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Comparative evaluation of immune response to secondary inoculation of sheep red blood cells in egg-type chicken

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ABSTRACT

Present study was conducted to evaluate humoral immune response of 3 White Leghorn (WL) and 2 Rhode Island Red (RIR) strains to booster inoculation of sheep red blood cells. Immune response to sheep red blood cells was measured as total, Mercapto-ethanol resistant (MER) and Mercapto-ethanol sensitive (MES) titre at 6, 10, 14 and 18 days post secondary (booster) inoculation. Haemagglutination titre gives indication of total antibodies while MER and MES titre represented level of immunoglobulin G (IgG) and immunoglobulin M (IgM) respectively. The overall antibody production was higher after booster inoculation when compared with birds not given booster. There were significant differences between the strains for response to booster, with WL being superior and differing significantly from RIR strains. Thus RIR strains had lower level of antibodies against SRBC booster. Of WL selected strains PL1 had better immune responsiveness compared to PL2 and control except for MER level, which was higher in PL2. The overall peak titer value was occurring during 6d PSI for total and MES titre and during 10d PSI for MER titre. The antibody titre after booster inoculation first peaked during 6–10d PSI and then gradually declined. There were significant differences between days PSI for total antibody titre in birds given and not given booster inoculation while MER and MES have no significant differences for days PSI.

Key words: Egg-type chicken, Immune response, Secondary inoculation, Sheep red blood cells

Incorporation of genetic resistance in poultry stocks has several advantages including the enhancement of the immune- response to vaccines (Gavora and Spencer 1979). Immuno-competence in poultry is evaluated by challenging the birds with antigens which may be causative agent of diseases including Marek's disease virus or New Castle disease virus or some other diseases, but disadvantage is that they are expensive, time consuming and will cause morbidity and mortality in flock due to their pathogenic nature. So the appropriate way to study immuno-competence in a species is to challenge it with non-pathogenic, non-specific antigen like sheep red blood cells (SRBC), mollusk haemocyanin, chicken egg white lysozyme and bacterial lipo polysaccharides but SRBC is the most commonly used antigen to study immuno-competence in poultry (Vanderzipp 1983). Parmentier *et al.* (1998) reported that birds having higher antibody response against non-specific, non-pathogenic and multi-determinant antigen SRBC also produced more antibodies to a variety of other antigens. In the present study SRBC were used as T dependent antigen to quantify the antibody response to its booster inoculation in White Leghorn (WL) and Rhode Island Red (RIR) strains.

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MATERIALS AND METHODS

The evaluation of immune response to secondary inoculation of T dependent antigen SRBC was done on 2 selected strains of Single Comb WL PL1 and PL2, a Single Comb WL random bred control line PL3 and 2 RIR (RIR-B and -C) strains. For the comparison of strains for cell mediated immune response to booster inoculation of SRBC, 70 birds of each genetic group were given *ad lib.* feed and water. For the preparation of SRBC suspension, about 30 ml blood was collected in anticoagulant Alsever solution. The red blood cells were washed 3- times with equal volume of phosphate buffer saline (PBS) pH 7.4. After final wash the packed cells were brought to 2.5% and 0.25% vol/vol solution in PBS. Prior to injection of SRBC suspension a blood sample was drawn from each chick to measure the base titer level. Each chick was injected intravenously with 0.25% SRBC suspension and blood samples were collected at 6, 10, 14

and 18 days PPI (post primary injection). At 28 days PPI half of the birds were given secondary injection of 0.25% SRBC suspension. Before giving secondary injection blood sample was drawn to measure base titre at 28 days PPI and post-secondary injection sampling was done at 6, 10, 14 and 18 days PSI (post secondary injection). Blood samples were collected from the wing vein without adding any anticoagulant. The blood samples were centrifuged at 6 000 rpm for 10 min to harvest serum and serum samples were stored at 4°C.

Total antibody titre against SRBC was determined by haemagglutination test as per Martin *et al.* (1989). Titre value was expressed as $\log_2$ of the reciprocal of the highest dilution giving agglutination. Antibodies resistant and sensitive to 2-Mercaptoethanol (2-ME) were determined by Microtiter method (Miller *et al.* 1992). Antibody titre was expressed as $\log_2$ of the reciprocal of the highest dilution giving agglutination. These titre values were recorded as 2-mercaptoethanol resistant (MER) antibody titre, which indicates the level of immunoglobin G (Ig G). 2-mercaptoethanol sensitive (MES) antibody titre, which indicates the level of immunoglobin M (IgM) was recorded as difference of total antibody titre and MER titre. The different strains were compared for total, MES and MER titre by using analysis of variance (Harvey 1988). The significance of differences between strains was evaluated by Duncan's multiple range Test (Duncan 1955).

RESULTS AND DISCUSSION

The antibody titres were greatly affected by the secondary inoculation of SRBC and were significantly higher than the primary titre. There is increased response to secondary inoculation of SRBC for total, MER and MES titre (Fig. 1). Secondary response for total and MES was highest at 6d PSI and declined with passage of time while for MER it increased from 6–10 d PSI and then declined till 18d. The peak titre of antibodies was obtained 3–7 days PPI of SRBC in many studies (Vanderzipp and Leenstra 1980; Ubosi *et al.* 1985, Martin *et al.* 1989, Miller *et al.* 1992). The titre values PPI and PSI showed wide differences at all stages indicating that the antibody response is small for primary inoculation but it increased widely with inoculation of booster. The differences between primary and secondary titre were greatest for MES titre indicating that the titre of Ig M was greatly affected by the secondary booster inoculation although the total and MER titre were also significantly affected.

Mean overall total titre in chicks given and not given secondary booster inoculation was 6.86 and 4.29 respectively (Table 1) indicating enhancement of response with booster inoculation. RIR-C has highest difference between titre value in chicks given and not given booster inoculation at 6 days PSI while PL1 has highest at 10 and 18 days PSI and PL2 at 14 days PSI. The lowest difference in titre values for the effect of booster inoculation was in PL3 at 6 days PSI, PL2 at 10 days PSI and RIR-C at 14 and 18 days PSI. The higher values of total titre in chicks given booster inoculation indicate that booster inoculation helped to improve the immune responsive ability of the birds.

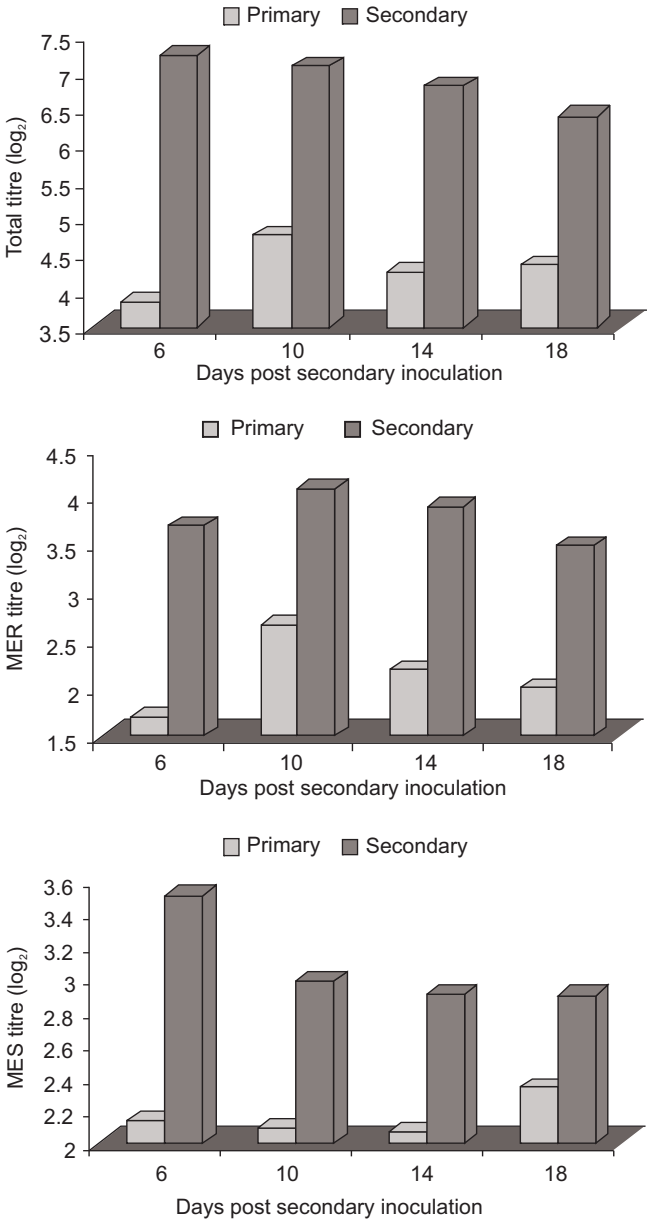

Fig. 1. Antibody titres ($\log_2$) post secondary inoculation of SRBC.

Mean total titre in chicks given booster inoculation was highest in PL1 strain and without booster it was highest in PL2 strain. The titre was lowest in RIR-B strain with and without booster inoculation and there were significant differences between the strains for secondary response with booster. White Leghorn selected strain PL1 has higher total titre with booster as compared to control line at 10, 14 and 18 days PSI. The superiority of chicks given booster inoculation for total titre in white leghorn selected strain PL1

Table 1. Means±SE of total antibody titre of chicks given or not given secondary inoculation of sheep red blood cells (SRBC) in different strains

DPSI	Overall	White Leghorn			Rhode Island Red		Superiority of	
		PL1	PL2	PL3	RIR-B	RIR-C	PL2 over control	WLh-S over RIR
6 NB	3.84±0.19^{X}	3.90±0.46	3.90±0.43	4.00±0.49	3.77±0.39	3.69±0.41	–0.10 (–2.5)	0.17 (4.6)
B	7.21±0.20^{Q}	7.05±0.41	7.35±0.42	7.05±0.46	7.00±0.41	7.58±0.54	0.30 (4.3)	–0.11 (–1.5)
10 NB	4.75±0.19^{Y}	5.10±0.31	5.10±0.35	4.90±0.60	4.15±0.49	4.30±0.15	0.20 (4.1)	0.68 (15.4)
B	7.06±0.06^{PQ}	8.00±0.36^{b}	7.05±0.35^{ab}	7.25±0.45^{ab}	6.12±0.44^{a}	6.74±0.35^{ab}	–0.20 (–2.8)	1.09 (16.9)
14 NB	4.25±0.18^{XY}	4.30±0.15	4.10±0.23	4.60±0.65	3.85±0.48	4.46±0.24	–0.50 (–10.9)	0.05 (1.2)
B	6.80±0.21^{PQ}	8.00±0.36^{c}	7.85±0.38^{c}	7.00±0.45^{bc}	5.29±0.44^{a}	5.58±0.34^{ab}	0.85 (12.1)	2.48 (45.6)
18 NB	4.34±0.22^{XY}	4.10±0.18	4.80±0.83	4.30±0.67	4.23±0.44	4.31±0.29	0.50 (11.6)	0.18 (4.2)
B	6.38±0.24^{P}	7.45±0.49	6.95±0.46	5.85±0.49	6.11±0.58	5.47±0.30	1.10 (18.8)	1.42 (24.6)
Mean NB	4.29±0.09	4.35±0.16	4.48±0.26	4.45±0.29	4.00±0.22	4.29±0.16	0.03 (0.7)	0.27 (6.5)
B	6.86±0.10	7.63±0.21^{b}	7.30±0.20^{b}	6.79±0.23^{ab}	6.13±0.24^{a}	6.34±0.25^{a}	0.51 (7.5)	1.22 (19.6) *

Means within a row given small letters in superscripts differ significantly (P≤0.05) while those given capital letters differ in column. DPSI, days post secondary inoculation; B, chicks given booster inoculation; and NB, chicks given no booster inoculation.

Table 2. Means±SE of mercaptoethanol resistant (MER) titre of chicks given and not given secondary inoculation of sheep red blood cells in different strains

DPSI	Overall	White Leghorn			Rhode Island Red		Superiority of	
		PL1	PL2	PL3	RIR-B	RIR-C	PL2 over control	WLh-S over RIR
6 NB	1.69±0.15^{X}	2.20±0.36	2.00±0.39	1.40±0.31	1.46±0.35	1.54±0.29	0.60 (42.9)	0.6 (40.0)
B	3.69±0.14	4.20±0.38	4.05±0.36	3.60±0.17	3.18±0.32	3.37±0.21	0.45 (12.5)	0.85 (25.9)
10 NB	2.66±0.16^{Y}	3.70±0.26^{b}	3.10±0.18^{b}	2.70±0.40^{b}	1.46±0.22^{a}	2.69±0.31^{b}	0.40 (14.8)	1.32 (63.5) *
B	4.07±0.11	4.10±0.14	4.30±0.24	4.50±0.30	3.59±0.15	3.79±0.29	–0.20 (–4.4)	0.51 (13.8)
14 NB	2.18±0.17^{XY}	2.70±0.26	2.00±0.21	2.70±0.65	1.92±0.33	1.77±0.26	–0.70 (–25.9)	0.5 (27.0)
B	3.90±0.12	4.15±0.23	4.15±0.28	4.15±0.29	3.53±0.27	3.42±0.28	0.00 (0.0)	0.68 (19.6)
18 NB	2.00±0.13^{Y}	2.00±0.36	2.20±0.42	1.50±0.22	1.92±0.21	2.31±0.29	0.70 (46.7)	–0.02 (–0.9)
B	3.49±0.11	3.65±0.21	3.90±0.18	3.50±0.25	3.24±0.26	3.11±0.26	0.40 (11.4)	0.61 (19.2)
Mean NB	2.13±0.07	2.65±0.18^{b}	2.33±0.17^{ab}	2.08±0.23^{ab}	1.69±0.14^{a}	2.08±0.15^{ab}	0.25 (12.0)	0.61 (32.4)
B	3.78±0.06	4.03±0.13^{b}	4.10±0.14^{b}	3.94±0.13^{b}	3.38±0.13^{a}	3.42±0.13^{a}	0.16 (4.1)	0.66 (19.4)

Means within a row given small letters in superscripts differ significantly (P≤0.05) while those given capital letters differ in column. DPSI, days post secondary inoculation; B, chicks given booster inoculation; and NB, chicks given no booster inoculation.

as compared to control indicates that selection for egg number and egg weight resulted in higher immune responsive ability in selected chicks after secondary inoculation. At 6 days PSI, RIR-C has highest titre with booster and WL control was having highest titre without booster at 6 and 14 days PSI. At 10 days PSI the titre value was highest in both WL selected strains while at 18 days PSI it was highest only in PL2. Overall superiority of PL2 over control line was higher (7.5%) with booster as compared to without booster (0.7%). RIR-B has poor response among all the strains with and without booster at all the stages except at 6 and 18 days without booster when RIR-C was having poor response.

Comparison of WL selected strains with RIR revealed that WL selected strains had higher total titre value at all stages PSI except for 6 days PSI. The overall superiority of WLh over RIR is 6.5% and 19.6% in chicks given and not given booster which indicated that the WL selected strains had greater immune responsiveness than RIR strains irrespective of whether secondary inoculation is given or not. Benda *et al.* (1990) reported significant breed differences for humoral and cellular immune responses in WL and RIR birds.

The mercaptoethanol resistant (MER) titre, which represents Ig G antibody level had the highest difference in chicks given and not given booster inoculation in strain PL3 at 6 day PSI, RIR-B at 10 days PSI and PL2 at 14 and 18 days PSI while the differences in MER titre were lowest in RIR-B at 6 days and 18 days PSI, PL2 at 10 days PSI and PL3 at 14 days PSI (Table 2). There were significant differences between strains for mean MER titre values for chicks given and not given booster inoculation. After booster inoculation, the highest mean MER titre was recorded in PL2 (4.10) while lowest was in RIR-B (3.38). Without booster inoculation the titre was highest in PL1 and was lowest in RIR-B. Among all the strains PL1 had higher MER titre with and without booster at 6 days PSI and without booster at 10

days PSI. At 10 days PSI, the MER titre was higher in control line chicks given booster inoculation. At 14 days PSI, the MER titre was highest and same in PL1 and control line chicks not given booster inoculation and was same in all WL strains given booster. At 18 days PSI, RIR-C has higher titre without booster and the WL selected line PL2 had higher titre with booster inoculation. There were nonsignificant differences between the strains for response to booster inoculation at all stages except at 10 days PSI in chicks given no booster which showed significant differences. The MER titre was lowest in RIR-B, RIR-C and PL3 strains. The WL strains were superior to control line but the superiority in titre values was more without booster (12.0%) and less with booster (4.1%) indicating that the selection for egg production is not effective for improvement of response to booster inoculation.

Comparison of WL selected strains with RIR for MER titre revealed that WL selected strains had higher MER titre value at all stages PSI except for 18 days PSI without booster. The overall superiority of WL over RIR is 19.4% and 32.4% in chicks given and not given booster respectively which indicates that the WL selected strains had greater immune responsiveness than RIR strains but the superiority was more without booster inoculation.

For mercaptoethanol sensitive (MES) titers in different strains, highest difference in MES titre in chicks given and not given booster inoculation was in RIR-C at 6 days PSI, in PL1 at 10, 14 and 18 days PSI (Table 3). The overall MES titre values in chicks given booster inoculation were higher than those not given booster in all strains and highest mean titre was in PL1 (3.60) and lowest in RIR-B (2.75). There were nonsignificant differences between the strains for MES response to booster inoculation. Kundu *et al*. (1999) has also reported significant variation to MER and MES response among various genetic groups for 2 Mercaptoethanol resistant (Ig G) and sensitive (Ig M) antibody responses to sheep erythrocyte on different days post SRBC immunization.

The MES titre was higher in selected line PL1 chicks given booster inoculation at 10, 14 and 18 days PSI. It indicated that selection affects the MES titre at 10, 14 and 18 days PSI and selected line chicks develop better immune responsiveness only due to booster inoculation. WL selected lines chicks have lower MES titre than RIR chicks given booster inoculation at 6 days PSI while PL3 was having highest titre without booster at 6 and 18 days PSI indicating that MES titre deteriorates due to effect of selection at these stages. At 10 and 14 days PSI, RIR chicks have higher MES titre in chicks not given booster inoculation. Thus the RIR chicks had better immune responsiveness at 6 days PSI as compared to WL selected strain and its performance deteriorated with time at subsequent ages. The temporal pattern of antibody response to sheep red blood cells is studied only in a few studies like that of Boa-Amponsem *et al.* (2000). The mean MES titre of WL selected strains was inferior as compared to control by 9.7% without booster but was superior by 12.3% with booster indicating that the favourable effect of selection in enhancement of MES response to booster.

Comparison of WL selected strains with RIR for MES titre revealed that WL selected strains had higher MER titre value at all stages PSI with booster except for 6 days PSI. Without booster inoculation the WL selected strains were superior to RIR only at 18 days PSI. The overall superiority of WL selected strains over RIR with booster is 19.7% but these selected strains were inferior to RIR without booster by 14.6%. It indicates that the WL selected strains had greater immune responsiveness than RIR strains with booster only.

Thus the booster inoculation of SRBC enhanced the antibody production against T dependent antigen, SRBC. Selected WL strains were superior for total and MER (Ig M) antibody production as compared to control line and RIR with and without booster. Selected WL strains were inferior to control line and RIR for MES (Ig G) antibody production

Table 3. Means±SE of mercaptoethanol sensitive (MES) titre of chicks given and not given secondary inoculation of sheep red blood cells in different strains

DPSI	Overall	White Leghorn			Rhode Island Red		Superiority of	
		PL1	PL2	PL3	RIR-B	RIR-C	PL2 over control	WLh-S over RIR
6 NB	2.14±0.13	1.70±0.33	1.90±0.18	2.60±0.31	2.31±0.29	2.15±0.25	–0.70 (–26.9)	–0.43 (–19.3)
B	3.51±0.21	2.85±0.41	3.30±0.43	3.45±0.45	3.82±0.49	4.21±0.50	–0.15 (–4.3)	–0.95 (–23.6)
10 NB	2.09±0.17	1.40±0.31	2.00±0.30	2.20±0.44	2.69±0.47	2.00±0.30	–0.20 (–9.1)	–0.64 (–27.4)
B	2.99±0.17	3.90±0.34	2.75±0.30	2.75±0.46	2.53±0.37	2.95±0.38	0.00 (0.0)	0.58 (21.1)
14 NB	2.07±0.13	1.60±0.22	2.10±0.18	1.90±0.23	1.92±0.35	2.69±0.30	0.20 (10.5)	–0.46 (–19.9)
B	2.91±0.18	3.85±0.31	3.70±0.46	2.85±0.39	1.76±0.38	2.16±0.29	0.85 (29.8)	1.81 (91.9)
18 NB	2.34±0.19	2.10±0.31	2.60±0.70	2.80±0.51	2.31±0.26	2.00±0.30	–0.20 (–7.1)	0.2 (9.3)
B	2.90±0.24	3.80±0.65	3.05±0.48	2.35±0.43	2.88±0.55	2.37±0.48	0.70 (29.8)	0.82 (31.4)
Mean NB	2.16±0.08	1.70±0.15	2.15±0.20	2.38±0.20	2.31±0.17	2.21±0.14	–0.23 (–9.7)	–0.33 (–14.6)
B	3.08±0.10	3.60±0.22	3.20±0.21	2.85±0.22	2.75±0.24	2.92±0.23	0.35 (12.3)	0.56 (19.7)

Means within a row given small letters in superscripts differ significantly ($P \leq 0.05$) while those given capital letters differ in column. DPSI, days post secondary inoculation; B, chicks given booster inoculation; and NB, chicks given no booster inoculation.

without booster but were superior with booster inoculation. Since poultry production depends upon prophylactic vaccination for control of various diseases, the booster inoculation of vaccines must be used to enhance the immune status of the birds and disease resistance.

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