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Calcium dependent or independent pathway for antibody response against sheep red blood cells in various indigenous and imported breeds of chicken

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ABSTRACT

Birds (433), 7-week-old, of both sexes belonging to Indian native breeds including Aseel, Kadakanath, Naked Neck and Frizzle fowl along with the exotic breeds like Dahlem red, White Leghorn, Synthetic Dam Line (SDL) and Naked Neck broiler were utilized to test the complement response (expressed as CH_{50} unit/ml) to sheep erythrocytes determined by means of both calcium dependent pathway or classical pathway (CPW) and calcium independent pathway or alternate pathway (APW). The effect of genotype (breed), sex and their interactions on antibody response was also studied. The results revealed the presence of natural CPW and APW antibodies on day of immunization in all groups under study irrespective of sex. The natural APW response was highest in Aseel and lowest in broilers. The highest CPW responses were observed in Dahlem red and Aseel followed by Kadakanath, Naked Neck, White Leghorn, Frizzle, SDL broiler and NN broiler on day 5 p.i. Aseel had the highest level followed by Kadakanath, but Dahlem Red showed much regressed value on day 12 p.i. and also on day 19 p.i. In all the genetic lines peak response was observed on 5th day p.i. and declined thereafter. Aseel, which showed highest response on day 5 p.i. for APW response also had the highest value on day 12 p.i. and 19 p.i. and lowest response was observed in broilers. Males excelled the females for APW response at all days of measurements post immunization in all genetic groups except Dahlem Red and NN broiler on 12th day p.i. Statistical analysis revealed significant variation in CPW and APW response among the various genetic groups and this remained consistent in all days of post immunization i.e. on day 0,5,12,19 p.i. Sex effects were not significant in any days of p.i. for C.P.W response. Sex effects were significant on days 12 and 19 p.i. only. However, interaction between breed and sex were significant on days 5, 12, and 19 p.i. for CPW and for APW, on days 0 and 19 p.i. It is concluded that the chicken populations utilized in this study differed widely with respect to their immunocompetence traits, on different days of post SRBC injection and observed higher antibody than young birds.

Key words: Antibody, Calcium dependent or classical pathway (CPW), Calcium independent pathway or alternate pathway (APW), Complement, Indigenous chicken

Indian native breeds of chicken have better general disease resistance for many tropical diseases than the exotic breeds as they evolved through natural selection over a long period in the tropical environment. Breed variations in humoral antibodies of native Indian chickens and exotic chickens were reported (Kundu *et al.* 1996, Kundu *et al*. 1999, Singh *et al*. 2010, Kumar and Kumar 2011). The complement system is a complex enzymatic system through which antigen is trapped on antigen presenting cells (Kinoshita 1991, Parmentier *et al*. 2002, Parmentier *et al*. 2004). The complement system is activated by 2 pathways, the classical pathway (CPW) which is antibody dependent and the alternate pathway (APW) rests on the covalent binding of C3 protein to foreign microbial particles and both leads to membrane attack complex. However, scientific support on this hypothesis is lacking, so in the present study we evaluated the complement response (complement hemolytic activity) both by CPW and APW against sheep red blood cells (SRBC) immunization, their natural levels and persistence in different Indian native breeds and compared with exotic breeds reared in the same tropical environment.

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MATERIALS AND METHODS

Experimental material: The indigenous and exotic breeds of chicken, viz. Aseel, Kadakanath, Naked Neck (NN), Frizzle, Dahlem Red, White Leghorn, Synthetic broiler dam line and Naked Neck broiler line were maintained at the experimental poultry farm of CARI and constituted the base material for this experiment. All the indigenous stocks were maintained without any deliberate selection while the White Leghorn and broiler populations have a history of long term selection for part period egg production and 6 -week body weight, respectively.

Chicks from all these genetic groups were obtained in a single hatch. They were wing banded and brooded under hover following standard management practices. They were shifted to grower house at 8 weeks of age. Other management and feeding regimens were kept identical as far as possible for all the genetic groups. Chickens (433) of both sexes and 8 lines comprising the Indian native breeds: Aseel (44), Kadakanath (69), Naked Neck (53) and Frizzle (49), Dahlem red (49) along with exotic breeds: White Leghorn (80), synthetic dam line (SDL) broiler (43) were tested for general immunocompetence against SRBC immunization at 7 weeks. All the experimental birds were healthy, apparently free from parasitic infestation and received normal routine health care. The Marek's Disease (MD) vaccine was given subcutaneously (S/C) at 1-d-old, infectious bursal disease (IBD) at days 14 and 35 by occulonasal route (O/N), Newcastle disease (ND) vaccination was with RD F strain at days 5 and 28 by O/N followed by RD R_2B i/m at day 56 and fowl pox (FP) at day 42 by wing web route.

Sheep red blood cell challenge: The SRBCs collected in Alsever's solution, were washed 3 times in phosphate buffer saline (PBS, pH 7.2). After the final wash the packed cells were brought to a 25% vol/vol solution in the PBS. The blood sample was drawn from each chick prior to injection (day 0) of SRBC. Each experimental bird (7 wk old) was injected intramuscularly in the thigh muscle with 1 ml of the 25% SRBCs suspension to induce a T-cell dependent antibody response. Additional blood samples were drawn at days 5, 12 and 19-post injection. Serum was recovered from clotted blood by centrifugation and was stored at –70°C until tested.

Assay technique: Complement activity (CPW and APW) against SRBCs was determined with a haemolytic technique as described by Demey *et al*. (1993) using an adapted light-scattering method. The method was used with some minor

Table 1. Means and standard errors for anti- SRBC CPW complement (ca-dependent pathway) response (CH_{50} U/ml) at 7 weeks of age

Genotypes	Sex	N	Complement (CPW) level (CH_{50}U/ml): response in days post- injection			
			0	5	12	19
Aseel	C	44	433.32±26.10^{ab}	812.20±61.19^{a}	458.20±39.50^{c}	178.43±29.71^{b}
	M	29	436.52±34.05	755.83±77.64^{ab}	430.76±50.49^{a}	163.62±36.75^{a}
	F	15	427.13±40.61	921.20±95.59^{A}	511.27±62.39^{A}	207.07±51.38^{A}
Kadakanath	C	69	470.83±26.35^{a}	746.20±38.02	433.18±31.17$^{d'}$	88.67±13.65^{c}
	M	36	491.03±37.10	890.33±59.57^{a}	546.14±45.51^{a}	120.50±21.37^{ab}
	F	33	448.79±37.60	588.97±26.58^{B}	311.00±30.70^{B}	53.94±14.51^{B}
Naked Neck	C	53	431.70±28.80$^{a\ c}$	578.09±24.6^{c}	232.08±22.65^{a}	25.17±16.29^{a}
	M	23	444.49±52.69	635.39±43.38^{bc}	249.52±37.30^{b}	18.74±36.26^{c}
	F	30	421.97±31.72	534.17±26.14^{B}	218.70±28.33^{CD}	30.10±8.61^{B}
Frizzle	C	49	314.31±20.13^{d}	309.63±21.07^{b}	176.67±18.01^{ab}	43.43±7.72^{a}
	M	25	302.64±29.81	261.08±24.53^{d}	134.24±20.64^{cd}	39.32±12.54^{c}
	F	24	326.46±27.34	360.21±31.92^{CD}	220.88±27.42^{CD}	47.71±9.03B
Averaged for	C	215	417.83±13.61	618.77±22.90	330.43±16.69	81.0±9.41
indigenous chicken	M	113	425.87±20.19	664.71±36.48	365.03±26.04	92.89±14.88
	F	102	408.93±18.04	567.88± 25.65	292.10±19.56	67.98±10.95
Dahlem	C	49	634.55±39.82^{e}	812.49±52.75^{a}	224.06±31.11^{a}	31.43±11.67^{a}
Red	M	23	605.13±56.10	757.74±87.37^{ab}	298.43±51.07^{b}	63.26±13.72^{bc}
	F	26	660.58±56.83	860.92±62.64^{A}	158.27±33.21^{DE}	3.27±16.69^{CD}
White	C	80	397.11±26.13$^{b'c'}$	478.61±24.77$^{d'}$	214.49±17.29a$^{'}$	47.74±7.72$^{a'}$
Leghorn	M	44	436.34±37.95	533.20±36.46^{c}	200.48±25.36bc	43.64±13.31^{c}
	F	36	349.17±33.82	411.89±29.15^{C}	231.61±22.79^{C}	52.75±5.62^{B}
SDL broilers	C	43	146.77±12.41^{f}	309.37±23.97^{b}	131.05±14.59^{b}	32.77±5.16^{a}
	M	20	137.35±16.20	248.55±26.97^{d}	92.95±19.03^{d}	24.70±5.63^{c}
	F	23	154.96±18.59	362.26±35.10^{CD}	164.17±19.49^{DE}	39.78±8.14^{ABC}
Naked Neck broilers	C	46	156.22±10.85f	226.35±16.10^{b}	102.76±10.32b	19.00±3.88^{a}
	M	23	136.09±13.54	169.78±14.80^{d}	104.65±14.25cd	18.43±4.72^{c}
	F	23	176.35±16.16	282.91±23.45^{D}	100.87±15.23E	19.57±6.26^{BD}
Averaged for broilers	C	89	151.65±8.18	266.46±14.85	116.43±8.91	25.65±3.26
	M	43	136.67±10.32	206.42±15.85	99.21±11.57	21.35±3.63
	F	46	165.65±12.29	322.59±21.69	132.52±13.11	29.68±5.30

C, Combined sex; M, male; F, female; n, number of birds; Means in the same column with different superscripts differ significantly ($P<0.05$); Lower superscripts apply to comparisons of genotypes within male sex; upper case superscripts apply to comparisons of genotypes within the female sex and lower case with sign (') superscripts apply to comparisons of genotypes within combined sex.

modification. Briefly, sera were diluted serially in appropriate buffers in flat-bottomed 96-well microtitre plates and incubated with sensitized sheep erythrocytes to measure CPW and APW. The plates were shaken in a Titertek every 30 min during the period of incubation. The results (the amount of light –scattering by erythrocytes upon lysis) were read at 655 nm in a microplate reader. The readings were log transformed, and the hemolytic activity was expressed as the titre that lysed 50% of the erythrocytes ($CH_{50}U$/ml). Calculation was done in computer as per the specific programme supplied.

Statistical analysis: Procedures described by Snedecor and Cochran (1994) were used for analysis of data. The titers were evaluated by analysis of variance with breed, sex and breed × sex interaction as main effects. The differences in the means of various genetic groups were compared by the multiple range test (Duncan 1955)

RESULTS AND DISCUSSION

Complement response to SRBC antigen: Complement response was determined by means of both calcium dependent pathway or classical pathway (CPW) and calcium independent pathway or alternate pathway (APW). The complement response values were expressed as $CH_{50}U$/ml.

CPW response: The means along with standard errors for CPW complement response of 7-week old chickens are presented in Table 1 according to sex and genetic groups. Data revealed the presence of natural CPW complement in all the groups under study. Mean titres ($CH_{50}U$/ml) for combined sex averaged over all the indigenous breeds of fowl on days 5, 12, 19 p.i. were 618.77 ± 22.90, 330.43 ± 16.69 and 81.07 ± 9.41, respectively. Among the 8 genetic groups studied, highest CPW responses were observed in Dahlem Red and Aseel followed by Kadakanath, NN, WLH, Frizzle, SDL broiler and NN broiler on day 5 p.i. On day 12 p.i. Aseel had the highest level followed by Kadakanath. But Dahlem Red showed much regressed value on day 12 and 19 p.i. In all the genetic lines peak response was seen on fifth day p.i. and declined (Fig.1) thereafter.

The results of analysis of variance presented in Table 2 revealed significant (P< 0.01) differences among genetic groups and those remained consistent through post

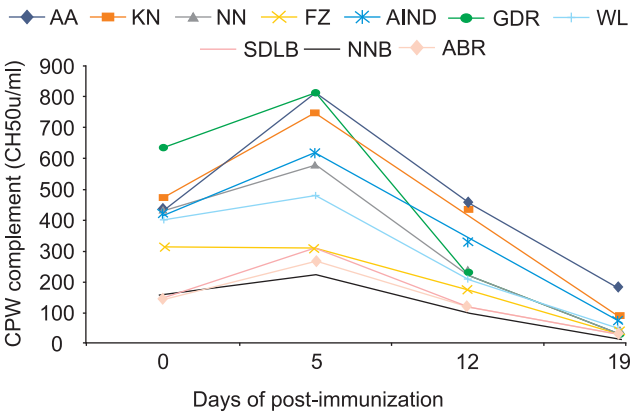

Fig. 1. The anti-SRBC CPW complement (Ca.dep.pw) response in various genotypes of chicken (combined sex) at 7 weeks of age. Aseel (AA), Kadakanath (KN), Naked Neck (NN), Frizzle (FZ), Averaged for Indigenous chicken (Aind), German Dahlem Red (GDR), White Leghorn (WL), SDL broiler (SDL-B), Naked Neck broilers (NN-B), averaged for broilers (Abr)

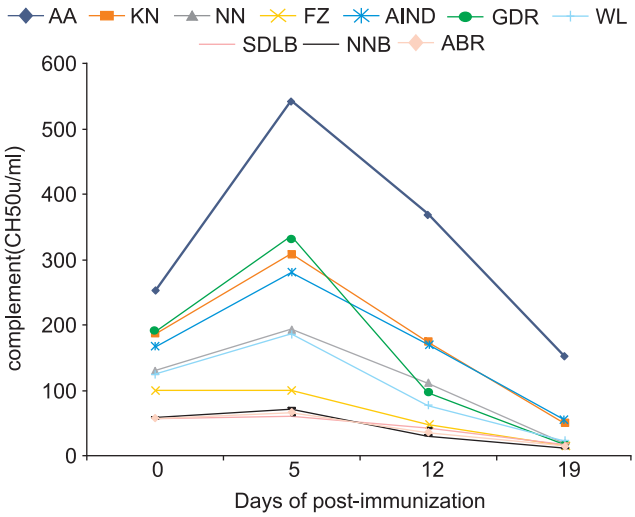

Fig. 2 The anti-SRBC APW complement response (Ca.indep.PW) in various genotypes of chicken (combined sex) at 7 weeks of age. Aseel (AA),Kadakanath (KN), Naked Neck (NN), Frizzle (FZ), Averaged for Indigenous chicken (Aind), German Dahlem Red (GDR), White Leghorn (WL), SDL broiler (SDL-B), Naked Neck broilers (NN-B), averaged for broilers (Abr)

Table 2. Mean squares from analysis of variance for anti-SRBC CPW complement levels ($CH_{50}U$/ml) at 7 weeks of age

Source of variation	DF	Mean squares (CPW)						
		Absolute level			Response			
		D0PI	D5PI	D12PI	D19PI	D5PI	D12PI	D19PI
Breed	7	12565.47**	7372468**	3326101.00**	1597205.00**	2818835**	876897**	129247.10
Sex	1	920.34	3433.56	42911.95	6119.70	7909.22	31263.53	2293.59
Breed × Sex interaction	7	34442.54	595031.20**	235140.50**	48818.99	392244.40**	192999.9***	19832.16*
Error	417	38605.81	116686.40	53587.45	34137.11	61843.53	31231.90	9702.26

**P<0.01; *P<0.05; DPI, days post immunization.

Table 3. Mean and standard errors for anti-SRBC APW complement (Ca- independent pathway) response (CH_{50} U/ml) at 7 weeks of age

Genotypes	Sex	N	Complement (CPW) level (CH_{50}U/ml) : response in days post - injection			
			0	5	12	19
Aseel	C	44	253.95±15.78$^{a'}$	543.00±44.48$^{d'}$	369.93±33.14$^{e'}$	152.89±24.23$^{b'}$
	M	29	230.86±17.81	534.59±62.31^{a}	398.86±45.00^{a}	185.41±30.62^{a}
	F	15	298.60±28.33	559.27±52.80^{A}	314.00±41.54^{A}	90.00±35.16^{A}
Kadakanath	C	69	187.29±12.94^{b}	308.65±20.08$^{e'}$	174.29±14.02$^{f'}$	49.99±6.64$^{c'}$
	M	36	212.61±20.02	304.08±30.19^{b}	187.08±22.10^{b}	47.44±10.50^{b}
	F	33	159.67±14.81	313.64±26.15^{C}	160.33±16.72^{B}	52.76±8.38^{B}
Naked Neck	C	53	130.45±8.39^{c}	194.02±14.41$^{b'}$	111.57±9.99$^{d'}$	18.49±4.29$^{a'}$
	M	23	137.43±15.60	180.48±21.46^{de}	139.04±14.76^{bc}	23.26±8.82^{b}
	F	30	125.10±8.91	204.40±19.53^{D}	90.50±12.41^{C}	14.83±3.45^{C}
Frizzle	C	49	101.10±5.47$^{c'}$	100.96±9.12$^{a'}$	47.57±6.27^{ab}	16.39±4.06$^{a'}$
	M	25	98.72±7.43	122.44±14.09ef	52.64±9.98^{e}	19.56±7.06^{b}
	F	24	103.58±8.19	78.58±9.77^{E}	42.29±7.53^{D}	13.08±3.90^{C}
Averaged for	C	215	167.28±6.86	281.02±15.79	169.99±11.46	55.62±6.56
indigenous chicken	M	113	176.80±9.89	297.89±24.12	201.91±18.24	71.76±10.84
	F	102	156.74±9.38	262.32±19.78	134.62±12.42	37.75
Dahlem	C	49	190.27±20.08^{b}	336.86±21.20^{c}	97.37±12.72$^{c'd'}$	17.98±5.18$^{a'}$
Red	M	23	164.70±18.51	281.09±26.54^{bc}	114.91±17.29^{cd}	27.22±7.50^{b}
	F	26	212.88±33.90	386.19±29.50^{B}	81.85±18.22^{CD}	9.81±6.90^{C}
White	C	80	125.46±7.35^{c}	186.59±11.62^{b}	77.71±6.48$^{a'c'}$	22.96±3.34$^{a'}$
Leghorn	M	44	136.20±11.14	213.95±18.43^{cd}	69.68±9.06^{de}	19.50±5.11^{b}
	F	36	112.33±8.68	153.14±10.46^{D}	87.53±9.09^{C}	27.19±3.96^{C}
SDL broilers	C	43	56.44±4.84$^{e'}$	61.56±2.92^{a}	41.93±3.17$^{a'b'}$	18.23±1.89$^{a'}$
	M	20	53.65±4.84	64.45±4.10^{f}	38.95±4.69^{e}	16.80±2.75^{b}
	F	23	58.87±2.94	59.04±4.15^{E}	44.52±4.33^{DE}	19.48±2.62^{C}
Naked Neck broilers	C	46	58.48±2.66$^{e'}$	71.63±17.76^{a}	30.74±2.91$^{b'}$	12.02±1.44$^{a'}$
	M	23	54.17±3.74	57.91±5.15f	37.09±4.24^{e}	13.78±2.33^{b}
	F	23	62.78±3.64	85.35±35.30^{E}	24.39±3.61^{E}	10.26±1.68^{C}
Averaged for broilers	C	89	57.49±1.90	66.76±9.25	36.15±2.22	15.02±1.22
	M	43	53.93±2.98	60.95±3.35	37.95±3.11	15.19±1.78
	F	46	60.83±2.33	72.20±17.68	34.46±3.17	14.87±1.69

C, Combined sex; M, male; n,number of birds; Means in the same column with different superscripts differ significantly (P<0.05); Lower superscripts apply to comparisons of genotypes within male sex, upper case superscripts apply to comparisons of genotypes within the female sex and lower case with sign (') superscripts apply to comparisons of genotypes within combined sex.

immunization periods i.e. on day 5,12, and 19 p.i. Sex effects were nonsignificant. Interaction (breed × sex) effects were significant on all the periods post immunization.

APW response: The sex wise means and standard errors for APW natural complement on day of immunization and complement response on days 5, 12 and 19 p.i. of various genetic lines are presented in Table 3. The natural APW complement was highest in Aseel and lowest in broilers, the overall means for natural complement level was 167.28 ± 6.86 pooled over four indigenous breeds. The corresponding values from higher to lower were for Dahlem Red, WLH and broilers, respectively. Among the indigenous fowl, the Frizzle showed lowest natural APC level. The overall response (CH_{50} U/ml) for the combined sex averaged over all indigenous fowl breeds on days 5, 12 and 19 p.i. showed gradual declined trend. APW response on day 5 was highest in Aseel followed by Dahlem Red, Kadakanath, Naked Neck, White Leghorn, Frizzle,NN broiler and SDL broiler in that order (Table 3). Aseel, which showed highest response on day 5 p.i. for APW response had also the highest value on day 12 and 19 p.i. Lowest response was observed in broilers (Fig. 2). The results of analysis of variance for APW response are presented in Table 4. Differences among group were significant (P< 0.01) on day's 0, 5,12 and 19 p.i. Significant sex effects were observed on days 12 and 19 p.i. only. Line × sex interaction effects was significant on days 0 and 19 p.i. Males excelled the females for APW response at all the days of measurement post immunization in all genetic groups except Dahlem Red and NN broilers on 12th day p.i.

Natural CPW and APW complement levels averaged for indigenous chickens breeds was 417.83 ± 13.61 and 167.28 ± 6.86 for combined sex with 425.87 20.18 and 176.80 ±9.89 in males and 408.93 ± 18.04 and 156.74± 9.38 in females. Both CPW and APW were higher for the indigenous breeds of chickens compared to exotic breeds of layers and broilers

Table 4. Mean squares from analysis of variance for anti- SRBC APW complement levels ($CH_{50}U/Ml$) at 7 weeks of age

Source of variation	DF	Mean squares (APW)						
		Absolute level			Response			
		D0PI	D5PI	D12PI	D19PI	D5PI	D12PI	D19PI
Breed	7	225836.6**	2436994.00**	1314370.00	506086.80**	1189665.0**	491436.80**	74803.38**
Sex	1	3278.43	25210.29	34445.72	7791.76	10306.27	58977.68**	21178.55**
Breed × Sex interaction	7	19516.07**	82726.07*	16795.93	8114.27	36155.18	13162.79	12199.75**
Error	417	6480.30	33271.10	15817.17	8885.65	20927.14	9381.70	3504.18

**P<0.01; *P<0.05; DPI, Days post immunizations.

(Tables 1 and 3). This was in agreement with the reported findings of Demey et al. (1996), Barta and Barta (1975) and Skeeles *et al.* (1980). All these workers reported higher complement levels for indigenous chickens than the exotic chickens studied by them.

Saxena (1993) reported normal serum complement levels for chickens and Guinea fowls, the hemolytic complement was 1.5 and bactericidal complement was 1.7 for the guinea fowl. The corresponding values were 1.7 and 1.54 for Kadakanath and 1.16 and 1.37 for broilers. These values were expressed as log 10 (n+1). Skeeles *et al.* (1979a) reported low complement levels in specific pathogen free chickens compared to indigenous chickens. Chanh *et al.* (1976) reported complement levels for 6-week-old chicken, which were comparable in magnitude to those realized in this investigation for 7- week- old birds. Increase in complement levels with age for APW complements were reported by Shen *et al*. (1984). CPW and APW complement levels recorded in this study for different genetic groups were higher than those reported by others (Chanh *et al.* 1976, Shen *et al.* 1984 and Dorney *et al*. 2005). Significant genetic differences for complement levels were in agreement with the findings of Shen *et al.* (1984) who reported that B haplotype influence 10% differences in complement activity. On the other hand, complement values reported by Chanh *et al.* (1976) for inbred line were lower than those recorded in this investigation. In his study F_1 hybrids exhibited higher complement titres whereas F_3 or back crosses had higher titres only, when they were either B^{17} or B^{18} genotypes. Individuals homozygous for B^{15} loci had the lowest titres. This suggested the role of B haplotype in regulations of complement levels, which is inherited as a recessive trait. Saxena (1993) however obtained no significant differences between the strains/ lines for hemolytic complement levels. Dorney *et al.* (2005) reported significant differences both in the calcium-dependent (classical, CPW) and the calcium-independent (alternate, APW) complement titres between various indigenous phenotypes of major genes.

The sex effects were not significant but the breed sex interaction effects were significant on day 5, 12 and 19 p.i. for CPW response. For APW response, sex effect was significant on day 12 and 19 p.i. and interaction effects on day 19 p.i. Nonsignificant sex effects for CPW confirm the finding of previous worker (Skeeles *et al.* 1979a, Skeeles *et al.* 1980 and Saxena 1993. Saxena (1993) also could not observe any significant interaction effects for complement levels, which was contrary to the observation made in this investigation.

Significant variation was observed in CPW and APW response among the various genetic groups and this remained consistent in all days of post immunization. Sex effects were not significant in any days of p.i. for C.P.W response but significant for APW on 12 and 19 dpi only. However, interaction between breed and sex were significant on all days of p.i. for CPW and and on 19 day p.i. for APW response, It is concluded that the chicken populations utilized in this study differed widely with respect to their immunocompetence traits, on different days of post SRBC injection and observed higher antibody than young birds. The variation in the immunocompetence traits can be utilized for the production of disease resistance birds.

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