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Evaluation of immune organ weight in a complete 3x3 diallel cross of Indian native chicken breeds with CARI-Red

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Abstract
A full 3x3 diallel cross of Aseel Peela (AP), Kadaknath (KN) and CARI-Red (CR) were used for comparison of performance of pure and crossbred chickens for immune organ weight and to estimate the relative importance of different types of gene action involved in the inheritance of this trait. Two different models commonly used for diallel analysis of poultry data were employed in this investigation. The analyses of variance among genetic groups revealed significant difference between crossbred and purebred in male, female and combined sex. Model B did not yield any result in case of reciprocal effects for immune organ except in combined sex for spleen, bursa and thymus percentage.

Keywords: Diallel cross, chicken breeds, SCA, GCA, immune organ weight

1. Introduction
Native breeds are reservoir of various major genes. Server researchers are trying to exploit their potential towards various poultry sector. Along with the production and reproduction traits, poultry breeding programme should also takes into account for general health status of the birds. Genetic disease resistance is complex and involves several systems of the body with immune system being an important component (Warner *et al.*, 1965; Male and Roitt, 1993) [7, 4]. Immune organs are main for storage of lymphocytes as well as their maturation. Thus, the collective weight of immune organs is a reliable measure of immune response as it offers the delineative picture by neglecting the variations in individual organs. The spleen is the largest peripheral lymphoid organ in chickens, and it plays a significant role in both antibacterial and antiviral immune responses against acquired antigens. Thymu also play a defined role in antibody response to antigens The bursa of Fabricius is the primary lymphoid organ in avian species. The bursa of Fabricius of birds has an essential role as a central lymphoid organ for the differentiation of B lymphocytes (Cooper *et al*., 1966) [1]. Review of immune-competence status suggested that high producing exotic stocks are least efficient with respect to immune system in comparison to native breeds. Therefopre efficient utilisation of the genetic variation in immunoresponse in poultry breeding requires an urgent attention. There is lack of systematic studies on crossbreeding parameters of immune organ in Indian native chicken breeds. Moreover, complete diallel mating system is the most efficien t to provide detail information about cross breeding parameters. Therefore, the current study aimed to estimate the cross breeding genetic parameters for immune organ weight (%) from a complete 3x3 diallel experiment using Aseel Peela, Kadaknath and CARI-Red and to find out the best cross combination to understand the additive and non-additive gene actions involved in immune organ weight (%) trait.

2. Materials and Methods
2.1 Experimental birds & Mating plan
2.1.1 Kadaknath (KN): The Kadaknath birds reveals appreciable degree of resistance to diseases compared to other exotic breeds of fowl, however it is more susceptible to Marek's disease under intensive rearing conditions, Kadaknath birds are also resistant to extreme climatic conditions like summer heat and cold winter stress.

2.1.2 Aseel Peela (AP): Aseel is a famous bird of Indian native chicken and is well known for its pugnacity, high stigma, majestic gait, dogged fighting and for their excellent meat

producing qualities. It is biggest in size among all the Indian native chickens, which measure 28 inches from back to toe.

2.1.3 CARI-Red (CR): This exotic fowl is dual purpose breed with heavy body weight. It is developed at Humboldt University, Berlin, Germany. The bird is adapted for temperate climatic condition. The most common colour is red, single comb and popular for brown shelled egg production.
Aseel Peela, Kadaknath and CARI-Red were utilized in a 3x3 full diallel cross experiment which resulted into three crossbred, three reciprocal and three purebred genetic groups (table 1).

Table 1: Mating design and genetic groups

Male Female	AP (23)	KN (20)	CR (16)
AP (138)	AP x AP	KN x AP	CR x AP
KN (120)	AP x KN	KN x KN	CR x KN
CR (96)	AP x CR	KN x CR	CR x CR

As per the mating plan, the hens were inseminated by intravaginal technique. First and Second insemination was done after a day interval and thereafter insemination was repeated after every five days till the required number of eggs were obtained from each genetic group. The chicks were brooded up to 6 weeks of age in four tiers battery cage brooder following standard brooding management practices, then shifted to grower house and managed with ad libidum feeding and watering up to 15 wk of age.

2.2 Measurement of traits
Immune organ weights (Thymus, Spleen, and Bursa of fabricius) were evaluated in 72 birds i.e. 8 from each genetic group (4 male and 4 female) after 15 wks of age. Birds were weighed before fasting and were starved for nearly 12 hours but water was provided ad libidum. On following morning, the birds were weighted and then sacrificed as per the standard practice. The different percent yields were calculated by digital weighing machine.

3. Result and Discussion
3.1 Performance of purebred and crossbred progeny
Cross bred CR x AP showed higher value for spleen weight % in case of female and combined sex. Cross bred CR x AP also showed higher value for bursa as well as thymus in case of female and combined sex (Table 2). According to Mayor, M. (2021) at 12 weeks, CR purebred showed significantly higher (p<0.001) spleen weight followed by CSML X CR while CSML purebred had the least relative spleen weight. Similar results were also reported by Thapa, (2018) [6]. The analyses of variance among genetic groups are revealed significant difference between crossbred and purebred in male, female and combined sex (table 3). The means and standard error for different mating groups are summarized in table (4), which revealed that no significant difference between crossbred and purebred at irrespective of sex.

Table 2: Mean ± S.E. of immune organ weight (%) in different genetic groups

		Weight (%)	
GG	Spleen (%)	Bursa (%)	Thymus (%)
	Male		
AP x AP	0.14±0.004^{bc}	0.12±0.004^{b}	0.13±0.003^{c}
KN x AP	0.13±0.004^{ab}	0.12±0.004^{b}	0.13±0.006^{abc}
CR x AP	0.14±0.003^{bc}	0.12±0.00^{b}	0.13±0.004^{bc}
AP x KN	0.14±0.003^{c}	0.12±0.003^{b}	0.13±0.003^{abc}
KN x KN	0.12±0.003^{a}	0.10±0.003^{a}	0.12±0.003^{a}
CR x KN	0.13±0.004^{ab}	0.11±0.004^{b}	0.12±0.003^{ab}
AP x CR	0.13±0.003^{abc}	0.11±0.005^{b}	0.13±0.004^{bc}
KN x CR	0.13±0.004^{ab}	0.11±0.004^{b}	0.12±0.006^{ab}
CR X CR	0.14±0.003^{c}	0.12±0.004^{b}	0.13±0.004^{bc}
	Female		
AP x AP	0.14±0.004^{bcd}	0.12±0.004^{ab}	0.14±0.005^{c}
KN x AP	0.12±0.004^{a}	0.11±0.005^{a}	0.12±0.004^{ab}
CR x AP	0.15±0.004^{cd}	0.14±0.005^{c}	0.14±0.004^{c}
AP x KN	0.12±0.003^{a}	0.12±0.003^{a}	0.12±0.005^{ab}
KN x KN	0.12±0.004^{a}	0.11±0.004^{a}	0.11±0.004^{a}
CR x KN	0.14±0.003^{bc}	0.12±0.003^{ab}	0.13±0.005^{bc}
AP x CR	0.13±0.006^{ab}	0.12±0.006^{ab}	0.12±0.003^{ab}
KN x CR	0.12±0.005^{a}	0.11±0.005^{a}	0.11±0.004^{a}
CR X CR	0.15±0.004^{d}	0.13±0.004^{b}	0.14±0.003^{c}
	Combined sex		
AP x AP	0.14±0.004^{cde}	0.12±0.004^{c}	0.13±0.003^{cde}
KN x AP	0.14±0.004^{cde}	0.12±0.004^{c}	0.14±0.005^{de}
CR x AP	0.13±0.004^{bc}	0.12±0.004^{c}	0.13±0.006^{abcd}
AP x KN	0.12±0.004^{ab}	0.11±0.005^{bc}	0.12±0.004^{abc}
KN x KN	0.14±0.003^{cd}	0.12±0.000^{c}	0.13±0.004^{bcde}
CR x KN	0.15±0.004^{e}	0.14±0.005^{d}	0.14±0.004^{e}
AP x CR	0.14±0.003^{de}	0.12±0.003^{bc}	0.13±0.003^{abcd}
KN x CR	0.12±0.003^{a}	0.11±0.003^{b}	0.12±0.005^{ab}
CR X CR	0.12±0.003^{ab}	0.10±0.003^{a}	0.12±0.003^{a}

Mean bearing common superscript column wise do not differ significantly (p<0.05)

Table 3: Analysis of variance for immune organ weight (%) among genetic group

Source of variation	d.f	Spleen (%)	Bursa (%)	Thymus (%)
		Male		
Between genetic group	8	0.00018**	0.00027**	0.00017*
Error	27	0.000044	0.000055	0.000071
		Female		
Between genetic group	8	0.00066**	0.00049**	0.00055**
Error	27	0.000069	0.000073	0.000068
		Combined sex		
Between genetic group	8	0.00057**	0.00056**	0.00057**
Error	63	0.000083	0.000089	0.00008

*Significant at P<0.05; ** Significant at P<0.01.

Table 4: Mean ± S.E for immune organ weight (%) in different mating system

		Organ weight (%)		
SV	**N**	**Spleen (%)**	**Bursa (%)**	**Thymus (%)**
		Male		
Pure breds	12	0.135±0.003[a]	0.112±0.004[a]	0.126±0.003[a]
crossbred	24	0.133±0.002[a]	0.115±0.002[a]	0.124±0.002[a]
		Female		
Pure breds	12	0.137±0.004[a]	0.12±0.003[a]	0.128±0.004[a]
crossbred	24	0.129±0.003[a]	0.119±0.003[a]	0.122±0.003[a]
		Combined sex		
Pure breds	24	0.14±0.003[a]	0.116±0.003[a]	0.127±0.003[a]
crossbred	48	0.131±0.002[a]	0.117±0.002[a]	0.123±0.002[a]

Mean bearing common superscript column wise do not differ significantly (p<0.05)

3.2 Estimation of crossbreeding genetic parameters by complete diallel analysis

Since the preliminary analysis revealed significant difference between the genetic groups for immune organ weight traits. Therefore, the data were subjected to further analysis using two different models of diallel analysis to estimate the relative importance of different type of gene action involved in the inheritance of this trait under consideration i.e. Model A-Griffing (1956), method 1 under model 1 and Model B-Hyman (1954) as given by Wearden (1964)

3.2 a Analysis of variance for combining ability and other effects

The analyses of variance for body immune organ (spleen, bursa and thymus) revealed highly significant difference for general combining ability at 15 wk of ages in male, female and combined sex in case of model A. In case of model B the GCA value are similar to GCA value of model A (table 5 and 6). The analyses of variance for SCA of body immune organ (spleen, bursa and thymus) are presented in table (5 and 6), for model A and model B respectively, which revealed significant difference for Spleen and bursa percentage at 15 wks of ages in male and female. There was no significant difference for spleen, bursa and thymus in combined sex. No significant difference was observed in case of thymus percentage, in irrespective of sex. Reciprocal effects were significant under model A (table 5) in female and combined sex for spleen, bursa and thymus weight percentage. No significant reciprocal effects were observed in male. Model B did not yield any result in case of reciprocal effects for immune organ except in combined sex for spleen, bursa and thymus weight percentage (table 6).

Table 5: Analysis of variance for combining ability of immune organ using model-A

Sv	**d.f**	**Spleen (%)**	**Bursa (%)**	**Thymus (%)**
		Male		
GCA	2	0.000086**	0.00018**	0.00016**
SCA	3	0.000037*	0.000048*	0.000011
RE	3	0.000027	0.000014	0
Error	27	0.00001	0.000013	0.000017
		Female		
GCA	2	0.00045**	0.00029**	0.00027**
SCA	3	0.000047*	0.000039	0.000029
RE	3	0.000094**	0.000093**	0.00016**
Error	27	0.000016	0.000017	0.000016
		Combined sex		
GCA	2	0.00022**	0.00022**	0.00021**
SCA	3	0.000016	0.0000037	0.000012
RE	3	0.000032*	0.000043*	0.000039*
Error	63	0.0000095	0.00001	0.0000091

*Significant at P<0.05; ** Significant at P<0.01.

Table 6: Analysis of variance for combining ability of immune organ using model-B

s.v	**d.f**	**Spleen (%)**	**Bursa (%)**	**Thymus (%)**
		Male		
a	2	0.00007**	0.0001**	0.00007**
b	3	0.000006*	0.00006*	0
c	2	0.00002	0.00002	0
d	1	0.00003	0.00003	0
error	27	0.00004	0.00006	0.00007
		Female		
a	2	0.0004**	0.0003**	0.0004**
b	3	0.00009*	0.000006	0.00009
c	2	0.0002	0.00012	0.0002
d	1	0.0001	0.0001	0.0001
error	27	0.000069	0.00007	0.00007
		Combined sex		
a	2	0.00005**	0.00004**	0.00002**
b	3	0	0.00009	0.00002
c	2	0.00005	0.00012	0.00007
d	1	0.0008**	0.0004*	0.0004**
error	27	0.00008	0.00009	0.00008

*Significant at P<0.05; ** Significant at P<0.01.
(a= Parental line, b = Genetic interaction, c = Maternal effect, d = Reciprocal effect)

3.2 b Estimation of different cross breeding genetic parameters on chicken

Different cross breeding genetic parameters were estimated by using model A. The effect of GCA, SCA and RE of different cross breeding parameters for immune organ are presented in table (7). On perusal of the table the CR had highest and positive GCA value in female and combined sex but AP had higher CGA in case of male. While, KN had lowest and negative value of GCA for immune organ percentage in male, female as well as combined sex. Mayur, M. (2021) reported that GCA variances for immune organ weight at both the ages (8 and 12 wks) were non-significant and Desi yielded higher and positive GCA estimates followed by CSML, while CARI-Red being negative at both the ages. Variances for SCA differed significantly (P<0.001) for immune organ weights were also reported by Mayur, M.,

2021 at 12 wks of age. Reciprocal effects value was obtained by model A. Positive but negligible reciprocal effects were observed in immune organ weight (%) in all crosses of measurement irrespective of sex.

Table 7: Mean of GCA, SCA and RE of different cross breeding parameters for immune organ using model –A

Parameters			Male			Female		Combined sex		
		Spleen (%)	Bursa (%)	Thymus (%)	Spleen (%)	Bursa (%)	Thymus (%)	Spleen (%)	Bursa (%)	Thymus (%)
GCA	g1	0.002	0.004	0.004	0.001	0.001	0.004	0.002	0.002	0.004
	g2	-0.004	-0.006	-0.006	-0.009	-0.008	-0.008	-0.007	-0.006	-0.006
	g3	0.001	0.000	0.001	0.007	0.006	0.003	0.005	0.004	0.002
SCA	S12	-0.004	-0.003	0.000	-0.001	0.005	-0.004	-0.002	0.005	-0.002
	S13	-0.001	0.001	-0.003	-0.002	-0.003	-0.001	-0.001	-0.001	-0.002
	S23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
RE	r12	0.001	0.004	0.000	0.010	0.011	0.013	0.006	0.007	0.006
	r13	0.000	0.000	0.000	0.006	0.003	0.009	0.003	0.001	0.004
	r23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*(1= AP, 2 = KN, 3 = CR)

4. Conclusion

Analysis under Griffing's model provides significant GCA and SCA suggesting that the improvement of crossbreds may be brought about by RRS method of selection. Additive gene action was slightly more importance as compared to non-additive gene action for inheritance of immune organ weight percentage. Desi and CARI-Red derived crosses had the superior immune organ weight.

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