Estimation of Crossbreeding Parameters for Serum Lysozyme Level in Broiler

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ABSTRACT : The main objective of the present study is to estimate the crossbreeding parameters in respect to serum lysozyme level in broilers. The experiment involved a complete 4×4 diallel design using 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). The lyophilised *Micrococcus lysodeikticus* suspension was used to detect the lysozyme level in the serum of birds. The data were analysed by least-squares method to find the effects of genetic and non-genetic factors using appropriate model. The crossbreeding parameters for this trait were estimated by complete diallel model assuming the effect of each synthetic line as fixed. The results indicated that additive and non-additive genetic variation attributed to minor genes at many loci is important for the genetic control of serum lysozyme level in chickens. Total non-additive components of variance also showed significant amount of heterosis in crossbred progenies, and therefore exploitation of non-additive component of variance is possible for improvement in serum lysozyme level in broilers. The overall results suggested that for commercial broiler production system, the selection for specialised line on the basis of serum lysozyme level and subsequent crossing of parent lines could enhance the immunocompetence status in relation to serum lysozyme level in crossbred chickens. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 2 : 166-171)***

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INTRODUCTION

Apart from the consideration of important production and reproduction traits, the broiler breeding programme also takes into account the general health status of the birds. Health is one of the important priorities for genetically improving the productivity of birds in varied systems of rearing (Sheldon, 2000). Genetic resistance to disease development is complex and involves several systems of the body with immune system being an important component (Warner et al., 1987; Roitt, et al., 1993). Many workers (Bacon, 1987; Gavora, 1990, 1998) reported the feasibility of improving the genetic resistance in poultry. Efficient utilisation of the genetic variation in immunoresponse in poultry breeding is therefore required an urgent attention.

Lysozyme is abundant in the body fluid and it is considered as a non-specific bactericidal substance. The estimation of serum or egg lysozyme levels in poultry (Coterill and Winter, 1954; Sato and Watanabe, 1976; Saxena, 1993) and cattle (Lie, 1980) were reported. Sohn et al. (2000) discussed the role of lysozyme as immunostimulant in monogastric animal and fish. However, reports on appropriate nature of genetic inheritance of serum lysozyme level are limited. Therefore, the present work envisages evaluation of crossbreeding parameters involved with inheritance of serum lysozyme level in different synthetic broiler lines and their crosses.

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MATERIALS AND METHODS

A complete 4×4 diallel experiment, involving four broiler lines namely Coloured Synthetic Male Line (CSML), White Synthetic Male Line (WSML), Coloured Synthetic Female Line (CSFL) and Naked Neck Line (NNL), was planned in the present study. These four synthetic lines can be assumed as independent populations according to the selection principle applied on these lines.

Experimental design

A complete 4×4 diallel experiment resulted into four purebreds, six crossbreds and six reciprocals of crossbred groups. A total of 96 sires (comprising 24 sires from each line) were used in the present experiment. For each genetic group, 6 sires and 42 dams were used and dams were randomly allotted for a particular genetic group from a line.

Management

The mating was done in the pen and all birds were provided uniform environment throughout. Eggs were collected twice each day. Dry and cleaned eggs were collected and stored for 10 days in the egg cooler before setting them in the incubator. The eggs were set in the automatic incubator and candled on 18th day of incubation. The chicks were taken out of the incubator on the 22nd day after setting the eggs. The chicks were wingbanded and vaccinated against New Castle disease (F_1 strain) vaccine immediately. All chicks in the present experiment were hatched in a single hatch.

The chicks were brooded on floor under hover type of brooder for a period of 6 weeks after which the hovers were

removed. The chicks belonging to the same genetic group were brooded together in a pen and continued in the same pen till the experiment was completed. Almost uniform numbers of chicks were maintained in each pen. The feeding and watering were provided *ad libitum*. Standard broiler management practices were followed throughout the experimental period and were kept same for all the genetic groups. The sexing was done at 6th week of age.

Lysozyme assay

The lysozyme assay was carried out according to the method described by Sato and Watanabe (1976) among 476 birds at the age group of eight to ten weeks from 16 genetic groups including 4 purebreds and 12 crossbreds (approximately 25 to 30 birds in each group).

Preparation of standard curve : A stock solution of 6 mg / ml of Egg White lysozyme (Sigma) was diluted to 0.0024 mg / ml in diphosphate buffer (0.066 M, pH 6.3). The above concentration of lysozyme was further serially diluted by two fold dilutions. Then, 0.5 ml of different concentrations of lysozyme was added to set of tubes containing 5 ml aliquots of lyophilised *Micrococcus lysodeikticus* (Sigma) suspension. The tubes were then kept in water bath at 37°C for 20 minutes. Then the optical densities were recorded at 520 nm. The curve was plotted between concentration of lysozyme and optical density.

Quantitation of serum lysozyme : The Lyophilised *Micrococcus lysodeikticus* (200 µg) was suspended into 1 ml of diphosphate buffer to obtain an optical density of 0.70 at 520 nm. In test tubes, 2 ml of *Micrococcus lysodeikticus* suspension (OD 0.70 at 520 nm) was taken. Then 200 µl of fresh test serum was added to that suspension in each tube. The test tubes were kept at 37°C for 20 minutes. The optical density readings were taken after 20 minutes at 520 nm wavelength. The concentrations of lysozyme in test sera samples were determined by the standard curve.

Statistical analyses

The data were analysed for the estimation of effects of genetic and non-genetic factors on serum lysozyme level under study, using least-squares techniques (Harvey, 1990). The mathematical model was used as follows:

$Y_{ijkl}=\mu+g_i+(s:g)_{ij}+c_k+(g\times c)_{ik}+e_{ijkl}$

Where,

Y_{ijkl}=value of trait under study on the l^{th} individual in i^{th} genetic group, j^{th} sire and k^{th} sex

μ=the overall population mean

g_i=the effect of i^{th} genetic group (i=1,2...,16)

$(s:g)_{ij}$=the random effect of the j^{th} sire within i^{th} genetic group

c_k=the effect of k^{th} sex

$(g\times c)_{ik}$=the interaction between i^{th} genetic group and k^{th} sex

e_{ijkl}=random error with NID $(0, \sigma^2_e)$

Besides, the estimation of different crossbreeding parameters for serum lysozyme level was undertaken by using the diallel model given by Eisen et al. (1983). The statistical model is given by:

$Y_{ijk}=\mu+\frac{1}{2}l_i+\frac{1}{2}l_j+m_j+\delta(h+h_i+h_j+s_{ij}+r_{ij})+e_{ijk}$

Where,

i(j)=1,2.....p; k=1,2,...n

Y_{ijk}=the k^{th} observation on the progeny of a mating of dam from the j^{th} line with a sire of i^{th} line

μ=the overall population mean

l_i (l_j)=the average direct line effect of the i^{th} (j^{th}) line

m_j=the average maternal genetic effect of the j^{th} line

h_{ij}=the direct heterosis obtained by crossing lines i and j = $h+h_i+h_j+s_{ij}$

h=the average or overall heterosis

h_i(h_j)=the line direct heterosis of the line i (j)

s_{ij}=the specific combining ability obtained by crossing lines i and j

r_{ij}=the residual reciprocal effect in the cross i and j

δ=0 for parental line progenies (i=j) and δ=1 for crossbred progenies ($i\neq j$)

e_{ijk}=the random error with NID $(0, \sigma_e^2)$

Restrictions imposed on the parameter estimates are:

$\Sigma l_i=\Sigma m_j=\Sigma h_i=\Sigma s_{ij}=\Sigma s_{ji}=\Sigma\Sigma s_{ij}=\Sigma r_{ij}=\Sigma r_{ji}=r_{ij}+r_{ji}=0$

The estimates of parameter z_i, w_{ij} and net line effect were calculated according to Eisen et al. (1983).

RESULTS AND DISCUSSIONS

The least-squares analysis of variance for the serum lysozyme level and the corresponding least-squares means and standard errors for combined sex of different genetic groups are presented in table 1 and 2, respectively.

The least-squares analysis of variance revealed that the genetic group had highly significant effect ($p<0.01$) on the serum lysozyme level in chicken. The other sources of variation were found to be non-significant. The significant differences among genetic groups have been reported for egg lysozyme by Coterrill and Winter (1954) and Pal (1992). However, Saxena (1993) observed significant sire and strain differences in the serum lysozyme level in guinea fowls.

Table 1. Least-squares analysis of variance for serum lysozyme level in broiler

Source of Variation	df	Mean Sum of Squares
Genetic group	15	9.00**
Sire: Genetic group	78	0.87
Sex	1	1.05
Sex×Genetic group	15	0.94
Error	366	0.64

** $p<0.01$.

Table 2. Least-squares estimates for serum lysozyme level in different genetic groups of pure and crossbred broilers (combined sex)

Genetic group	Code	Serum lysozyme level (μg/ml)
Purebred		
CSML×CSML	11	5.38 ± 0.17^{g}
WSML×WSML	22	4.05 ± 0.18^{abcd}
CSFL×CSFL	33	4.52 ± 0.19^{cdef}
NNL×NNL	44	4.67 ± 0.17^{def}
Crossbred		
CSML×WSML	12	3.95 ± 0.19^{abc}
CSML×CSFL	13	4.61 ± 0.18^{cdef}
CSML×NNL	14	4.49 ± 0.17^{cdef}
WSML×CSML	21	5.43 ± 0.19^{g}
WSML×CSFL	23	4.31 ± 0.18^{bcde}
WSML×NNL	24	3.62 ± 0.20^{ab}
CSFL×CSML	31	4.96 ± 0.18^{efg}
CSFL×WSML	32	3.79 ± 0.18^{ab}
CSML×NNL	34	3.55 ± 0.21^{a}
NNL×CSML	41	5.03 ± 0.17^{fg}
NNL×WSML	42	4.58 ± 0.18^{cdef}
CSML×CSFL	43	4.09 ± 0.18^{abcd}

1. CSML: Coloured Synthetic Male Line; 2. White Synthetic Male Line; 3. CSFL: Coloured Synthetic Female Line; 4. NNL: Naked Neck Line.
Means having at least one common superscripts columnwise do not differ significantly ($p<0.01$).

From the least-squares means, it was observed that CSML (line 1) had the highest level of serum lysozyme (5.38±0.17 μg/ml) among the purebreds, which was also significantly different from the other purebreds.

Among the crossbreds, the highest level of serum lysozyme was noted in the cross 2×1 (5.43±0.19 μg/ml), which was not significantly different from the crosses 3×1 (4.96±0.18 μg/ml) and 4×1 (5.03±0.17 μg/ml). It was observed that line 1 as dam had higher level of serum lysozyme in its cross progenies.

The mean serum lysozyme level in the present investigation ranged from 3.55 to 3.43 μg/ml. The estimates were higher than the earlier studies reported in chicken populations (Greenfield and Bigland, 1971; Saxena, 1993), which might be due to effect of age, sex or strain of birds involved.

Since the genetic group effect was found significant for the serum lysozyme level, the diallel analysis of the data using complete diallel model (Eisen et al., 1983) was considered for further analysis. The least-squares analysis of variance under the model of Eisen et al. (1983) for the serum lysozyme level is presented in table 3. The least-squares means and standard errors of different crossbreeding parameters for the serum lysozyme level are presented in table 4. The estimates of z_i, w_{ij} and net line effect are given in table 5.

Table 3. Least-squares analysis of variance for serum lysozyme level using complete diallel model of Eisen et al. (1983)

Source of variation	df	Mean Sum of Squares
Direct genetic effect	3	0.11**
Maternal genetic effect	3	0.47**
Overall heterosis	1	0.25**
Line direct heterosis	3	0.10*
Specific combining ability	2	0.04
Residual reciprocal effect	3	0.21**
Pooled error	460	0.02

* $p<0.05$, ** $p<0.01$.

On perusal of the above tables, it was observed that the effects of various crossbreeding parameters were highly significant on the serum lysozyme level. The average direct line effect, average maternal effect, average or overall heterosis, line direct heterosis and residual reciprocal effects were found to contribute significantly to serum lysozyme level in chicken.

The significant effect of average direct line and average maternal genetic effect indicated the importance of additive and dominance gene action, while the significant average and direct line heterosis indicated the influence of total non-additive gene action on the serum lysozyme level in chickens.

Lie (1980) and Bessarabov and Krykanov (1985) reported that additive genetic control is important for the trait. On the other hand, Saxena (1993) observed that a partial dominance along with additive genetic variation was important for the serum lysozyme level in birds. All these studies are in agreement with the present findings.

The least-squares estimates of average direct line effect showed that additive genetic effect along with dominance effect might be important for the inheritance of serum lysozyme level in chicken. The line 4 contributed most significantly to the total variation due to lysozyme level and this line can be used as one of the parents for better exploitation of average direct line effect in crossbred progenies.

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Assuming no epistasis or inter-allelic gene actions, the l_i

Table 4. Least-squares estimates and standard error of different crossbreeding parameters for serum lysozyme level using complete diallel model of Eisen et al. (1983)

Parameter	Symbol	Least-squares Estimates
Overall mean	μ	4.66±0.07
Direct genetic effect	l_1	0.13±0.16^{ab}
	l_2	-0.35±0.16^{a}
	l_3	-0.31±0.16^{a}
	l_4	0.53±0.16^{b}
Maternal genetic effect	m_1	0.59±0.09^{c}
	m_2	-0.26±0.09^{a}
	m_3	0.18±0.09^{b}
	m_4	-0.51±0.09^{a}
Overall heterosis	h	0.29±0.08
Line direct heterosis	h_1	0.20±0.09^{b}
	h_2	0.17±0.09^{b}
	h_3	-0.16±0.09^{a}
	h_4	-0.22±0.09^{a}
Specific combining ability	s_{12}	-0.11±0.06
	s_{13}	0.07±0.06
	s_{14}	0.04±0.06
	s_{23}	0.04±0.06
	s_{24}	0.07±0.06
	s_{34}	-0.11±0.06
Direct heterosis	h_{12}	0.55±0.10^{b}
	h_{13}	0.40±0.10^{b}
	h_{14}	0.31±0.10^{b}
	h_{23}	0.34±0.10^{b}
	h_{24}	0.31±0.10^{b}
	h_{34}	-0.20±0.10^{a}
Residual reciprocal effect	r_{12}	-0.31±0.07^{a}
	r_{13}	0.03±0.07^{b}
	r_{14}	0.28±0.07^{b}
	r_{23}	0.04±0.07^{b}
	r_{24}	-0.35±0.07^{a}
	r_{34}	0.07±0.07^{b}

Means having at least one common superscripts columnwise do not differ significantly ($p<0.01$).

includes additive and dominance direct effects of nuclear genes summed over all loci, while the m_i contains additive and dominance maternal effects of genes (Gardner and Eberhart, 1966; Eisen et al., 1983). Variation among lines in m_i was higher than in l_i for serum lysozyme level. The results showed the importance of maternal genetic effects and indicated that transmitted genetic effects are equally as important for the serum lysozyme level.

Table 5. Summary of z_i, w_{ij} and net line effect (n_i) for serum lysozyme level

Parameters		Estimate±SE
z_i	z_1	-0.01±0.05^{b}
	z_2	-0.02±0.05^{b}
	z_3	-0.19±0.05^{ab}
	z_4	-0.22±0.05^{a}
w_{ij}	w_{12}	-0.001±0.02^{a}
	w_{13}	-0.01±0.02^{a}
	w_{14}	0.02±0.02^{a}
	w_{23}	0.01±0.02^{a}
	w_{24}	0.01±0.02^{a}
	w_{34}	0.19±0.02^{b}
n_i	n_1	0.38±0.02^{b}
	n_2	-0.09±0.02^{a}
	n_3	-0.15±0.02^{a}
	n_4	-0.14±0.02^{a}

Means having at least one common superscripts columnwise do not differ significantly ($p<0.01$).

The overall heterosis (h) was significant for the serum lysozyme level in broilers. This indicated that crossbreds performed better than the purebreds and hence non-additive genetic component is also important for serum lysozyme level.

The line direct heterosis component, which was estimated as a deviation from overall heterosis, was also found highly significant for serum lysozyme level in chicken. The highest estimate of line direct heterosis was observed in line 1 (0.20±0.09), which did not differ significantly from that of line 2 (0.17±0.09).

Line direct heterosis has the clearest genetic interpretation when expressed as z_i (Casas and Wellhausen, 1968). The estimate of z_i (table 5) showed that the lowest and negative estimate of z_i was observed for the line 4 (z_4), which was not significantly different from that of z_3. The highest estimate of z_i was observed in line 1, though the direction was negative. The low and negative direction of z_i values for serum lysozyme level in chicken indicated the deviations from mean gene frequency and dominant effects were non-significant.

The effect of specific combining ability of a cross (s_{ij}) was not significant for the serum lysozyme level. The highest estimate of s_{ij} was observed in the crossbred progenies of 1×3 and 2×4, while the lowest estimate was noted in 1×2 and 3×4.

Values of w_{ij} (table 5) provide a more definitive picture of genetic effects than s_{ij}, it being function of dominance effects and deviation of parental line gene frequency from mean gene frequency (Eisen et al., 1983). The highest estimate of w_{ij} was observed in the cross 3×4 (0.19±0.02).

which was significantly different from other estimates of w_{ij}. The lowest estimate of w_{ij} was observed in the cross 1×2 (-0.001±0.02), which was not significantly different from other estimates of w_{ij} except w_{12}. It can be noted that most of these estimates are positive in direction or almost close to zero. The negative estimates of w_{ij} generally indicate that for loci contributing dominance effects, the gene frequency deviation in each parental line were of opposite sign (Eisen et al., 1983).

The estimates of direct heterosis for the serum lysozyme level were significant for all the crosses except 3×4. Percent direct heterosis (estimated as 100 times h_{ij} divided by mid-parent mean) for progenies of cross 1 and 2 was the highest (5.3%). The percent heterosis among the crosses ranged between 3.0 to 5.3% except the cross 3×4, which showed the negative heterosis.

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 the serum lysozyme level. Significant reciprocal effects are due to differences between lines in gene frequency for loci contributing additive or dominance maternal genetic effects (Eisen et al., 1983).

A perusal of the estimates of residual reciprocal effects showed that the dams of line 4 had superior performance in crossbred progenies in respect to the serum lysozyme level except with line 2. In general, the difference in maternal performance for a particular line was not uniform and differed among crosses significantly.

Gregory et al. (1978) suggested that the net line effect is the best estimate of the contribution of a line in crosses, where means were calculated by excluding the parental lines. Net line effect (table 5) for the serum lysozyme level was the highest in line 1, which was significantly different from net line effects of others. The net line effects in descending order for other lines were lines 2, 4 and 3, which did not differ significantly among themselves. Expectation of net line effects as a deviation from the mean of all crosses incorporates average maternal genetic effects with general combining ability. The highest estimate of net line effect for the line 1 supported this statement. The line 1 showed the highest estimate of maternal genetic effect, which is mentioned earlier. The magnitude of general combining ability for line 1 was determined by additive direct line effects, dominance direct effects, the deviation of gene frequency of the line from mean gene frequency at each locus, and the number of lines in the diallel.

CONCLUSION

It can be concluded that the lysozyme level in serum is genetically controlled both by additive and dominance components of genes, since direct line effect was significant for the trait. Total non-additive components of variance also showed significant amount of heterosis in crossbred progenies, and therefore exploitation of non-additive component of variance is possible for improvement in serum lysozyme level in broilers.

The present investigation indicated clearly that additive and non-additive genetic variation attributed to minor genes at many loci is important for the genetic control of serum lysozyme level in chickens. Both direct line effect (l_i) and maternal effect (m_i) were important for the serum lysozyme level in broilers. It can be said that since the study was undertaken comparatively at younger age, therefore further works could be carried out to confirm the importance of maternal effect in chickens at later stage of life. The overall heterosis for serum lysozyme level is significant. However, the expression of heterosis for a specific cross, depended on the particular genes which showed dominance effects. Direct heterosis of a cross was associated with the significant line heterosis and negative estimates of w_{ij}, the negative sign being the deviation of gene frequency in opposite directions for each line. The overall results suggested that for commercial broiler production system, the selection for specialised line on the basis of serum lysozyme level and subsequent crossing of parent lines could enhance the immunocompetence status in relation to lysozyme level in crossbred chickens.

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