

## IMMUNOSUPPRESSION IN BROILERS EXPOSED TO INCREASED TEMPERATURE

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High ambient temperature is known to increase the susceptibility of the domestic fowl to various diseases like Newcastle disease (Sinha *et al.*, 1957), fowl cholera (Juskiewicz, 1967) and Coli-septicemia (Cheville, 1979) due to associated immunosuppression. Immunosuppressive effect of heat applied for short duration has been reported (Regnier *et al.*, 1980., Siegal and Latimer, 1984., Donker *et al.*, 1990) Zschiesche (1986) opined that chronic and repeated heat stress for longer duration (temperature more than 40°C) led to striking impairment of humoral immunity. Both *in vivo* and *in vitro* cell-mediated immune response were found to be suppressed in heat and cold stressed birds and with phytohaemagglutinin skin test, the wattle swelling was significantly reduced in birds exposed to higher temperature (Jeffrey *et al.*, 1981). Reports regarding long time repeated exposure of heat on immunity were found to be scanty. Hence the present work was undertaken to study the same.

### Materials and methods

One hundred and fifty four broilers were reared on deep litter system with commercial starter and finisher mash. Vaccination with LaSota was done on eighth day. The birds were randomly distributed in two groups on 14th day. Group P was maintained at prevailing room temperature ( $25.5 \pm 3.5^\circ\text{C}$ ). Group T was maintained at  $40 \pm 1^\circ\text{C}$  for 12 h from 7 A.M. to 7 P.M. During the remaining period, i.e., from 7 P.M. to 7 A.M., they were maintained at the prevailing room temperature ( $25.5 \pm 3.5^\circ\text{C}$ ).

Concanavalin A (Con A) skin test was performed as per the method described by Carrier and Dehoech (1989) in six birds of each group on 0, 7, 14, 21 and 28 days of heat exposure. Adequate controls were maintained by injection of normal saline in the opposite leg. Skin thickness was measured 12, 24, 36 and 48 h after injection.

Birds were sacrificed 1, 8, 15, 22 and 29 days after heat exposure and serum and lymphoid organs (Spleen, bursa of fabricius and thymus) were collected for Haemagglutination Inhibition (H.I.) titre against Newcastle disease (ND) and histological examination respectively. Effect of heat on lymphoid organs was estimated by counting the medullary lymphocytes from randomly selected bursal follicles (Jalnapurkar, 1977). Organ body weight ratio and moisture content of lymphoid organs were also estimated.

### Results and discussion

The skin thickness increased due to Con A injections from 12 h. onwards, reaching a peak after 24 h. It reduced and became negligible in 48 h. Hence 24 h. readings were considered to study the effect of heat on cell-mediated immunity. It was found that the mean values in group T were 1.76, 1.16, 1.65, 0.73 and 0.78 mm on 0, 7, 14, 21 and 28 days of heat exposure respectively. The parallel values for Group P were 1.88, 1.40, 1.80, 0.90 and 1.13 mm. The differences in values were highly significant (Table 1a and 1b).

Table 1 Con A Skin Test  
(a) Absolute increase in skin thickness of Group P and Group T (mm)

Hours	15th day (0)		22nd day (7th)		29th day (14th)		36th day (21st)		43rd day (28th)	
	P	T	P	T	P	T	P	T	P	T
12 h	1.12±0.03	0.88±0.04	1.03±0.03	1.03±0.04	1.16±0.061	1.15±0.03	0.36±0.06	0.26±0.03	0.68±0.04	0.32±0.03
24 h	1.88±0.02	1.76±0.04	1.4±0.02	1.16±0.03	1.8±0.02	1.65±0.04	0.9±0.08	0.73±0.04	1.13±0.15	0.78±0.15
36 h	0.8±0.08	0.78±0.04	1.38±0.03	1.38±0.03	1.13±0.03	1.12±0.03	0.88±0.04	0.52±0.05	0.75±0.05	0.52±0.04
48 h	0.22±0.03	0.10±0.03	0.13±0.02	0.06±0.02	0.22±0.03	0.05±0.02	0.1±0.03	0.03±0.02	0.06±0.02	0.05±0.02

(b) Comparison of Group means (increase in skin thickness)

Hours	15th day (0 day)	22nd day (7th day)	29th day (14th day)	36th day (21st day)	43rd day (28th day)
12 h	4.33**	0.00	1.42	13.51**	6.39**
24 h	30.92**	4.28**	3.00**	17.89**	15.21**
36 h	1.70	0.00	2.69*	5.06**	5.86**
48 h	2.42*	2.24*	5.27**	1.58	0.325

\* Significant at 5% level

\*\* Significant at 1% level

Table 2 Organ weight to body weight ratio in percent (Mean  $\pm$  SE)

DPHE	Spleen			Bursa			Thymus		
	Group P	Group T	t <sub>10</sub>	Group P	Group T	t <sub>10</sub>	Group P	Group T	t <sub>10</sub>
1	0.09 $\pm$ 0.01	0.08 $\pm$ 0.00	2.36 <sup>b</sup>	0.25 $\pm$ 0.01	0.21 $\pm$ 0.01	3.92 <sup>d</sup>	0.34 $\pm$ 0.01	0.32 $\pm$ 0.01	1.78 <sup>ns</sup>
8	0.13 $\pm$ 0.00	0.11 $\pm$ 0.01	2.93 <sup>c</sup>	0.37 $\pm$ 0.01	0.32 $\pm$ 0.01	3.57 <sup>d</sup>	0.40 $\pm$ 0.00	0.35 $\pm$ 0.01	5.33 <sup>d</sup>
15	0.17 $\pm$ 0.01	0.12 $\pm$ 0.01	5.56 <sup>d</sup>	0.53 $\pm$ 0.00	0.29 $\pm$ 0.01	2.35 <sup>b</sup>	0.45 $\pm$ 0.00	0.40 $\pm$ 0.01	5.75 <sup>b</sup>
22	0.16 $\pm$ 0.00	0.12 $\pm$ 0.01	5.16 <sup>d</sup>	0.23 $\pm$ 0.01	0.19 $\pm$ 0.01	2.17 <sup>a</sup>	0.51 $\pm$ 0.00	0.35 $\pm$ 0.01	12.62 <sup>d</sup>
29	0.13 $\pm$ 0.00	0.10 $\pm$ 0.00	7.32 <sup>d</sup>	0.18 $\pm$ 0.00	0.10 $\pm$ 0.01	10.31 <sup>d</sup>	0.41 $\pm$ 0.00	0.28 $\pm$ 0.01	12.63 <sup>d</sup>

a - P &lt; 0.1; b - P &lt; 0.05; c - P &lt; 0.025; d - P &lt; 0.01

t<sub>10</sub> - Student 't' statistic value at 10 degree of freedom

DPHE - Days post initiation of heat exposure for Group T and days post initiation of experiment for Group P

There was significant reduction in the organ body weight ratio of spleen and bursa. In thymus too, these ratios were significantly reduced except for the first day (Table 2). Histologically bursa of Fabricius, spleen and thymus revealed mild to moderate congestion in addition to varying degrees of degeneration, necrosis and depopulation.

The average medullary lymphocyte count of the follicles of bursa of Fabricius (Table 3) reached the peak of 452 in Group P on the 15th day and reduced to 212 on 29th day. In the Group T, the peak, 299, was observed on the eighth day which declined to 94 on the 29th day. It was noticed that there was a significant reduction in these counts throughout the experiment.

The weekly (GMAT) geometric mean of antibody titre (Table 4) of Group P reached ten units on 22nd day and declined to 8.16 units on the 29th day. The parallel values of Group T were 5.5 and 4.5 units respectively. The highest reading of 7.16 units was reached on eighth day in this group, which was however lower than any of the values of Group P. The above differences were highly significant from eighth day onwards.

In the present experiment a correlation between GMAT, medullary lymphocyte count of bursal follicle and organ body weight ratio was observed.

Significant reduction in average lymphocyte count of Group T birds indicated progressive degenerative and necrotic changes in the bursa. Exposure to high temperature of 41°C to 46°C for 30 min. to six h. either intermittently or continuously reduced antibody responses to antigenic challenges (Regnier *et al.*, 1980., Donker *et al.*, 1990). A parallel reduction in the lymphoid organ

body weight ratio was noted. The moisture per cent of the bursa of Fabricius of Group T was 81.07 and 78.2 on first day and 29th day respectively whereas that of Group P was 81.6 and 78.78 during the same period which indicated that reduction in bursa body weight ratio was due to the reduction in the lymphocyte count of that organ.

The increase in skin thickness after injection of mitogens may be due to the stimulation of T cells which in-turn causes the migration and retention of the migrated cells, through lymphokines (Davis *et al.*, 1972). In the birds that were exposed to heat, T-cells stimulation might have been to a lesser extent resulting in reduction in the liberated lymphokines. This reduction in increased skin thickness was more marked in the birds after 22 and 29 days of heat exposure. Similar depression of cell-mediated immunity due to heat exposure has been recorded by Jeffrey *et al.* (1981). Histologically too, a marked depopulation of lymphocytes in the thymus supported the findings.

### Summary

Effect of prolonged and intermittent exposure to increased atmospheric temperature on the immune responses was studied experimentally in 154 broilers. It was observed that  $40 \pm 1^\circ\text{C}$  for 29 days, 12 h. a day depressed both humoral and cell-mediated immunity. There was a reduction in the weight of the lymphoid organs

Table 3 Average medullary lymphocyte count of follicles of bursa of fabricius

DPHE		Bird number										Mean $\pm$ SE			
		1*		2*		3*		4*		5*		6*		P	T
1	356	298	366	284	370	309	381	299	379	292	371	291	370.5 $\pm$ 3.71	295.5 $\pm$ 3.49	14.73 <sup>d</sup>
	400	299	386	300	393	292	402	298	405	307	400	301			
8	428	223	411	215	469	192	469	178	458	199	479	207	452 $\pm$ 10.92	202.33 $\pm$ 6.63	19.55 <sup>d</sup>
	377	160	382	160	386	145	392	163	378	166	392	157			
22	231	104	196	103	230	78	207	91	194	94	213	95	212.17 $\pm$ 6.14	94.17 $\pm$ 3.86	15.79 <sup>d</sup>
	400	299	386	300	393	292	402	298	405	307	400	301			

\* -Average of medullary lymphocyte count of five bursal follicles

H -Group H; d - p < 0.01;  $t_{10}$  - Student's 't' at 10 degree of freedom; P - Group P

DPHE -Days post initiation of heat exposure for Group T and days post initiation of experiment for Group P

Table 4 Geometric mean antibody titres

DPHE	Group P	Group T	t <sub>10</sub>
1	8	6.66	0.6899
8	8.83	7.16	2.6797 <sup>c</sup>
15	9.33	5.83	7.7262 <sup>d</sup>
22	10	5.5	8.0071 <sup>d</sup>
29	8.16	4.5	6.9487 <sup>d</sup>

Mean sum of squares (MSS)

0.8293<sup>NS</sup>

Error mean sum of squares (EMSS)

0.9296

C.P. &lt; 0.025; d.P. &lt; 0.01

DPHE - Days post initiation of heat exposure for Group T and days post initiation of experiment for Group P

(bursa of Fabricius, spleen and thymus) consequent to depletion of medullary lymphocytes of bursa of Fabricius and a decrease in haemagglutination inhibition titres against N.D. Cell-mediated immunity as assessed by skin test with Con A showed considerable depression.

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