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Effect of increased incubation temperature on juvenile growth, immune and serum biochemical parameters in selected chicken populations

U RAJKUMAR[1], M SHANMUGAM[2], K S RAJARAVINDRA[3], A VINOTH[4] and S V RAMA RAO[5]

Directorate of Poultry Research, Rajendranagar, Hyderabad, Telangana 500 030 India

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ABSTRACT

The present experiment was conducted to evaluate reproductive performance, juvenile growth, immune response and serum biochemical parameters in Naked Neck (NN), Punjab Broiler-2 (PB-2) and Dahlem Red (DR) chicken exposed to 2°C increased incubation temperature for 3 h each on 16th, 17th and 18th day of incubation in a randomized block design. The birds were reared at high ambient temperatures (32°C–45°C) during summer. Higher incubation temperature had no effect on hatchability. There were no significant differences between the *in ovo* heat exposed or normal incubated chicks in weekly body weight, feed intake (FI) and feed conversion ratio (FCR) except NN chicken. The cell mediated immune response to Phytohaemagglutinin-P (PHA-P) was significantly higher in heat exposed birds in NN and DR chickens. There were no significant differences between the treatments in other immune and serum biochemical parameters. There was significant difference between the genotypes in body weight, feed intake and feed conversion ratio. PB-2 birds recorded significantly higher body weight from 14th day to till 42nd day. The NN birds had significantly higher FRAP (ferric reducing ability of plasma) value and cell mediated immune response to PHA-P. The lipid peroxidation was significantly higher in PB-2 birds indicating high stress. In conclusion, prenatal exposure of 2°C increased incubation temperature had positive effect on juvenile growth in NN; cell mediated immune response (PHA-P) in NN and DR, while no effect was observed in all the parameters in PB-2 chicken.

Key words: Growth, Heat exposure, Immune response, Thermal adaptation

Poultry species are more vulnerable to heat stress due to increased global temperature as birds can tolerate a narrow zone of temperature, 18–24°C which is the thermoneutral zone for the birds. Increase in temperature beyond this range due to environment will lead to cascading effects on thermoregulation and could be harmful to the birds. High environmental temperature exerts a negative influence on the performance of poultry in terms of loss of productivity, reduced reproductive efficiency, increased stress, reduced immune competence and increased investment costs to mitigate the climate change (Rajkumar *et al*. 2011). Thermal manipulation during embryogenesis (pre-natal) induces physiological memory due to epigenetic adaptation to high temperature eliciting the improved thermotolerance during the post-natal life (Yahav 2008). Pre-exposure of embryo to high or low temperatures during incubation improves the adaptabilities to hot and cold environments respectively, during the post-natal life (Yahav *et al*. 2004 a, b). Changes in incubation temperatures during critical period of development of the thermoregulatory system can result in long-lasting modifications to the cellular and molecular neuronal mechanism of temperature regulation (Janke and Tzschentke 2010). Increased incubation temperature during later stages of incubation (critical period) favours the sex ratio towards male (Tzschentke and Halle 2009). Daily cyclical higher incubation temperatures, depending on the length of exposure and the days of the temperature modification, appear to improve tolerance of chickens to higher ambient temperatures (Yahav 2009).

In the present study, the effect of increased incubation temperature between embryonic day 16 and 18 on post- natal juvenile performance, immune and biochemical parameters was evaluated in selected chicken populations

MATERIALS AND METHODS

The experiment was conducted at Directorate of Poultry Research, Hyderabad, India. The ambient temperature ranged from 32 to 45°C during the experiment period. The experiment was approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Population: Three chicken populations i.e., Naked Neck (NN), Punjab Broiler-2 (PB-2) and Dahlem Red (DR) were utilized for studying the effect of epigenetic adaptation to higher temperature during the pre-natal life. These lines were considered for the study keeping in view of their utility in breeding programs for development of

Present address: [1,5] Principal Scientist (ullengala@yahoo.com, svramarao1@gmail.com), [2,3] Scientist (dr_shan@rediffmail.com, ksrravindra@gmail.com), [4] Senior Research Fellow.

improved chicken varieties for backyard poultry.

About 733 eggs from three breeds were exposed to higher temperature (39.5°C), 2°C above the normal incubation temperature (37.5°C) at 16th, 17th and 18th day of incubation for 3 hours. The relative humidity was maintained at 65% during the exposure. About 726 eggs were incubated with normal incubation temperature and humidity. A total 1072 chicks (539 normal and 533 heat exposed) representing three populations were utilized for the experiment. The chicks were randomly distributed at the rate of 6 birds per battery brooder cage (60´75 cm) placed in an open sided house in a randomized block design with two effects and nine replicates.

Rearing and management: The chicks were reared from day old to six weeks of age under standard management practices with a decreasing temperature schedule from 34±1°C during the first week which was gradually reduced 26±1°C by third week of age, thereafter chicks were maintained at room temperature. The broiler chicks (NN and PB-2) were fed *ad-libitum* with broiler starter (2,900 cal: ME, 22%: CP) and finisher (3,000 cal: ME, 20%: CP) diets based on maize-soybean meal from 0–4 and 5–6 weeks of age, respectively. The DR chicks were offered layer ration (2,700 cal: ME, 22%: CP) *ad libitum* throughout the experiment period. The feed conversion ratio (FCR) was calculated at weekly intervals by taking the ratio of feed consumed to weight gain. The chicks were vaccinated against Marek's disease (1st day), Newcastle disease (7th and 30th day) and Infectious Bursal disease (14th and 24th day). The minimum and maximum shed temperature at the time of blood sample collection for immune and biochemical parameters was 24°C and 38°C, respectively, with a relative humidity of 62 %.

Immune response: The cellular (Phytohaemagglutinin-P: PHA-P) and humoral (NDV) immune responses were studied in different set of birds consisting of 36 (3 breed x 2 treatments x 6 birds) birds on 42nd day of age. Blood samples were collected from wing vein and serum was separated and stored for further analysis.

PHA-P: The birds were injected with 100 mg PHA-P in 0.1 ml sterile saline solution in the left wattle at 6 weeks of age. The thickness was measured with a thickness gauge before and 24 h after injection. The wattle swelling was calculated as the difference between the thickness of the wattle before and after the injection.

Antibody response to NDV: Antibody titre against NDV was determined by HI assay using 4 HA units of NDV. The highest dilution where complete inhibition of agglutination observed was read as titer (Thayer and Beard 1998) and expressed as $\log_2$ values.

Blood biochemical parameters

Lipid peroxidation: Lipid peroxidation (LP) in the serum was assessed by the protocol described by Ohkawa *et al*. (1979). Absorbance of test samples was measured at 532 nm against blank and the total amount of lipid peroxidation was calculated in terms of nmol malondialdehyde (MDA)/ml.

Alkaline Phosphatase: Alkaline phosphatase (ALP) enzyme activity was measured by p-nitrophenol method (Bowers and Mc Comb 1975).

FRAP: The ferric reducing ability of plasma / ferric antioxidant power (FRAP) of the serum was estimated according to the procedure described by Benzie and Strain (1996). Absorbance was measured at 595 nm using a micro plate reader and antioxidant capacity of the sample was calculated using standards.

Statistical analysis: The experiment was carried out in randomized block design (RBD) with two effects, genotype and heat exposure with 9 replicates and 6 birds for each replicate. The replicate group from each cage was considered as the experimental unit for analyzing the data on body weights and feed efficiency, while for immune and biochemical parameters, the data on individual birds were considered for analysis. The data were analyzed separately for each breed to study the treatment effects as the breeds are heterogeneous and their feeding schedules are different. The data were subjected to General Linear Model (PROC GLM) procedure in SAS 9.2 Package. The significance of means was tested using Tukey's criterion (SAS institute 2009).

RESULTS AND DISCUSSION

The present results showed nonsignificant effect of 2°C increased temperature exposure for 3 h over the normal incubation temperature (37.5°C) on fertility and hatchability. The mean fertility and hatchability (fertile egg set) percentage in normal and heat exposed birds was 87.60 and 87.23 and 74.24 and 72.71, respectively. However, there were breed variations in fertility and hatchability (Table 1). It was well established that higher incubation temperature reduces the hatchability but it mainly depends on the duration of the temperature and strength of exposure in addition to the stage of embryo development (Halle and Tzschentke 2011). The present nonsignificant variations in fertility and hatchability might be attributable to the shorter duration and marginal increase in incubation temperature. Similar to the present findings other workers have also reported no difference in fertility and hatchability (Yahav *et al*. 2004a, Werner *et al*. 2010, Loyau *et al*. 2013) in broiler and turkeys with different exposure time and temperature schedules. Short term heat exposure during the later stages of incubation increased the hatching performance in chicken (Tzschentke and Halle 2009) contrary to the present findings. Halle and Tzschentke (2011) observed no negative effects on hatchability after exposing embryos to chronic as well as short term increase in temperature during the last 4 days of incubation similar to the present findings. Moraes *et al*. (2003) recorded decreased hatchability and delayed hatching due to increased incubation temperature (39° C) for 2 h/day from 13th –17th day of incubation. The marginal non significant decrease in hatching percentage might be due to the depressed corticosterone levels in the thermal conditioned embryos at the time of internal pipping (Halle and Tzschentke 2011). The central and peripheral

Table 1. Fertility (%) and hatchability (%) in normal and heat treated experimental groups

	Normal group			Heat treated group	
	Fertility	Hatchability Fertile egg set (FES)	Fertility Total egg set (TES)	Hatchability	Fertile egg set (FES)	Total egg set (TES)
Naked Neck	90.63	79.87	68.8	90.12	77.55	65.03
PB-2	79.34	94.00	70.89	84.10	92.85	74.50
Dahlem Red	92.90	91.50	83.33	87.50	90.00	76.29
Over all	87.60	88.46	74.34	87.23	86.80	71.94

Table 2. Body weight (g), feed intake (g) and feed conversion ratio in normal and heat exposed birds in different chicken populations

	Naked Neck				PB-2				Dahlem Red			
	Normal	Heat	SEM	P	Normal	Heat	SEM	P	Normal	Heat	SEM	P
Body weight												
0 day	49.40	49.56	0.44	0.86	41.25	41.33	0.19	0.82	34.04	34.14	0.22	0.82
7th	91.94	94.22	0.84	0.18	91.05	88.77	0.83	0.18	49.09	46.76	0.98	0.25
14th	192.34	199.71	2.26	0.10	218.82	208.90	2.34	0.08	90.18	79.78	3.39	0.13
21st	339.40 [b]	353.75 [a]	3.84	0.05	382.35	383.13	4.05	0.92	134.20	120.05	6.15	0.26
28th	537.36[b]	576.36 [a]	8.81	0.02	625.11	620.83	6.14	0.11	195.01	179.27	10.56	0.47
35th	734.92 [b]	751.14 [a]	12.18	0.05	813.02	838.99	7.66	0.09	263.14	245.44	12.04	0.47
42nd	1017.0	1022.9	14.25	0.83	1111.0	1086.9	11.94	0.32	346.89	348.58	18.87	0.96
Feed intake												
7th	63.70	66.77	1.11	0.17	70.39	6.38	1.25	0.11	29.54	27.7	1.23	0.34
14th	244.15	250.06	3.75	0.44	278.26	266.14	3.76	0.11	119.12	110.71	4.26	0.33
21st	544.34	599.46	10.27	0.47	613.89	602.12	9.25	0.53	278.35	265.57	8.78	0.48
28th	980.73	994.29	15.58	0.67	113.79	111.60	16.71	0.52	429.91	418.37	17.57	0.75
35th	1589.8	1570.2	49.94	0.84	178.93	172.51	39.90	0.43	674.23	702.81	28.39	0.62
42nd	2228.1	2207.6	51.15	0.84	2520.7	2423.1	51.73	0.35	956.74	958.46	36.28	0.98
FCR												
7th	1.50	1.51	0.02	0.90	1.41	1.40	0.02	0.64	2.02	2.17	0.05	0.16
14th	1.27	1.25	0.01	0.73	1.27	1.27	0.01	0.83	1.32	1.39	0.02	0.11
21st	1.60	1.58	0.02	0.71	1.61	1.57	0.02	0.35	2.12	2.21	0.03	0.26
28th	1.83	1.73	0.03	0.16	1.82	1.79	0.02	0.61	2.25	2.34	0.05	0.42
35th	2.19	2.10	0.08	0.64	2.21	2.05	0.05	0.16	2.62	2.88	0.09	0.16
42nd	2.19	2.16	0.03	0.72	2.26	2.24	0.04	0.84	2.83	2.82	0.07	0.93

Means within a row with a common superscript do not differ significantly (P<0.05).

nervous thermoregulatory mechanism and other body functions are well developed during the later stage of incubation (Tzschentke 2007) which enables the embryos to adjust to the short term increase in temperature without any negative effects on hatching performance. However, a higher hatch rate in broilers was reported when eggs were exposed to higher temperature for 3h/day between embryonic day 8 and 10 (Collin *et al*. 2007).

The pre-natal heat exposure could not cause any significant variation in growth, feed intake and FCR in PB-2 and DR birds, while NN chicken had significant differences in body weight during 21st to 35th days of rearing (Table 2). However, the body weight significantly differed (P<0.01) in three chicken breeds studied (Table 3). The body weights in NN chicken exposed to high temperature were significantly higher than nonexposed (normal) Naked Neck birds during 21–35 days period, however body weights were similar in early as well as later stage of rearing (Table 2). The significant higher performance in Naked Neck chicken may be because of its high heat tolerance ability due to major gene effect (Merat 1986, Cahaner 1993, Rajkumar *et al*. 2011). Heat exposed NN chicken recorded higher body weights throughout the experiment period, which might be due to the epigenetic adaptation to the increased temperature during thermal conditioning of embryos. The short term increase in incubation temperature might have stimulated the muscle growth (Halevy *et al*. 2006) leading to higher body weights in NN chicken, however the positive effect on growth was not observed in PB-2 and DR birds. Tzschentke and Halle (2009) observed positive effect of

Table 3. Body Weight (g), feed intake (g) and feed conversion ratio in three selected chicken populations

	Naked neck	PB-2	Dahlem Red	SEM	P
Body weight					
0 day	49.49[a]	41.28 [b]	34.08[c]	0.17	0.00
7th day	93.08 [a]	90.10 [a]	58.12 [b]	0.53	0.00
14th day	196.02[b]	214.66 [a]	85.87 [c]	1.62	0.00
21st day	346.57 [b]	382.67 [a]	128.33 [c]	2.93	0.00
28th day	556.85 [b]	623.31 [a]	188.48 [c]	5.08	0.00
35th day	743.02 [b]	823.88 [a]	255.79 [c]	6.25	0.00
42nd day	1019.94 [b]	1100.93 [a]	347.59 [c]	9.26	0.00
Feed intake					
7th day	65.24 [a]	68.39 [a]	28.36 [b]	0.72	0.00
14th day	247.10 [b]	272.20 [a]	114.92 [c]	2.35	0.00
21st day	551.90 [b]	608.00 [a]	271.96 [c]	5.59	0.00
28th day	987.51 [b]	1126.93 [a]	424.14 [c]	10.15	0.00
35th day	1580.00 [b]	1757.22 [a]	688.52 [c]	23.13	0.00
42nd day	2217.86 [b]	2471.89 [a]	957.60 [c]	27.87	0.00
Feed conversion ratio					
7th day	1.49 [a]	1.43 [a]	1.17 [b]	0.01	0.00
14th day	1.26 [b]	1.28 [b]	1.36 [a]	0.01	0.00
21st day	1.59 [b]	1.59 [b]	2.17 [a]	0.02	0.00
28th day	1.78 [b]	1.81 [b]	2.30 [a]	0.02	0.00
35th day	2.15 [b]	2.14 [b]	2.75 [a]	0.05	0.00
42nd day	2.18 [b]	2.26 [b]	2.83 [a]	0.03	0.00

Means within a row with a common superscript do not differ significantly (P<0.01).

Table 4. Immune and serum biochemical parameters in selected chicken populations

	PHAP (mm)	ND (log2)	ALP (U/L)	LP (nmoles of MDA/ml)	FRAP (µmoles/L)
Breed					
Naked neck	2.33[a]	5.50	827.54	0.64 [b]	2019.58 [a]
PB-2	1.94[b]	6.58	800.79	0.87 [a]	1904.56 [ab]
DR	1.96 [b]	6.0	801.57	0.66 [b]	1691.48 [b]
SEM	0.13	0.29	26.69	0.05	58.53
P	0.03	0.30	0.88	0.04	0.05

Means within a column with a common superscript do not differ significantly (P<0.05).

increased incubation temperature on growth as it favoured the male chick production. Piestun *et al*. (2013) observed significantly or numerically higher body weights in thermal conditioned chicks during incubation similar to the present results in NN chicken. The positive effect of preexposure to high temperature during incubation on post natal growth was reported by many authors (Yahav *et al*. 2004a, Hammond *et al*. 2007) in poultry. The present non significant differences among the heat exposed and normal birds in PB-2 and DR chicken were in agreement with findings of Werner *et al*. (2010) for growth traits in broilers who reported no influence of increased temperature during incubation. Collin *et al*. (2007) observed no variation among the body weights from 28 to 41 days in broilers which were similar to the present study, however the period of exposure is from 8–10 days while time of exposure is similar to the present study. Melesse *et al.* (2013) observed nonsignificant variations in body weight in heat stressed and normal commercial layers similar to the present findings in DR birds.

The FCR and feed intake (FI) estimates are presented in Table 2 and 3. During the first week DR recorded significantly (P<0.01) better FCR compared to NN and PB-2 chickens. The FCR was significantly (P<0.01) higher in DR layers from 14th day to 42nd day (Table 3) compared to NN and PB-2 chicken. The FCR was almost similar in Naked Neck and PB-2 birds throughout the experiment period. The FI showed similar trend during the experimental period in NN and PB-2 chicken. The FI was significantly lower in DR which could be due to breed character as layer birds consume less feed. The FCR and FI were similar in both the treatment groups without any significant variation. The preexposure to 2°C higher temperature during 16th to 18th day of incubation would not have been sufficient to create any impact on the chicks either on growth or on feed intake. Therefore, the FCR was almost similar in both the treatments in spite of the pre exposure to high temperature (Table 3). Similar findings of no effect of heat treatment during incubation on feed consumption and FCR was reported by Piestun *et al*. (2011).

There were significant breed differences for reproductive parameters, growth and FCR which is a well established fact (Rajkumar *et al*. 2010) which might be due to the genetic architecture of the various chicken populations

Table 5. Immune and serum biochemical parameters in two experiment groups

	Naked Neck				PB-2				Dahlem Red			
	Normal	Heat	SEM	P	Normal	Heat	SEM	P	Normal	Heat	SEM	P
PHA-P, mm	2.01[b]	2.57[a]	0.25	0.05	1.90	1.97	0.21	0.87	1.48 [b]	2.44 [a]	0.14	0.05
ND, log2	5.66	6.33	0.53	0.56	6.16	7.00	0.28	0.15	4.83	6.16	0.57	0.26
ALP, (U/L)	821.52	833.56	36.28	0.87	874.19	727.40	55.13	0.19	772.44	830.70	34.28	0.42
LP, (n moles of MDA/ml)	0.49	0.78	0.17	0.08	1.11	0.62	0.17	0.17	0.55	0.76	0.05	0.13
FRAP (µ moles/L)	1913.3	2125.86	124.78	0.27	1778.21	2031.9	135.19	0.33	1615.4	1767.56	109.44	0.52

Means within a row with a common superscript do not differ significantly (P<0.05).

under study. The feed intake and FCR was similar in PB-2 and NN chicken as both are broiler based compared to the DR which is a layer chicken. The feed intake varied significantly in three breeds which might be attributable to the breed characteristics and basal metabolic requirements of the birds. The body weights of NN and PB-2 observed in the present study was in accordance with reports of Rajkumar *et al*. (2011), up to 4 weeks of age, however, non significant differences in 6 week body weights were also observed (Rajkumar *et al*. 2011), which might be attributable to breed differences and feeding regime followed during the course of experiment.

The cell mediated immune (CMI) response to phyto heamagglutinin-P (PHA-P) was significantly higher in NN chicken compared to other two breeds (Table 4). The CMI response was significantly ($P \leq 0.05$) higher in heat exposed groups in NN and DR chicken, while there was no significant variation in PB-2 chicken (Table 5). The humoral response to ND virus was similar across the breeds and between the treatments without any significant variation (Table 4). The significant variations in immune response in thermo stressed chicken were due to release of corticosterone leading to lymphoid tissue involution resulting in suppression of humoral and cell mediated immune responses (Maiorka and Dahlke 2006). The higher response in heat exposed birds in the present study might be due to the epigenetic adaptation of chicks during the embryonic stage to higher temperature. The CMI response to PHA-P was significantly higher in NN chicken indicating the better immune status of the birds which might be an added advantage for this chicken to be utilized for the development of heat tolerant chicken varieties in tropical countries (Galal 2008, Rajkumar *et al*. 2010). The antibody response to ND virus was not significant between the breeds similar to the findings of Rajkumar *et al*. (2010) who reported nonsignificant variation between normal and NN broiler chickens. The variation between the reports may be attributable to the status of the birds, experimental and environmental conditions prevailed during the study. Significant variation in CMI response between the heat exposed and normal birds in NN and DR chickens indicated the positive effect of short term increase in incubation temperature on immune response; however this was not the case in PB-2, a broiler.

Lipid peroxidation and FRAP varied significantly ($P \leq 0.05$) among the breeds (Table 4). LP was significantly higher in PB-2 compared to other two breeds, while FRAP was higher in NN chicken. NN recorded significantly ($P \leq 0.05$) lower lipid peroxidation. The ALP, LP and FRAP estimates were similar between the heat exposed and normal birds without any significant variation in different chickens (Table 5). The lipid peroxidation was higher in PB-2 indicating the increased lipid oxidation under stress condition resulting in the higher concentration of MDA in serum. The heat stress increased the lipid peroxidation because of free radical generation under stress condition especially in broilers which are more prone to stress (Rajkumar *et al*. 2010) which substantiate the present findings wherein PB-2 broilers were in more stress compared to other birds studied. The present study substantiates the fact that the NN chicken are more tolerant to heat stress which was in accordance with earlier reports (Rajkumar *et al*. 2011). The increased activity of antioxidant enzymes has been considered as the protective response against the oxidative stress (Altan *et al*. 2003). Heat exposure could not produce any significant effect on lipid peroxidation status in the chicken. Alkaline phosphatase (ALP) is present in all tissues, but is particularly concentrated in liver, bile duct, kidney and bone. Higher levels of ALP in the circulation may indicate bone growth or hepatobillary disorders. In the present study there was no significant difference between the breeds and treatments for ALP indicating no adverse reaction due to heat stress/ exposure. The FRAP assay measures the nonenzymatic defenses in biological fluids and is related linearly to the concentration of the antioxidants present. Among the three chicken populations used in the present experiment the NN has significantly higher FRAP level indicating that this population is well positioned to counteract the oxidative stress as observed by the lower lipid peroxidation level compared to PB-2 birds. On the other hand the DR population has the lowest FRAP value and may be susceptible to oxidative stress. Apart from the breed differences the *in ovo* heat exposure seems to have no effect on FRAP level.

The present study concluded that increasing the incubation temperature by 2°C for 3 h each on 16^{th}, 17^{th} and 18^{th} day of incubation does not affect hatchability, had positive effect on juvenile growth in NN; cell mediated immune response (PHA-P) in NN and DR, while no effect was observed in all the parameters in PB-2 chicken reared at high ambient temperature. The NN chicken is having better antioxidant capacity and immune status in comparison to other populations utilized in the experiment. Further studies with high incubation temperature or long duration of exposure during later stages of incubation may be conducted, which may influence the adaptation to high ambient temperature and post-natal performance and useful in combating heat stress in tropical environments.

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