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Diallel analysis of immune competence and biochemical traits in selected coloured broiler lines

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ABSTRACT

The combining ability analysis of immune and biochemical traits was studied in a full diallele mating involving 4 coloured broiler lines (NN, Naked neck; DD, Dwarf; PB-1; PB–2). The data on 320 birds (immune response) and 160 birds (biochemical traits) from 16 genetic groups was analyzed and significant parameters were further analyzed for estimation of crossbreeding parameters. The genetic group had significant influence on the antibody response to sheep red blood cells (SRBC) and *in vivo* cell mediated immune (CMI) response to phytohaemagglutinin-P (PHA-P). The entire biochemical variables except breast fat varied significantly among the crosses. The heterosis was significant for both immune response and biochemical variables studied. PB-1 as male and DD, NN, PB-2 as female lines performed better with respect to majority of the immune response and biochemical parameters. The purebred effect (PE) was significant for SRBC and PHA-P, while general combing ability (GCA) was significant for SRBC only. The results suggested that purebred effect and GCA was important in the inheritance of immune competent traits, therefore, pureline selection and crossing will improve the immune response in the coloured broilers.

Key words: Biochemical traits, Cholesterol, Diallel analysis, Immune competence, Protein

Diallel mating has been the proven method of breeding for exploitation of heterosis among the parental populations and crosses (Nath *et al.* 2007) for various economic traits. A full diallel mating is a common practice to identify superior cross combinations and nicking ability of specific cross combinations (Wearden *et al.* 1965) to suggest the breeding strategies to be followed for exploitation of additive and non additive genetic variation. The information involving Naked neck (NN), Dwarf (DD), PB-1 and PB-2 lines in a diallel mating is very much useful under tropical hot-humid climate as the first two major genelines are proved to be heat tolerant (Merat 1990, Rajkumar *et al*. 2011). The presence of naked neck (*na*) and dwarf (*dw*) genes in the crosses will be useful in the scenario of predicted increasing temperatures in the years to come. Though lot of information is available on general immune competence and biochemical traits of chicken, the data on crossbreeding parameters and combining ability analysis for immune competence and biochemical traits is limited. The full diallel mating experiment was conducted to study the heterotic effect, relative importance of general and specific combining abilities, maternal ability and sex linked effects on immune competence and biochemical traits and to find out the best cross combination with respect to immune comptence and biochemical traits.

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MATERIALS AND METHODS

Experimental birds: A full diallel crossing involving four broiler lines (NN, DD, PB-1 and PB-2) maintained at the Project Directorate on Poultry was carried out to study the combining abilities and other crossbreeding parameters. The PB-1 and PB-2 are synthetic coloured broiler lines under long term intensive selection for high 5-week body weight and 40-week part period egg production, respectively. The NN and DD are broiler based major gene lines maintained under mild selection pressure for 6-week body weight for the last seven generations. The mating was carried out with pooled semen involving 10 sires and 40 dams from each line and a total of 2280 chicks were produced in 16 different cross combinations comprising of 4 purebreds, six F_1 crossbreds and six reciprocals. A random sample of 20 birds for immune response and 10 birds for biochemical composition from each cross were selected at 6 weeks of age. All the chicks were hatched in a single hatch and reared under standard managemental practices throughout the experimental period. The chicks were fed *ad-libitum* with broiler starter (2900,

ME; 22, CP) and finisher (3000, ME; 20, CP) diets based on maize-soybean meal from 0–4 and 5–6 weeks of age, respectively. The chicks were vaccinated against Marek's disease, Newcastle disease, infectious bursal disease and fowl pox on 1st, 5th, 14th and 21st day, respectively. The experiment was approved by the Institute Animal Ethics Committee.

Immune response: The humoral (SRBC: sheep red blood cells and NDV: New Castle disease virus) and cellular (PHA-P: phyto heamagglutinin-P) immune responses were studied in 20 birds from each cross on 42 day of age.

Antibody response to SRBC: Twenty birds from each group were injected intravenously with 0.1 ml of 0.5% suspension of packed sheep red blood cells (SRBC) in normal saline on 42nd day. Five days later, blood was collected from wing vein and sera were collected and stored at – 20°C. The total antibody titre was determined by haemagglutination test (Wegman and Smithies 1966). The reciprocal of highest dilution showing complete agglutination was expressed as titre (log_2).

Antibody response to NDV: Antibody titre against NDV was determined by haemagglutination inhibition (HI) assay using 4 HA units of NDV. The highest dilution where complete inhibition of agglutination observed was read as titre (Thayer and Beard 1998) and expressed as log_2 values.

In vivo CMI response to PHA-P: The birds were injected with 100 µg PHA-P in 0.1 ml sterile saline solution in the left wattle at 6 weeks of age. The thickness was measured with a thickness gauge before and after 24 h of injection. The wattle swelling was calculated as the difference between the thickness of the wattle before and after the injection.

Biochemical traits

Biochemical traits like protein and total cholesterol levels were measured in the plasma samples of 160 birds 10 from each genetic group on 42nd day. The serum protein and cholesterol concentrations were estimated by Lowry *et al.* (1951) and Zlatkis *et al.* (1953) methods, respectively. The muscle protein and fat perecentages on dry matter basis were estimated by chemical method described by AOAC (1990).

Statistical analysis

The data were analyzed to estimate the effect of genetic and non-genetic factors on different immune competence traits and biochemical traits using least squares techniques (Harvey 1990). A mixed effect nested model was utilized with sire as random effect and genetic group, sex and their interaction as fixed effects. The traits which showed significant effect of genetic group were subjected to full diallel analysis using Henderson (1948) cited as model 8 by Harvey (1966) to estimate different crossbreeding parameters for immune competence and biochemical parameters. The mathematical model used was

$$y_{hijk} = \mu + a_h + p_{lii} + g_i + g_j + m_j + s_{ij} + r_{ij} + e_{hijk}$$

where, i(j), 1,2,…,4; k = 1,2,…n; y_{hijk} is the k^{th} observation on progeny of j^{th} dam line and i^{th} sire line in h^{th} type of breeding (purebred or crossbred); µ is the overall population mean; a_h is an effect common to all progenies of h^{th} type of breeding; p_{lii} is the purebred effect common to all progeny of a mating between i^{th} sire line and i^{th} dam line; g_i (g_j) is the general combining ability effect for the i^{th} (j^{th}) line; m_j is the maternal effect of j^{th} line of dam; s_{ij} is the specific combining ability effect in progeny of i^{th} and j^{th} line; r_{ij} is the residual reciprocal effect or sex linked effect in progeny of i^{th} sire line and j^{th} dam line; e_{ijk} is random error, NID (0, σ^2_e).

RESULTS AND DISCUSSION

Immune parameters: The antibody response to SRBC and NDV; and *in vivo* CMI response to PHA-P in crosses is summarized in Table 1. The humoral response to SRBC significantly (P≤0.05) varied among the crosses. The immune response to SRBC ranged from 4.25±1.12 log_2 (PB-1 X PB-2) to 9.12±0.63 log_2 (NN X DD), while NDV titres were not significantly different in the crosses. The cell mediated immune (CMI) response to PHA-P varied significantly (P≤0.05) in the crosses. The highest (1.35±0.08 mm) response was obtained in DDXPB-2 cross, while lowest (0.49±0.07 mm) response was recorded in PB-2 X PB-1 cross. Similar findings for SRBC and PHA-P were observed by many authors (Patra *et al*. 2004; Fathi *et al*. 2008; Rajkumar *et al*. 2010a, b) in chicken. However, Rajkumar *et al*. (2011)

Table 1. Antibody response to SRBC and NDV and *in vivo* CMI response to PHA-P in diallele crosses.

Cross	SRBC titre* (log_2)	ND titre (log_2)	*In vivo* CMI response (mm)*
NN	7.55±0.72^{ab}	8.00±0.76	0.67±0.13^{bc}
ND	9.12±0.63^{a}	7.62±0.67	0.59±0.14^{bc}
NB_1	7.37±0.67^{ab}	9.25±0.70	0.67±0.19^{bc}
NB_2	7.37±0.98^{ab}	9.00±0.98	1.38±0.21^{a}
DN	6.28±0.80^{ab}	8.28±0.91	0.57±0.11^{bc}
DD	7.57±0.75^{ab}	9.71±1.16	0.54±0.04^{c}
DB_1	7.57±0.68^{ab}	9.00±0.69	1.35±0.21^{a}
DB_2	7.62±1.03^{ab}	8.87±0.87	1.45±0.08^{a}
B_1N	5.28±1.65^{bc}	9.28±0.64	0.79±0.05^{bc}
B_1D	7.57±1.47^{ab}	9.00±0.81	0.51±0.06^{c}
B_1B_1	7.14±1.01^{ab}	9.28±0.60	0.58±0.05^{bc}
B_1B_2	4.28±1.12^{c}	9.42±0.64	0.55±0.14^{c}
B_2N	5.25±1.38^{bc}	9.37±0.73	0.55±0.11^{c}
B_2D	8.12±1.02^{ab}	8.50±0.65	0.87±0.15^{bc}
B_2B_1	7.00±0.80^{ab}	8.20±0.57	0.49±0.07^{c}
B_2B_2	7.66±0.61^{ab}	8.16±0.83	0.98±0.10^{b}
Average	7.05±0.59	8.81±0.61	0.78±0.11

* P≤0.05; Means with same superscript within the column do not differ significantly; NN, Naked neck; DD, Dwarf; B_1B_1, Pb1; B_2B_2, Pb2; ND, Naked neck × Dwarf, NB_1, Naked neck × Pb1; NB_2, Naked neck × Pb2; DN, Dwarf × Naked neck; DB_1, Dwarf × Pb1; DB_2, Dwarf × Pb2; B_1N, Pb1 × Naked neck; B_1D, Pb1 × Dwarf; B_1B_2, Pb1 × Pb2; B_2N, Pb2 × Naked neck; B_2D, Pb2 × Dwarf; B_2B_1, Pb2 × Pb1.

Table 2. Biochemical parameters in serum and muscle in diallele crosess

Cross	Serum cholesterol (mg/dl) *	Muscle Fat (g)%		Serum protein (g/dl)*	Muscle protein (g)%	
		Breast	Thigh*		Breast*	Thigh**
NN	170.64±10.33^{c}	3.01±0.31	4.94±1.07^{c}	5.97±0.32^{ab}	92.65±1.50^{a}	87.82±1.97^{ab}
ND	238.88±17.25^{abc}	3.65±0.52	8.97±1.67^{a}	5.73±0.99^{ab}	89.36±1.13^{ab}	87.44±2.05^{ab}
NB_1	198.15±10.24^{abc}	1.99±0.73	6.87±2.04^{ab}	5.88±0.14^{ab}	90.77±0.49^{a}	83.96±1.69^{ab}
NB_2	191.68±16.29^{abc}	3.82±0.59	8.47±5.10^{ab}	7.37±0.59^{a}	88.56±1.11^{ab}	80.65±5.74^{abc}
DN	208.52±9.92^{abc}	3.50±0.65	9.96±0.82^{ab}	6.25±0.29^{ab}	95.01±1.25^{a}	89.55±1.96^{a}
DD	184.41±11.21^{abc}	4.01±0.34	7.51±1.39^{ab}	5.62±0.56^{ab}	90.08±2.23^{ab}	87.20±1.24^{ab}
DB_1	251.00±6.72^{abc}	2.10±0.45	4.85±0.43^{c}	5.33±0.28^{b}	87.95±0.12^{ab}	84.95±1.92^{ab}
DB_2	279.77±9.21^{a}	1.73±0.86	12.61±1.0 ^{a}	5.37±0.65 ^{b}	80.12±0.73^{ab}	68.39±5.47^{de}
B_1N	207.68±11.77^{abc}	4.89±0.59	8.16±0.92^{ab}	7.37±0.19 ^{a}	88.41±1.85^{ab}	84.12±1.48^{ab}
B_1D	174.67±15.04^{c}	3.03±0.80	6.61±0.96^{ab}	5.46±0.09^{b}	94.48±1.21^{a}	91.12±0.10^{a}
B_1B_1	215.77±5.61^{abc}	2.62±0.67	7.70±3.21^{ab}	6.72±0.46^{ab}	85.58±3.16^{ab}	60.07±1.96^{e}
B_1B_2	187.32±14.01^{abc}	2.26±0.92	8.09±0.73^{ab}	6.44±0.10^{ab}	85.93±1.26^{ab}	81.32±0.71^{abc}
B_2N	192.01±14.93^{abc}	6.17±0.37	14.74±1.03^{a}	5.58±0.23^{ab}	73.02±1.50^{b}	73.79±3.37^{cd}
B_2D	198.29±12.36^{abc}	2.24±0.47	6.83±2.19^{ab}	7.19±0.21^{ab}	91.17±0.54^{a}	89.08±0.76^{ab}
B_2B_1	275.09±10.34^{ab}	3.14±0.16	10.83±1.53^{a}	5.67±0.61^{ab}	83.87±3.04^{ab}	78.67±6.21^{abc}
B_2B_2	225.14±14.25^{abc}	3.85±0.49	7.28±0.84^{ab}	5.60±1.38^{ab}	80.24±1.65 ^{b}	67.24±1.54^{de}
Average	212.43±8.86	3.25±0.39	8.37±0.96	6.10±0.41	87.34±1.19	80.96±1.72

* P≤0.05; ** P≤0.01; Means with same superscript within the column do not differ significantly.

reported non significant variations for SRBC and PHA-P contrary to the present results.

Serum biochemical parameters: The serum cholesterol concentration ranged from 170. 64±10.33 mg/dl in NN to 279.77±9.21 mg/dl in PB-2. The cholesterol concentration significantly (P≤0.05) differed between the crosses (Table 2). The cholesterol concentration was lower in NN similar to the earlier reports (Patra *et al*. 2002, Rajkumar *et al*. 2010a, 2011), however, the magnitude was higher in the present study. Panda *et al*. (2009) reported lower cholesterol estimates in broiler chicken which might be attributable to the breed differences and nutritional regime followed in the experiment. The average muscle fat in breast and thigh muscle was 3. 25±0.39 and 8.37±0.96%, respectively, in diallele crosses. The fat in breast meat did not show any significant variation among the crosses. The proportion of fat in thigh muscle significantly (P≤0.05) varied in the crosses, which ranged between 4.85±0.43 (DD X PB-1) and 14.74±1.03 (PB-2×NN). The muscle fat and serum cholesterol concentrations were positively correlated indicating the direct reflection of serum cholesterol in the muscle.

The protein concentration in serum and muscle significantly (P≤0.05) varied among the diallel crosses (Table 2) indicating the effect of genetic group. The average serum protein concentration (g/dl) in broiler crosses was 6.10±0.41, which ranged from 5.33±0.28 in DDXPB-1 to 7.37±0.59 in NN×PB-2 cross. The protein concentration observed were in accordance with findings of Rajkumar *et al*. (2011), however, lower estimates were reported by Panda *et al*. (2009) in broiler chicken. The breast muscle protein ranged between 73.02±1.50 (PB-2×NN) and 95.01±1.25% (DD × NN) with an average of 87.34±1.19% on dry matter basis in broiler crosses. The average thigh muscle protein was 80.96±1.72% with highest concentration (91.12±0.10) in PB-1×DD and lowest (60.07±1.96) in PB-1 pureline. The variations in the estimates might be due to the breed differences and feeding schedule followed during the experiment. The serum and muscle protein concentrations showed a direct positive relationship.

Crossbreeding parameters

Heterosis: The overall heterosis estimates were significant (P≤0.05) for all the immune and biochemical traits studied in the broiler crosses. However, the heterosis estimates between lines in a cross showed high degree of variation without any specific trend (Table 3). The high heterosis

Table 3. Heterosis estimate for various parameters in diallele crosses

Cross	SRBC	NDV	PHA-P	Serum cholesterol	Serum protein
ND	20.6	–13.9	–3.3	34.6	–1.2
NB_1	0.37	7.1	6.4	2.6	–7.4
NB_2	–3.1	11.4	66.2	–3.1	27.3
DB_1	3.1	–5.2	97.6	25.4	–13.6
DB_2	0	–0.8	90.1	36.6	–4.2
B_1B_2	–45.1	8.0	–34.2	–15.0	4.5
DN	–16.9	–6.5	–6.6	17.5	7.8
B_1N	–28.2	7.4	25.4	7.4	16.1
B_1D	3.1	–5.2	–8.9	–12.7	–11.5
B_2N	–31.3	16.0	–33.7	–2.9	3.6
B_2D	6.56	–4.9	14.5	–3.2	28.2
B_2B_1	–5.4	–6.0	–37.2	24.7	–8.0

for SRBC was observed in NN×DD cross and low in PB-1× PB-2 cross (Table 4). The high and low estimates in these crosses might be due to the high immune response in genelines (naked neck and dwarf) compared to the broilers (Rajkumar *et al*. 2010a; b). The heterosis for serum cholesterol was positive and significant (P≤0.05) indicating the relatively high cholesterol concentration in broiler birds. The negative significant heterosis for thigh muscle fat was in desirable direction as low fat chicken has the consumer preference.

Purbred effect (PE): The PE for SRBC and PHA-P was significant (P≤0.05) with higher estimate in PB-2 chicken.

Table 4. Crossbreeding parameters for immune parameters in broiler crosses

Parameter	SRBC	PHA-P
Overall mean	7.17±00.30	0.75±00.03
Heterosis	0.26±00.52*	–0.06±00.06*
Purebreds	*	*
NN	–0.06±00.98[b]	–0.05±00.12[b]
DD	0.13±01.05[ab]	–0.13±00.13[b]
PB–1	–0.29±01.05[c]	–0.10±00.13[b]
PB-2	0.22±01.13[a]	0.30±00.14[a]
General combining ability (GCA)		*
NN	0.72±00.40 [a]	0.01±00.05
DD	0.82±00.41 [a]	0.30±00.05
PB-1	–1.22±00.41 [c]	–0.22±00.05
PB-2	–0.32±00.39 [b]	–0.09±00.05
Maternal ability (MA)		
NN	–1.67±00.58	–0.17±00.07
DD	0.80±00.58	–0.36±00.07
PB-1	0.27±00.56	0.16±00.07
PB-2	1.14±00.58	0.37±00.07
Specific combining ability (SCA)		
S12	–0.15±00.69	–0.26±00.09
S13	0.18±00.71	0.12±00.09
S14	–0.03±00.69	0.13±00.09
S23	–0.03±00.77	0.13±00.09
S24	0.18±00.18	0.12±00.09
S34	–0.15±00.67	–0.26±00.08
Sex linked effects (SE)		
R12	0.06±00.98	0.08±00.12
R 13	–0.36±00.98	–0.23±00.12
R 14	0.35±00.98	0.14±00.12
R 23	–0.28±01.13	0.16±00.13
R 24	0.29±00.98	–0.07±00.12
R 34	–0.65±01.05	–0.06±00.13

*Significant at P≤0.05; Estimates with different superscripts in a column within the parameter differ significantly; SRBC, Sheep red blood cells; PHA-P, phytohaemagglutinin-P; NN, Naked neck, DD, Dwarf; PB-1, Punjab Brown-1; PB-2, Punjab Brown-2, S12, SCA of NNXDD; S13, SCA of NNxPB-1; S14, SCA of NNxPB-2; S23, SCA of DDxPB-1; S24, SCA of DDxPB-2; S34, SCA of PB-1xPB-2; R12, SE of NNXDD; R13, SE of NNxPB-1; R14, SE of NNxPB-2; R23, SE of DDxPB-1; R24, SE of DDxPB-2; R34, SE of PB-1xPB-2

The purebred effect of all biochemical parameters did not significantly varied among the crosses. All other lines recorded negative purebred effect except DD for SRBC. PB-1 and PB-2 lines had high PE for cholesterol and PB-1 for serum protein concentration. The PE for muscle protein (breast and thigh) was higher in genelines. The genetic interpretation of complete diallel model (Eisen *et al*. 1983) describes the purebred effect as the sum of direct genetic effects and average heterosis of a line while Harvey (1990) inferred it as the manifestation of high GCA or maternal effects or both.

General combining ability (GCA): The GCA reflects the amount of additive genetic variance of the breed/line. The GCA estimate of antibody response to SRBC was significant (P≤0.05) among the crosses indicating the importance of additive genetic effects in the inheritance of the trait (Table 4). The GCA estimates for SRBC and PHA-P were higher in DD followed by NN indicating the higher additive genetic variation which can be transmitted to the offsprings. The lower GCA estimates for immune traits in PB-1 and PB-2 indicated lower immune status of the broiler birds which was in accordance with the established fact that broilers are more susceptible to diseases compared to other birds (Swaggerty *et al*. 2009). The less GCA in PB-1 and PB-2 might be because of long term selection for higher body weight which has negative correlation with immune response. The GCA estimate of cholesterol and fat was lower in NN (Table 5) indicating less additiveness confirming the lower fat proportion in NN (Fathi *et al*. 2008, Rajkumar *et al*. 2010a, 2011). NN showed high additive genetic variation for serum protein, however, the muscle protein GCA estimates was higher in DD. PB-2, the broiler female line had high fat and low protein GCA estimates indicating the high fat and low protein concentration in broilers. The GCA represents the additive effects of all gametes, which was the breeding value of the individual bird. It might be considered as the summation of all the expression effects contributed by each gamete (Kiekens *et al*. 2006) in the context of gene expression.

Maternal ability (MA): The MA estimates were higher in PB-2 for SRBC and PHA-P and lower in NN chicken (Table 5). The MA for cholesterol concentration was higher in PB-1 line. The MA for muscle fat, serum protein and muscle protein was higher in PB-2 line than the other lines (Table 5). The higher MA estimates in PB-2 for all the traits indicating its suitability as female line, which was true as PB-2 was established coloured broiler female line. Barbato and Vasilatos (1991) reported that the incidence of MA in chicken was sporadic and generally of little importance beyond hatching. MA of line is proportional to the contribution of maternal, additive and dominance gene effects as well as difference in allele frequency between favorable and unfavorable alleles. MA determines the pre- and postnatal mothering ability of a line, which is a function of the genotype

Table 5. Least squares means for crossbreeding parameters for biochemical parameters in broiler crosses.

Parameter	Serum cholesterol	Muscle fat (thigh)	Serum protein	Muscle protein (breast)	Muscle protein (thigh)
Overall mean	209.28±8.15	09.49±01.16	06.06±00.16	87.79±01.40	79.16±00.84
Heterosis	6.78±14.12*	–01.89±02.01*	00.28±00.05*	–00.34±02.43*	03.59±01.47*
Purebreds					
NN	–17.84±28.24	00.33±04.33	–00.05±00.56	05.20±04.86	12.24±02.94
DD	–18.08±28.24	–00.09±04.33	–00.35±00.56	02.63±04.86	11.63±02.94
PB-1	13.27±28.24[a]	00.09±04.33	00.74±00.56	–01.86±04.86	–15.50±02.94
PB-2	22.64±28.24	–00.32±04.33	–00.37±00.56	–05.96±04.86	–08.38±02.94
GCA					
NN	–13.09±11.52	–01.35±01.64	00.31±00.23	00.62±01.98	01.32±01.20
DD	05.41±11.53	–00.37±01.64	–00.56±00.23	04.15±01.98	00.40±01.20
PB-1	21.44±11.53	–05.69±01.64	00.12±00.23	01.42±01.98	03.03±01.20
PB-2	29.12±11.53	07.42±01.64	00.12±00.23	–06.18±01.98	–04.75±01.20
Maternal ability (MA)					
NN	–06.71±16.30	03.39±02.32	00.07±00.32	–03.02±02.80	–01.14±01.69
DD	–32.87±16.30	–03.66±02.32	00.35±00.32	00.76±02.80	06.19±01.69
PB-1	39.64±16.30	–00.07±02.32	–00.59±00.32	–01.55±02.80	–02.25±01.69
PB-2	–0.7±16.30	07.13±02.32	00.16±00.32	03.81±02.80	02.79±01.69
SCA					
S12	11.38±19.96	–01.92±02.85	–00.12±00.39	00.40±03.43	01.49±02.08
S13	04.91±19.96	–00.35±02.85	00.30±00.39	01.69±03.43	–01.36±02.08
S14	-16.30±19.96	02.27±02.85	–00.18±00.39	–02.09±03.43	–00.12±02.08
S23	–16.30±11.38	02.27±02.85	–00.18±00.39	–02.95±03.43	00.12±02.08
S24	04.91±19.96	–00.35±02.85	00.30±00.39	01.69±03.43	–01.36±02.08
S34	11.38±19.96	–01.92±02.85	–00.12±00.39	00.40±03.43	
Sex linked effects (SE)					
R12	28.26±28.24	04.91±04.03	–00.39±00.56	–04.71±04.86	–04.72±02.94
R 13	–27.94±28.24	02.96±04.03	–00.40±00.56	00.45±04.86	00.46±02.94
R 14	–0.32±28.24	–07.87±04.03	00.80±00.56	04.26±04.86	04.25±02.94
R 23	03.91±28.24	–02.67±04.03	00.41±00.56	–02.10±04.86	01.13±02.94
R 24	24.35±28.24	07.58±04.03	–00.81±00.56	02.61±04.86	–05.85±02.94
R 34	–24.03±28.24	00.28±04.03	00.03±00.56	–01.65±04.86	01.59±02.94

*Significant at P≤0.05; Estimates with different superscripts in a column within the parameter differ significantly; NN, Naked neck, DD, Dwarf; PB-1, Punjab Brown-1; PB-2, Punjab Brown-2; S12, SCA of NNXDD; S13, SCA of NNxPB-1; S14, SCA of NNxPB-2; S23, SCA of DDxPB-1; S24, SCA of DDxPB-2; S34, SCA of PB-1xPB-2; R12, SE of NNXDD; R13, SE of NNxPB-1; R14, SE of NNxPB-2; R23, SE of DDxPB-1; R24, SE of DDxPB-2; R34, SE of PB-1xPB-2.

of a line rather than the genes transmitted to the male progeny of the line (Harvey 1966); the difference in egg size may be responsible for MA at early age, which reduces as age advances.

Specific combining ability (SCA): The SCA determines the degree of non-additive genetic effects in the lines, which is a result of either dominance or epistatic effects or a combination of both. The SCA estimates were positive in NNXDD and DDXPB-2 for SRBC and in NNXPB-1, NNXPB-2 and DDXPB-1 and DDXPB-2 for PHA-P response indicating the importance of non additive genetic effects. NNXPB-2 and DDXPB-1 had the negative SCA estimates for serum cholesterol revealing the low non additive nature of the trait.

Sex linked effects (SE): Least squares analysis of variance revealed non significant sex-linked effects for immune and biochemical traits indicating the less importance of 'Z' chromosome in the inheritance of these traits. Sex liked effects are determined by chromosomal as well as cytoplasmic inheritance, thus the limited effects of chromosomal inheritance for the immune and biochemical traits. The existence of reciprocal cross differences for economic traits (Nath *et al*. 2007) in broiler lines was well established. Harvey (1990) termed sex-linked effect as paternal effect, while Eisen *et al*. (1983) reported that the significant reciprocal differences indicated the presence of additive or dominant paternal effects.

The results suggest that, PB-1 as male and DD, NN, PB-2 as female lines performed better with respect to most of the immune response and biochemical parameters. Purebred effect and GCA was important in the inheritance of immune competent traits, therefore, pureline selection and crossing will improve the immune response in the coloured broilers.

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