



Evaluation of Growth, Carcass, Immune Response and Stress Parameters in Naked Neck Chicken and Their Normal Siblings under Tropical Winter and Summer Temperatures

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ABSTRACT : The performance of naked neck and normal chicken was evaluated with respect to growth, carcass, immune, biochemical and stress parameters under winter and summer seasons to assess the suitability of naked neck birds under high temperatures in the global scenario of climate change. The growth performance was significantly ($p \leq 0.05$) higher in naked neck chicken in the summer season. The dressing percentage was significantly ($p \leq 0.05$) higher in naked neck birds in both winter and summer season because of reduced plumage. The thigh, gilet and feather proportion significantly ($p \leq 0.05$) varied between naked neck and normal chickens in summer season. The humeral immune response to sheep red blood cells (SRBC), Newcastle disease vaccine (NDV) and cutaneous basophil hypersensitivity (CBH) did not show any significant differences among the chicken groups. The protein and cholesterol concentration observed was within the normal ranges. The total cholesterol levels in plasma were significantly ($p \leq 0.05$) lower in naked neck birds in both the seasons. H:L ratio was significantly ($p \leq 0.05$) lower in summer season indicating less stress in naked neck chicken. Basophil and eosinophil concentration was significantly ($p \leq 0.05$) higher in normal chicken in summer. The lipid peroxidation was higher in full feathered birds under summer stress. The enzyme glutathione reductase (GR) levels were significantly higher during the summer and varied significantly ($p \leq 0.05$) between the normal and naked neck chicken in both seasons. The results indicated that the naked neck birds performed significantly better at high ambient temperatures with respect to growth, carcass and biochemical parameters. It was concluded that the ability of the naked neck chicken to adapt to high temperatures foresees a viable option for the biological mitigation of climate change. (**Key Words :** Naked Neck Chicken, Growth, Carcass, Stress, Climate Change)

INTRODUCTION

Poultry flocks are more vulnerable to climate change because birds can only tolerate narrow temperature ranges, 18-24°C, the thermo neutral zone for chicken (Weaver Jr., 2002). Climatic conditions including temperature, relative humidity and ventilation will influence production performance, health and product quality of poultry (Freeman, 1987; Lin et al., 2006; Yahav, 2009) particularly when the birds are reared in open sided poultry houses. In the 20th century, there was an increase of 0.65°C in the average global temperature and 0.2 to 0.3% increase of precipitation in the tropical region. Through mathematical models based on data recorded from oceans, atmosphere

and biosphere has foreseen an increase of 1.4-5.8°C in global average temperature by the end of 21st century (IPCC, 2001).

Poultry are not well adapted to high ambient temperatures because they lack sweat glands. The effect of relative humidity on bird performance is closely associated with temperature. Air saturated with water vapor prevents loss of heat from birds through panting. Since poultry is reared in open sided houses in most of the tropical countries, any variation in these climatic conditions would greatly impact their production. In many tropical countries like India, the ambient temperature varies between -5°C to 50°C and humidity ranges from 55-98%. The body temperature of chicken (40.6-41.7°C) tends to rise as ambient temperature goes above or below the thermoneutral zone of birds (Weaver Jr., 2002). However, the impacts of such wide variation in temperature on poultry are not known. The physiological limits for body temperature in chicken

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vary from 23.5°C (Sturkie, 1946) to 47.0°C (Weaver Jr., 2002) beyond which the birds succumb to death. The challenges posed by climate change especially temperature variation are loss of productivity, reduced reproductive efficiency, increased stress, reduced immune competence and increasing investment costs to mitigate the climate changes. The impact of heat stress primarily depends on breed, strain and variety of the bird. The selection for fast growth was associated with increased heat stress in broilers (Cahaner et al., 1995; Singh et al., 1998; Altan et al., 2003). Excessive heat causes muscular strain and this diverts the metabolisms of the body. The naked neck birds are more tolerant to heat stress compared to their normal feathered siblings because of reduced feather mass leading to better heat dissipation (Merat, 1986; Patra et al., 2002). The lower feather mass increases the effective surface area for heat dissipation and increases the sensible heat loss from the neck region (Yahav et al., 1998). The *Na* gene received greater attention in broiler production because of its association with heat stress tolerance (Merat, 1986; Cahaner et al., 1993), which inhibits the poultry productivity and immunity in hot tropical climate (Horst, 1987). In broiler chickens the '*Na*' gene results in a relatively higher growth rate and meat yield than the normal birds at normal temperature and the effect is more pronounced at high temperature (Cahaner et al., 1993).

Mitigation strategies to combat heat stress like managerial, biological and nutritional interventions are required to be studied under the predictions of increased temperatures over the years. One of the strategies is biological mitigation to identify alternate breeds/varieties of chicken which can perform under the global scenario of increased temperatures due to climate change. The present study was conducted to evaluate the performance and stress status of naked neck (NaNa/Nana) and normal birds (nana) under winter and summer temperatures.

MATERIAL AND METHODS

Location

The experiment was conducted at Project Directorate on Poultry, Hyderabad, Andhra Pradesh, India. Hyderabad is located in Deccan plateau in southern region of India positioned between 17° 23' N and 78° 28' E at height of 500 m from sea level. The temperature ranges from 12°C in winter to 45°C in summer seasons. The experiment was approved by the Institutional Animal Ethics Committee (IAEC).

Birds

Naked neck gene was introduced into a synthetic broiler breeder line by crossing with a homozygous naked neck

(NaNa) population for improving the broiler production under tropical environment. The base population of naked neck line was developed after four successive generations of backcrossing and is maintained under mild selection pressure for six week body weight for the last six generations. Synthetic broiler line, a coloured broiler population under selection for six week body weight being used for production of commercial coloured broilers which look like *desi* birds. A total 240 birds (90 normal and 150 naked neck) during winter and summer were hatched out from the above population in a single hatch and segregated into two groups based on the presence of feathers on the neck region. The chicks were randomly distributed at the rate of 5 birds per battery brooder pen (60×75 cm) placed in an open sided house.

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 chicks 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 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), infectious bursal disease (14th and 24th day). The ambient temperature ranged from 12 to 30°C in winter and from 32 to 45°C in summer season, respectively.

Carcass traits

At 43rd day of age, 45 birds (30 naked neck and 15 normal) were selected randomly and sacrificed by cervical dislocation for evaluating the carcass traits during summer and winter season. The relative weights of dressed carcass, legs, breast, giblets (gizzard, liver and heart), feather and abdominal fat were recorded and expressed as percentage of live weight.

Immune response

The cellular (Phyto haemoagglutinin-P: PHA-P) and humoral (SRBC and NDV) immune responses were studied in different set of birds consisting of 45 (30 naked neck and 15 normal) birds per each set on 42nd day of age.

Cell mediated immune response

PHA-P: The birds were injected with 100 µg PHA-P (Bangalore Genei, Pvt. Ltd., Bangalore) in 0.1 ml sterile saline solution in the left wattle at 6 weeks of age. The thickness was measured with a thickness gauge (Mitutoyo,

Japan) before and after 24 h of injection. The wattle swelling was calculated as the difference between the thickness of the wattle before and after the injection.

Humoral immune response

Antibody response to SRBC : Twenty birds from each group were injected intravenously with 0.1 ml of 0.5 percent suspension of packed sheep red blood cells (SRBC) in normal saline at 6 weeks of age. Five days later, blood was collected from wing vein of each bird. Sera were collected and stored at -20°C . The total antibody titre was determined by haemagglutination test (Wegman and Smithies, 1966). The reciprocal of highest dilution showing complete agglutination was expressed as titre (\log_2)

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 titre (Thayer and Beard, 1998) and expressed as \log_2 values.

Nitric oxide : Nitric oxide in blood plasma was determined by the method described by Miranda et al. (2001). Briefly, the samples were deproteinised by treating with equal volume of ethanol and centrifuged at 3,000 rpm for 10 minutes. The supernatant was used for total nitrite determination by Griess assay after conversion of nitrate to nitrite by Vanadium (III) chloride reduction and the colour intensity was measured at 540 nm in an ELISA reader.

Blood biochemical parameters

Blood biochemical parameters like protein and total cholesterol in plasma and complete blood picture and heterophil/lymphocyte (H/L) ratio from whole blood were estimated from 45 birds at 6 weeks of age. The protein was estimated by chemical method described Lowry et al. (1951) and cholesterol by Zaks and Henly's method.

Lipid peroxidation : Lipid peroxidation in the haemolysate was assessed by the protocol described by Placer et al. (1966). Briefly, 200 μl diluted (1:20) RBC lysate was mixed with 1.3 ml of Tris KCl buffer and 1.5 ml of TBA reagent and boiled for 10 minutes. The reagents were cooled and then added 3 ml of pyridine-butanol mixture and 1 ml of 1 N NaOH. Absorbance of test samples was measured at 548 nm against blank and the total amount of lipid peroxidation was calculated in terms of nmol malondialdehyde (MDA)/mg protein.

Glutathione peroxidase (GPx): Activity of GPx in the haemolysate was assessed by the method Paglia and Valentine (1967). Two ml of 0.1 M PBS was added to 100 μl of 1:20 diluted RBC lysate. To this solution 100 μl each of reduced glutathione and H_2O_2 buffer was added. The reaction mixture was incubated for 5 min at room temperature and then 100 μl NADPH was added. The absorbance was measured in a spectrophotometer at 320 nm

for 5 min at every 60 sec interval. The enzyme activity was calculated using extinction coefficient of 3,781 and expressed as U/ml.

Glutathione reductase (GR) : Activity of GR in the blood lysate was assessed by the method developed by Cohen et al. (1970). Briefly, 2 ml of 0.1 M PBS, 100 μl of 1:20 diluted RBC lysate was mixed then 100 μl of 350 mM oxidized glutathione and 50 μl of EDTA were added. The mixture was incubated for 15 min at 37°C and then 100 μl of NADPH was added. The absorbance was measured at 340 nm for 5 min at every 60 sec interval. The enzyme activity was expressed as U/ml.

Experimental design and Statistical analysis

The experiment was carried out in randomized block design (RBD) with two effects genotype and season. The replicate group from each pen was considered as the experimental unit for analyzing the data on body weights and feed efficiency, whereas for slaughter, immune and stress variables, the data on individual birds were considered for analysis. 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). The interaction was not significant for all the parameters except for Glutathione reductase, hence only main effects were considered for analysis. The data on carcass traits were analyzed after *arc-sine* transformation of percentage values to study the variations among the genetic groups at different temperatures.

RESULTS

Growth performance

In summer, naked neck birds recorded significantly ($p \leq 0.05$) higher body weights during all the weeks except day old body weight (Table 1) compared to the normal birds. The body weights were similar between the normal and naked neck chicken during the winter season except for 1st and 3rd weeks which showed significant ($p \leq 0.05$) difference, in which the normal birds recorded higher body weights. The FCR did not show any significant variations among the normal and naked neck chicken either in winter or in summer.

Carcass traits

The live weight was significantly ($p \leq 0.05$) higher in naked neck chicken than the normal siblings during summer season, whereas it was not significant during the winter season (Table 2). The dressing yield was significantly higher in the naked neck birds in both the seasons. However, breast yield and fat had not shown any significant variation

Table 1. Performance of naked neck and normal birds in winter and summer seasons

Variable	Winter				Summer			
	Normal	Naked neck	SEM	p	Normal	Naked neck	SEM	p
Body weight (g)*								
Day old	46.12	46.05	0.31	0.935	43.36	44.20	0.30	0.303
Age, weeks								
1	96.00 ^a	88.40 ^b	1.49	0.042	99.33 ^b	108.81 ^a	1.41	0.008
2	232.40	212.24	4.81	0.105	197.30 ^b	226.74 ^a	4.02	0.001
3	436.26 ^a	393.66 ^b	6.93	0.010	376.26 ^b	401.53 ^a	4.26	0.015
4	663.38	630.41	10.31	0.226	549.53 ^b	594.94 ^a	7.81	0.017
5	862.88	832.86	13.99	0.442	811.21 ^b	873.18 ^a	10.78	0.019
6	1,191.09	1,133.54	16.17	0.192	972.08 ^b	1,079.28 ^a	17.65	0.012
FCR								
Age, weeks								
1	1.34	1.38	0.020	0.691	1.10	1.09	0.014	0.790
2	1.51	1.53	0.018	0.816	1.42	1.41	0.013	0.659
3*	1.61 ^b	1.65 ^a	0.019	0.031	1.52	1.53	0.011	0.377
4	1.74	1.75	0.017	0.921	1.66	1.66	0.015	0.918
5	1.90	1.90	0.014	0.981	1.70	1.75	0.015	0.199
6	1.93	1.95	0.015	0.321	1.90	1.88	0.016	0.669

* Significant at ($p \leq 0.05$). Means with different super scripts along the rows within the parameter differ significantly.

between the two groups in both the seasons. The thigh proportion was significantly ($p \leq 0.05$) higher in naked neck birds in summer but such difference was not observed during the summer. The giblet weight was significantly higher in naked neck birds compared to the normal in both the seasons. Feather proportion was significantly ($p \leq 0.05$) lower in naked neck chicken in both winter and summer seasons compared to the full feathered birds.

Immune response

The antibody response to sheep RBC was significantly ($p \leq 0.05$) higher in naked neck birds during the winter season, whereas no difference was observed during summer (Table 3). All other immune parameters such as ND titre, CMI response to PHA-P and Nitric Oxide were similar in both the seasons. The lymphoid organs (spleen and bursa)

weights were similar in both the groups during both winter and summer seasons.

Biochemical parameters

The concentrations of protein and cholesterol were within the normal ranges. The total cholesterol levels in plasma were significantly ($p \leq 0.05$) lower in naked neck birds compared to their normal siblings in winter and summer seasons. H:L ratio was significantly higher in naked neck birds during winter whereas significantly ($p \leq 0.05$) lower in summer season. Basophil and eosinophil concentration is significantly ($p \leq 0.05$) more in normal chicken in summer. The lipid peroxidation was higher in normal birds under summer stress and lower in naked neck birds under cold stress (Table 4) though not significant. GPx enzyme levels were also not significant among the two

Table 2. Slaughter variables (% live weight) in naked neck and normal chicken during winter and summer

Variable	Winter				Summer			
	Normal	Naked neck	SEM	p	Normal	Naked neck	SEM	p
n	15	30			15	30		
Live weight	1,189.33	1,191.96	19.45	0.271	1,051.46 ^b	1,135.48 ^a	12.41	0.031
Dressing yield (%)	69.08 ^b	72.95 ^a	0.24	0.035	71.12 ^b	74.23 ^a	0.231	0.027
Breast	19.35	19.49	0.53	0.904	16.08	16.61	0.19	0.203
Thigh	15.71	14.91	0.46	0.918	20.72 ^a	21.74 ^b	0.17	0.002
Giblets	5.06 ^b	5.80 ^a	0.13	0.007	4.49 ^b	4.74 ^a	0.05	0.017
Abdominal fat	1.75	1.64	0.12	0.674	1.62	1.29	0.10	0.503
Feather	5.26 ^a	3.92 ^b	0.16	0.000	5.24 ^a	3.45 ^b	0.19	0.005

* Significant at ($p \leq 0.05$). Means with different super scripts along the rows within the parameter differ significantly.

Table 3. Immune response and lymphoid organs (% live weight) in naked neck and normal chicken during winter and summer at 6 weeks of age

Variable	Winter				Summer			
	Normal	Naked neck	SEM	p	Normal	Naked neck	SEM	p
n	15	30			15	30		
SRBC (\log_2)	2.6 ^b	3.5 ^a	0.11	0.012	6.67	6.73	0.19	0.347
NDV (\log_2)	6.2	6.7	0.25	0.412	5.00	5.2	0.24	0.652
PHA-P (mm)	2.34	2.74	0.14	0.218	1.42	2.06	0.09	0.157
NO (μ M)	96.21	88.61	5.29	0.509	132.19	137.43	4.89	0.622
Lymphoid organs								
Spleen	0.19	0.21	0.01	0.845	0.15	0.20	0.01	0.321
Bursa	0.13	0.15	0.01	0.561	0.13	0.14	0.01	0.204

SRBC = Sheep red blood cells; NDV = New castle disease vaccine; PHA-P = Phyto haemagglutinin-P; NO = Nitric Oxide.

* Significant at ($p \leq 0.05$). Means with different super scripts along the rows within the parameter differ significantly.

groups of chickens. The stress enzyme Glutathione reductase (GR) levels were significantly higher during the summer and varied significantly ($p \leq 0.05$) between the normal and naked neck chicken in both the seasons.

DISCUSSION

Climate change has been a burning issue globally and the research is aimed at mitigating the strategies to overcome the impact of climate change especially under the predicted scenario of increased temperature. Naked neck (*Na*) gene is one of the potential resources which can tolerate high ambient temperatures (Merat, 1986). The findings revealed that naked neck chickens were significantly ($p \leq 0.05$) heavier than the normal birds in summer (Table 1) but not in winter, though overall growth performance was higher during the winter season. The increased performance of naked neck birds in summer may be attributable to the expression of *Na* gene under high

temperatures during the summer. The *Na* gene reduces the feather cover upto 20 to 40% (Merat, 1986; Singh et al., 2001; Fathi et al., 2008; Rajkumar et al., 2010a) thus leading to better heat dissipation because of increased surface area. Therefore, minimizes the stress in birds and ultimately reflects in increased growth performance. This characteristic feature of naked neck chicken plays an important role in its heat tolerance capacity by providing more surface area for heat dissipation thus increasing the sensible heat loss from the birds and reduces the heat stress. The present findings were in consistent with the earlier reports that the *Na* gene had a favourable effect on the growth performance of chicken reared under high temperatures (Merat, 1986; Cahaner et al., 1993; Galal and Fathi, 2001; Patra et al., 2002; Fathi et al., 2008; Reddy et al., 2008; Rajkumar et al., 2010b). However, significantly higher performance of naked neck birds was reported in both winter and summer seasons by Singh et al. (1998). Magothe et al. (2010) observed lower body weights in

Table 4. Blood biochemical parameters in naked neck and normal chicken during winter and summer at 6 weeks of age

Variable	Winter				Summer			
	Normal	Naked neck	SEM	p	Normal	Naked neck	SEM	p
n	15	30			15	30		
Protein, g/dl	6.86	7.30	0.12	0.082	8.35	8.22	0.17	0.732
Cholesterol, mg/dl	135.16 ^a	119.64 ^b	1.71	0.028	135.80 ^a	120.24 ^b	1.65	0.023
H/L ratio	0.39 ^b	0.45 ^a	0.02	0.004	0.71 ^a	0.62 ^b	0.03	0.012
Eosinophils	1.20	0.97	0.15	0.216	1.04 ^a	0.63 ^b	0.14	0.021
Monocytes	1.33	1.20	0.10	0.387	0.73	0.93	0.15	0.124
Basophils	1.98	2.23	0.14	0.617	2.10 ^a	1.61 ^b	0.21	0.005
Enzymes								
LP, nmoles of MDA/mg prot	1.12	1.25	0.02	0.315	3.48	2.48	0.31	0.278
GPx, U/ml	34.55	27.45	2.29	0.288	358.43	395.12	19.24	0.189
GR, U/ml	52.59 ^a	33.7 ^b	2.58	0.009	1,089.44 ^a	731.15 ^b	29.57	0.000

LP = Lipid peroxidation; MDA = Malondialdehyde; GPx = Glutathione peroxidase; GR = Glutathione reductase.

* Significant at ($p \leq 0.05$). Means with different super scripts along the rows within the parameter differ significantly.

naked neck birds from Kenya under free range conditions. Almeida and Zuber (2010) reported lower body weights in naked neck chicken than their normal counterparts under temperate conditions which might be due to the cold stress condition of naked neck birds.

FCR though not significant among the normal and naked neck chicken in either of the seasons, the magnitude varied numerically. The significant variation during the third week (Table 1) may be because of the chance which could not be explained scientifically. However, in winter the FCR was more which may be due to the high energy requirements for the basal metabolism of the birds. The FCR in naked neck chicken was higher in winter lower in summer than their normal siblings indicating the better adaptability of birds to the summer stress.

The significant ($p \leq 0.05$) higher live weights in naked neck chicken during summer season indicated the pronounced effect of *Na* gene under high temperatures. The dressing percentage was significantly ($p \leq 0.05$) higher in naked neck chicken in both the seasons which might be due to the significant reduction in feather content and increase in giblet weight and numerical reduction in abdominal fat content in naked neck birds compared to the normal birds which was in accordance with the earlier studies (Patra et al., 2002; Rajkumar et al., 2010a). The muscle proportion (thigh and breast) was higher in naked neck chicken because of the availability of higher levels of dietary protein for muscle development with lesser requirements for plumage development (Merat, 1990). The giblet proportion was significantly higher in naked neck chicken indicating the increased activity of liver and heart to meet the metabolic rate in these birds to combat the heat stress. In winter, it was higher in both the chicken suggesting the increased activity of vital organs to meet the higher basal metabolic requirements. The abdominal fat is lower in naked neck chicken though not significant substantiating the negative correlation between fat and heat tolerance (Macleod and Hocking, 1993). The significant influence of the *Na* gene in reducing the subcutaneous and intramuscular fat is well established (Raju et al., 2004; Fathi et al., 2008; Rajkumar et al., 2010a).

The present results suggested that there was no variation in cell mediated immune (CMI) and antibody response to NDV among the naked neck and normal chicken in both summer and winter seasons. The antibody response to SRBC was significantly ($p \leq 0.05$) higher in naked neck birds during winter, however there was no variation in summer season. The significant difference in SRBC titres during winter season could not be explained and needs further confirmation. Reddy et al. (1998) observed similar results of significant higher antibody response to SRBC in winter in naked neck chicken. Contrary to these findings,

Patra et al. (2004), El Safty et al. (2005), Fathi et al. (2008) and Rajkumar et al. (2010a) observed significant CMI response for PHA-P and antibody response for SRBC in naked neck chicken. The weight of spleen, an immune organ observed in the present study was in accordance with the finding of Fathi et al. (2008) whereas significant difference was observed in bursa weight contrary to the present results.

The cholesterol levels were significantly lower in naked neck chicken indicating the lower proportion of fat compared to the normal birds. Significantly lower cholesterol concentration in naked neck chicken were also reported by Patra et al. (2002) and Rajkumar et al. (2010a). The H:L ratio has been considered as the indicator of stress condition in chicken (Davison et al., 1983; Al Murrani et al., 2002; Fathi et al., 2008). The H:L ratio was significantly lower in naked neck chicken during the summer season indicating the better adaptability of these birds at higher temperatures compared to normal birds whereas significantly higher in winter because of cold stress. Increased temperatures in summer significantly decreased the lymphocytes and increased the heterophills and resulted in increased H:L ratio. The increased H:L ratio was reported in birds which were exposed to heat stress (Zulkifli et al., 1999; Altan et al., 2000; 2003). Fathi et al. (2008) observed similar findings in naked neck chicken under low temperatures. Basophills were significantly more in normal birds during summer season indicating the higher concentration of these cells under stress. The significant increase in basophilia in summer indicated severe stress condition in normal birds similar to the findings of Altan et al. (2003) and Maxwell et al. (1992).

The lipid peroxidation was higher in normal birds in summer indicating the increased lipid oxidation under stress condition resulting in the higher concentration of MDA in blood. The heat stress increased the lipid peroxidation because of free radical generation under stress condition in summer, thus the significant increased lipid peroxidation whereas the oxidation was similar between the genotypes in winter. The increased activity of antioxidant enzymes has been considered as the protective response against the oxidative stress (Mc Cord, 2000; Altan et al., 2003). The GR activity was significantly higher in stressed birds in both winter and summer seasons.

The present results revealed that naked neck chicken was performing significantly higher with respect to growth performance in summer under increased temperatures. The naked neck chicken were combating the heat stress efficiently and performing better than the normal birds in terms of growth, carcass and biochemical parameters. It is concluded that these chicken foresees a viable options as one of the alternatives for biological mitigation of climate change.

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