	1317 ± 12^{abc}
2×2	25	4.34 ± 0.63^{a}	4.73 ± 0.19^{ab}	4.05 ± 0.18^{abcd}	2.20 ± 0.16^{ab}	1308 ± 12^{abc}
3×3	30	6.51 ± 0.59^{abcd}	5.37 ± 0.20^{bc}	4.52 ± 0.19^{cdef}	2.05 ± 0.17^{a}	1303 ± 12^{abc}
4×4	32	7.82 ± 0.54^{def}	4.42 ± 0.17^{a}	4.67 ± 0.17^{def}	3.39 ± 0.15^{e}	1397 ± 12^{f}
Cross						
1×2	25	5.43 ± 0.64^{abc}	6.37 ± 0.20^{d}	3.95 ± 0.19^{abc}	2.77 ± 0.17^{bcd}	1306 ± 11^{abc}
1×3	31	6.85 ± 0.56^{bcd}	4.86 ± 0.18^{ab}	4.61 ± 0.18^{cdef}	2.15 ± 0.16^{a}	1290 ± 13^{ab}
1×4	34	5.09 ± 0.52^{ab}	4.74 ± 0.17^{ab}	4.49 ± 0.17^{cdef}	2.17 ± 0.15^{a}	1340 ± 12^{cd}
2×1	27	9.44 ± 0.62^{fgh}	4.78 ± 0.20^{ab}	5.43 ± 0.19^{g}	2.88 ± 0.17^{cde}	1326 ± 12^{bc}
2×3	29	7.22 ± 0.58^{cde}	5.01 ± 0.19^{abc}	4.31 ± 0.18^{bcde}	2.09 ± 0.16^{a}	1320 ± 11^{abc}
2×4	31	7.45 ± 0.62^{cdef}	5.11 ± 0.21^{abc}	3.62 ± 0.20^{ab}	2.44 ± 0.17^{abc}	1322 ± 11^{abc}
3×1	32	10.09 ± 0.60^{gh}	4.43 ± 0.18^{a}	4.96 ± 0.18^{efg}	2.58 ± 0.16^{abcd}	1287 ± 11^{a}
3×2	30	6.25 ± 0.58^{abcd}	5.65 ± 0.18^{c}	3.79 ± 0.18^{ab}	2.82 ± 0.15^{cd}	1311 ± 11^{abc}
3×4	25	8.00 ± 0.68^{defg}	4.98 ± 0.21^{abc}	3.55 ± 0.21^{a}	2.64 ± 0.18^{abcd}	1340 ± 13^{cd}
4×1	28	10.59 ± 0.53^{h}	4.58 ± 0.17^{a}	5.03 ± 0.17^{fg}	2.79 ± 0.15^{cd}	1285 ± 12^{a}
4×2	32	7.31 ± 0.57^{cdef}	4.40 ± 0.19^{a}	4.58 ± 0.18^{cdef}	3.16 ± 0.16^{de}	1391 ± 12^{ef}
4×3	34	7.40 ± 0.56^{cdef}	5.30 ± 0.18^{bc}	4.09 ± 0.18^{abcd}	2.22 ± 0.15^{ab}	1374 ± 12^{de}

[A] Means with at least one common superscript columnwise do not differ significantly ($P < 0.05$). Genetic Group : 1. CSML : Coloured Synthetic Male Line ; 2. White Synthetic Male Line ; 3. CSFL : Coloured Synthetic Female Line ; 4. NNL : Naked Neck Line.

The estimates of direct genetic effect were higher for l_4 for all traits except for HC, whereas l_2 showed comparatively lower estimates for all traits. Assuming no epistasis or inter-allelic gene actions, the component of l_i includes additive and dominance direct effects of nuclear genes summed over all loci (Eisen *et al.*, 1983). Hence it can be said that significant amount of additive and dominance gene action is responsible for variation in immunocompetence traits among parental lines. In case of maternal genetic effect, the estimates of m_1 showed higher and m_4 showed lower values, respectively for all traits except for HC. The maternal genetic effect (m_i) contains additive and dominance maternal effects of genes (Gardner and Eberhart, 1966). From the least-squares analysis of variance, it was observed that the variation in m_i was higher than that of l_i for all immunocompetence traits, except for CON response, which indicated that in general, contribution of maternal genetic effect is important for expression of immunocompetence traits in purebred and crossbred progenies. The overall heterosis (h) was also found significant for all immunocompetence traits except for CON. This indicated that crossbred progenies performed better than purebred progenies and hence non-additive genetic component is also important for immunocompetence traits. The line direct heterosis component, which was estimated as a deviation from overall heterosis, was also found highly significant for all traits. The estimates of line direct heterosis showed that h_2 had better

estimates for all immunocompetence traits. The significant effect of direct line heterosis is indicative of the influence of total non-additive gene action on the immunocompetence traits in chickens. There was no trend in the estimates of specific combining ability (SCA) among the immunocompetence traits but the effect of SCA was significant for CON response.
Residual reciprocal effects (r_{ij}) are assumed to indicate the difference in maternal performance between the reciprocal crosses of two lines. In the present investigation, the residual reciprocal effect was found significant for all traits except for CON response. The estimates of correlation coefficients between the immunocompetence traits and the average six weeks body weight in purebred and crossbred groups were non-significant in all cases.

CONCLUSION

From the overall results, it may be concluded that both additive and non-additive genetic gene actions are important for the expression of immunocompetence traits in broilers. Different immunocompetence traits are probably governed by different set of genes and therefore, it is difficult to identify a single purebred or crossbred genetic group, which is ideal with respect to all traits. Hence, to achieve simultaneous improvement in several traits, the selection strategy might be directed to select individuals on the basis of optimum index values based on various immunocompetence traits (Kean *et al*., 1994) or alternatively, commercial crosses could be produced by developing parallel parental lines, each selected for a single immunocompetence trait (Pinard-van der Laan *et al*., 1998). In general, it can be said that there is possibility of using general immunocompetence traits in present broiler breeding programme, which would take into account the multiple facets of immune systems and might result into development of birds having better general disease resistance ability against pathogens.

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