

Effects of genetic group \times ambient temperature interactions on performance and physiological responses of Naked-neck chickens and their F_1 crosses with Lohmann White and New Hampshire laying hens

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ABSTRACT

The objective of this study was to evaluate the effects of genotype \times temperature interactions on hormonal heat stress indicators and performance traits. Two-hundred forty female one-day-old chicks were randomly assigned to a completely randomized design in a 5×2 factorial arrangement with 5 genotypes (Naked-neck, Na, from Ethiopia; New Hampshire, NH; Lohmann White, LW and F_1 crosses of Na with NH and LW) and 2 ambient temperatures (thermo-neutral, 18–20°C; heat stress, 30–32°C). Blood samples were taken from 12 randomly selected birds per genotype and ambient temperature at 22, 38, 51 and 65 weeks of age. Levels of corticosterone (CS) and 3,5,3'-triiodothyronine (T_3) were determined in blood plasma. Heat stress effects on egg production traits were most severe in LW and NH, least severe in Na and Na \times LW, intermediate in Na \times NH. Plasma T_3 level was significantly reduced by 27.9% in heat stressed genotypes. Plasma CS increased by 12.6% in heat exposed genotypes. However, inconsistent responses of CS and T_3 levels were observed at different ages. The Na and their F_1 crosses demonstrated relatively better thermotolerance than LW and NH hens. Levels of plasma T_3 hormone might be considered as indicator of long-term heat stress in hens.

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KEY WORDS: Naked-neck chicken, F_1 crosses, heat stress, genotype x temperature interaction, corticosterone, 3,5,3'-triiodothyronine

INTRODUCTION

The effects of hot environment have been found to vary in magnitude among different chicken breeds (Mashaly et al., 2004; Lu et al., 2007). The use of in temperate climate developed chicken breeds in tropical environments may result in large economic losses because genotypes selected in temperate climates may respond differently to hot regions due to genotype and environment interactions (Falconer and McKay, 1996). Thus, the identification of genetic groups (or genotypes) having superior performance in warm environments is of extreme importance for the development of suitable chicken breeds in the tropics. Studies conducted on the Naked-neck chicken genotype have been associated with increased laying rate, egg size and egg weight in hot environments (Yunis and Galal, 2006; Rajkumar et al., 2011). Adrenocorticotrophic hormone stimulates the adrenal cortex, which in turn releases corticosteroids, primarily corticosterone (CS) in birds. Since increased levels of circulating CS have been observed under various stress situations (Davis et al., 2000; Piestun et al., 2008), the response to heat exposure is considered primarily as a reaction to stress. Heat stress stimulates the release of CS from the adrenal glands and increases plasma concentrations of CS in chickens (Zulkifli et al., 2009). Thyroid hormones have been known to be involved in the control of thermoregulation in birds and mammals (Collin et al., 2005). Warm-blooded animals respond to ambient temperature by decreasing thyroid hormone secretion rate as ambient temperature increases and *vice versa* (Silva, 2003). While it is generally accepted that 3,5,3'-triiodothyronine (T_3) stimulates metabolic rate and that both T_3 and thyroxin (T_4) concentration depress following heat stress, this pattern is not universally observed (Chiang et al., 2008). Therefore, this study aimed to investigate the effect of the genetic group \times ambient temperature interaction on physiological indicators of heat stress and performance of local Naked-neck and its F_1 crosses with Lohmann White and New Hampshire chicken genotypes.

MATERIAL AND METHODS

Animals and management

A total of 240 female one-day-old chicks were randomly assigned to a completely randomized design in a 5×2 factorial arrangement consisting of five genetic groups: Naked-neck (Na) from Ethiopia; Lohmann White (LW), New

Hampshire (NH) and their F_1 crosses (Na \times LW and Na \times NH) and two ambient temperatures (heat stress 30-32°C; thermo-neutral 18-20°C) (Table 1). Chickens reared at thermo-neutral and heat stress ambient temperatures were considered as control and experimental groups, respectively. Both LW and NH genotypes were used as a maternal line whereas the local Na was used as a paternal line to produce the F_1 crosses using artificial insemination (AI). The birds were hatched at the same time and the Lohmann female chicks were separated from male by feather sexing methods while those of NH breed and F_1 crosses by Japanese vent-sexing method. Twenty-four female chicks from each genotype were randomly assigned to thermo-neutral and heat stress ambient temperatures. From hatch to 20 week of age, they were raised on floor pens and then transferred to conventional individual layer cages with a dimension of 1000 cm² per hen (20 cm width \times 50 cm length) until the end of the experimental period (week 68). The temperature in both houses was thermo-regulated.

Table 1. Genetic structure and distribution of birds among experimental ambient temperatures

Ambient temperatures	Na and commercial layers ¹			F_1 crosses	
	Na	LW	NH	Na \times LW	Na \times NH
Thermo-neutral, 18-20°C	24	24	24	24	24
Heat stress, 30-32°C	24	24	24	24	24

¹ Na - Naked-neck (from Ethiopia); LW - Lohmann White; NH - New Hampshire; Na \times LW - F_1 crosses of Na males with LW females; Na \times NH - F_1 crosses of Na males with NH females

Ambient temperature and relative humidity of the house was measured at 2 h interval using a Tinytalk™ II Data Logger device. Relative humidity could not be controlled but was monitored continuously and ranged from 50 to 75% and 65 to 85% in the experimental and thermo-neutral houses, respectively. The hens were kept under 12-h light programme, which corresponds to the natural conditions in the equatorial tropics. The management practices in both houses were essentially the same. Mortality was recorded as it occurred. The age at first egg was used to determine the sexual maturity of birds. Eggs were collected once daily from start to end of laying period for 48 weeks. Egg weight and feed consumption were determined at 28-d intervals. Percentage hen-housed production, egg mass production and feed conversion ratio (FCR) were calculated using conventional methods.

During the growing period, the birds had *ad libitum* access to feed and water. Standard starter (11.4 MJ/kg and 18% CP) and grower rations (11.4 MJ/kg and 15% CP) were provided to all chickens and pullets, respectively (Table 2). Hens kept in individual cage were fed in-group *ad libitum* (4 hens/feed pan) and supplied with water using individual nipple drinkers. As presented in Table 2, they were fed on commercial laying feed containing 11.4 MJ/kg energy and 17% crude protein.

Table 2. Nutrient composition of commercial feed used for chickens, pullets and layers during the experimental period

Item	Chicks	Pullets	Layers
<i>Nutrients</i>			
energy, MJ/kg DM	11.4	11.4	11.4
crude protein, %	18.0	15.2	17.4
methionine, %	0.35	0.30	0.37
crude ash, %	7.3	6.5	12.5
crude fibre, %	5.0	5.5	5.3
crude fat, %	4.0	3.5	7.0
Ca, %	1.0	0.9	3.5
P, %	0.7	0.6	0.6
Na, %	0.12	0.12	0.12
<i>Feed additives per kg feed mixture</i>			
vitamin A, IU	9000	9000	12000
vitamin D ₃ , IU	1500	1500	2500
vitamin E, mg	15	15	20

Blood sampling

A total of 480 blood samples were collected from randomly selected 12 birds of each genotype and ambient temperature at 22, 38, 51 and 65 week of age. Blood samples (2-3 ml) were taken by a qualified veterinarian from the wing vein using disposable syringes and directly collected into ethylenediaminetetra acetic acid (EDTA) coated test tubes. Blood was taken in the morning between 8.00 and 10.00 a.m., and the time needed between handling of each hen and bleeding was less than one minute. Collected blood samples were centrifuged and plasma was stored at -20°C until further processing.

Hormones determination

The CS level was assayed with Radio Immuno Assay (RIA1364; DRG). Plasma T₃ was determined with an ELISA test (EIA1780, DRG, Marburg, Germany). In this procedure, a micro plate reader capable of readings at 450 nm wavelength was used. Moreover, a software package was used for facilitating data generation, analysis, reporting and quality control. Assaying was performed within four weeks of blood collection. Both analyses (CS and T₃ levels) were performed essentially as described in the manufacturer's manuals. Each sample was prepared in duplicate to enhance precision.

Statistical analysis

The experimental design was completely randomized using a 2 × 5 factorial that consisted of two ambient temperatures (thermo-neutral and heat stress) and five

genotypes (Na, LW, NH, Na \times LW and Na \times NH). Data analysis was done with the SAS procedures (2004) with the model including the main effects of genotype and ambient temperature and all two-way interactions. Comparisons of multiple means were made by using Duncan Multiple Range Test. All statements of statistical differences were based on $P < 0.05$ unless noted otherwise.

RESULTS

Performance traits

As shown in Table 3, except hen-housed egg production and age at sexual maturity, other production traits were severely affected by heat stress with significant genotype \times temperature interactions. In general, age at first egg of F_1 crosses was significantly earlier than of local Na chickens. Among heat exposed genotypes, age at sexual maturity was significantly earlier in LW and Na \times LW genotypes compared with other genotypes. At 20 week of age, the body weight in heat exposed NH was significantly higher than Na \times NH, while that of LW did not differ significantly from Na \times LW. However, at 68 week of age, the body weight was similar between high performing genotypes and their F_1 crosses. Surprisingly, compared with controls, the body weight of heat stressed Na \times LW increased by 1.7 and 2.8% at 20 and 65 week, respectively.

The effect of heat stress on percentage hen-housed egg production was most severe in LW and NH (-12% reduction), least severe in Na (-2%), intermediate in Na \times NH (-6.8%), but favours egg production in Na \times LW (by +0.2%). Similar trends were observed in total egg mass production as well. The effect of genotype \times heat stress interaction on feed consumption was significant ($P < 0.01$) and was more pronounced in LW and NH genotypes than in Na and their F_1 crosses. The average decline in feed consumption was the highest in NH (22.7%) and the lowest in Na \times LW genotypes (12.1%), whereas the corresponding figures in Na, LW and Na \times NH were 17.8, 18.3 and 16.7%, respectively. Although not significant, feed conversion ratio (FCR, kg feed/kg egg mass) slightly reduced in all heat exposed hens. Among heat stressed genotypes, FCR was lowest in LW (2.23 kg/kg egg mass) and highest in Na (4.99).

Among heat stressed genotypes, the mortality rate was 4.2, 16.7, 4.2 and 4.2% in Na, LW, NH and Na \times NH, respectively. No mortality was observed in heat stressed Na \times LW genotype. At thermo-neutral environment, no mortality was observed except in Na \times LW and LW genotypes, which had respectively 4.2 and 8.3% mortality rates.

Table 3. Least-square means of performance traits in Naked-neck, Lohmann White and New Hampshire laying hens and their F₁ crosses at thermo-neutral and heat stress ambient temperatures (values in parenthesis indicate \pm SD of the mean)

Genotypes (G)	Thermo-neutral						Heat stress						ANOVA [P (F)]		
	Ambient temperatures			Thermo-neutral			Heat stress			ANOVA [P (F)]					
	Na	LW	NH	Na×LW	Na×NH	Na×NH	Na	LW	NH	Na×LW	Na×NH	Na×NH	T	G	T×G
Age at sexual maturity, d	173 ^a (9.56)	154 ^d (6.22)	162 ^b (8.52)	156 ^{cd} (4.98)	160 ^{bc} (6.01)	166 ^a (5.65)	151 ^d (4.20)	162 ^b (6.83)	154 ^d (3.80)	159 ^c (5.38)	159 ^c (5.38)	159 ^c (5.38)	**	***	NS
Body weight, kg	0.95 ^d (0.12)	1.35 ^b (0.13)	1.58 ^a (0.17)	1.17 ^c (0.15)	1.26 ^c (0.14)	0.82 ^d (0.16)	1.25 ^b (0.14)	1.36 ^a (0.13)	1.19 ^{bc} (0.01)	1.10 ^c (0.14)	1.10 ^c (0.14)	1.10 ^c (0.14)	***	***	**
20 weeks, kg	1.27 ^c (0.21)	1.59 ^b (0.19)	2.03 ^a (0.20)	1.58 ^b (0.25)	1.72 ^b (0.26)	1.23 ^b (0.21)	1.55 ^a (0.28)	1.69 ^a (0.30)	1.63 ^a (0.22)	1.64 ^a (0.28)	1.64 ^a (0.28)	1.64 ^a (0.28)	***	**	**
Body weight, kg	0.21 (0.21)	0.19 (0.19)	0.20 (0.20)	0.25 (0.25)	0.26 (0.26)	0.21 (0.21)	0.28 (0.28)	0.30 (0.30)	0.22 (0.22)	0.28 (0.28)	0.28 (0.28)	0.28 (0.28)	**	***	NS
68 weeks, kg	39.2 ^c (10.3)	85.3 ^a (10.2)	73.5 ^b (11.6)	66.8 ^b (11.1)	67.6 ^b (9.38)	38.4 ^c (10.7)	76.1 ^a (18.5)	63.6 ^b (14.8)	66.9 ^{ab} (10.5)	63.0 ^b (9.80)	63.0 ^b (9.80)	63.0 ^b (9.80)	**	***	NS
Hen-housed egg production, %	10.3 (10.3)	10.2 (10.2)	11.6 (11.6)	11.1 (11.1)	9.38 (9.38)	10.7 (10.7)	18.5 (18.5)	14.8 (14.8)	10.5 (10.5)	9.80 (9.80)	9.80 (9.80)	9.80 (9.80)	***	***	***
Mean egg weight, g	44.4 ^c (2.95)	62.4 ^a (2.82)	63.9 ^a (3.24)	55.0 ^b (3.09)	53.5 ^b (2.23)	41.0 ^c (2.63)	54.7 ^a (5.13)	53.9 ^a (4.73)	51.6 ^a (3.43)	48.2 ^b (3.35)	48.2 ^b (3.35)	48.2 ^b (3.35)	***	***	***
Total egg mass	5888 ^d (1741)	17899 ^a (2341)	15809 ^b (2678)	12330 ^c (2087)	12113 ^c (1649)	5289 ^c (1484)	14115 ^a (4070)	11620 ^b (3088)	11588 ^b (1902)	10191 ^b (1629)	10191 ^b (1629)	10191 ^b (1629)	***	***	***
production, g/hen	77.0 ^c (4.94)	121 ^a (7.66)	116 ^a (6.09)	101 ^b (5.05)	105 ^b (5.12)	63.3 ^c (3.60)	98.8 ^c (8.11)	89.7 ^b (5.09)	88.8 ^b (5.41)	87.3 ^b (6.28)	87.3 ^b (6.28)	87.3 ^b (6.28)	***	***	***
Feed consumption, g/d/hen	5.29 ^a (1.22)	2.28 ^c (0.30)	2.49 ^{bc} (0.23)	2.78 ^b (0.34)	2.89 ^b (0.31)	4.99 ^a (0.86)	2.23 ^d (0.25)	2.44 ^{cd} (0.26)	2.80 ^{bc} (0.41)	2.88 ^b (0.31)	2.88 ^b (0.31)	2.88 ^b (0.31)	NS	***	NS
Feed conversion ratio, kg feed/kg egg mass	1.22 (1.22)	0.30 (0.30)	0.23 (0.23)	0.34 (0.34)	0.31 (0.31)	0.86 (0.86)	0.25 (0.25)	0.26 (0.26)	0.41 (0.41)	0.31 (0.31)	0.31 (0.31)	0.31 (0.31)	NS	***	NS

^{a-d} means between genotypes within each ambient temperature having different letters are significantly (P<0.05) different

Na - Naked-neck (from Ethiopia); LW - Lohmann white; NH - New Hampshire; Na × LW - F₁ crosses of Na males with LW females; Na × NH - F₁ crosses of Na males with NH females

* - P<0.05; ** - P<0.01; *** - P<0.001; NS - not significant

Physiological responses of corticosterone (CS) level

As presented in Table 4, the effect of genotype on CS levels was ($P < 0.01$) significant at all times measured, whereas that of temperature was significant at 38, 51 and 65 week, but not at 22. In general, the CS level significantly increased by 12.6% in heat stressed hens compared with those of controls. Nevertheless, the magnitude of heat stress on the response of CS level was inconsistent at different ages. In heat stressed genotypes, the overall mean values of CS concentration at 22 weeks slightly decreased compared with those of control groups. However, compared with thermo-neutral genotypes, the overall mean values of CS concentration increased in heat exposed genotypes by 19, 24 and 12.0% at 38, 51 and 65 week, respectively.

In heat exposed genotypes, the CS concentration at 22 week was only significantly higher for Na \times NH genotype (Table 4). Similar trend has been also observed in those genotypes held at thermo-neutral temperature. At 38 weeks,

Table 4. Level of plasma corticosterone (ng/dl) in Naked-neck, Lohmann White and New Hampshire laying hens and their F_1 crosses at thermo-neutral and heat stress ambient temperatures

Ambient temperatures (T)	Genotypes (G)	Age of birds, weeks			
		22	38	51	65
Thermo-neutral	Na	4.06 ^{ab}	4.28 ^b	3.96 ^a	3.04 ^c
	LW	3.60 ^b	3.30 ^b	2.73 ^c	2.78 ^c
	NH	3.42 ^b	4.19 ^b	3.83 ^{ab}	4.02 ^b
	Na \times LW	3.37 ^b	3.43 ^b	3.08 ^{bc}	2.42 ^c
	Na \times NH	5.00 ^a	5.88 ^a	4.39 ^a	5.31 ^a
Heat stress	Na	3.93 ^b	5.88 ^a	6.65 ^a	5.36 ^a
	LW	3.13 ^b	3.33 ^b	3.05 ^b	2.25 ^c
	NH	3.18 ^b	6.39 ^a	3.89 ^b	3.99 ^{ab}
	Na \times LW	2.79 ^b	3.09 ^b	4.45 ^b	3.78 ^b
	Na \times NH	5.48 ^a	6.40 ^a	4.28 ^b	4.28 ^{ab}
Pooled SEM		0.32	0.39	0.33	0.29
<i>ANOVA significance level [P (F)]</i>					
T		NS	**	***	*
G		***	***	***	***
T \times G		NS	**	***	***
Overall means					
Genetic group	Na	LW	NH	Na \times LW	Na \times NH
ng/dl	4.65	3.02	4.11	3.30	5.13
Temperatures	Thermo-neutral	Heat stress			
ng/dl	3.80	4.28			

^{a-c} means between genotypes within each ambient temperature and age having different letters are significantly ($P < 0.05$) different; Na - Naked-neck (from Ethiopia); LW - Lohmann White; NH - New Hampshire; Na \times LW - F_1 crosses of Na males with LW females; Na \times NH - F_1 crosses of Na males with NH females; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS - not significant; SEM - standard error of mean

increased in CS concentration was noted in heat stressed Na, NH and Na × NH genotypes which were significantly different from LW and Na × LW genotypes. However, at similar age only the Na × NH at thermo-neutral temperature had significantly higher ($P < 0.05$) CS level than other genotypes. At 51 week, the highest CS was only noted in heat stressed Na and was significantly different from other heat exposed genotypes. At 65 week, the highest CS was noted in Na, Na × NH and NH genotypes and the least in LW that were held under heat stress environment. The trend in CS concentration with increasing age was similar at both ambient temperatures. The maximum CS concentration was observed at 38 week of age and then declined sharply at 51 and thereafter slightly until 65 week of age.

Physiological responses of 3,5,3'-triiodothyronine (T_3) level

As presented in Table 5, the interactions between genotype and ambient temperature in T_3 level were significant ($P < 0.001$) at all ages. The effect of heat

Table 5. Plasma levels of 3,5,3'-tri-iodothyronine (nmol/l) in Naked-neck, Lohmann White and New Hampshire laying hens and their F_1 crosses at thermo-neutral and heat stress environments

Ambient temperatures (T)	Genotypes (G)	Age of birds, weeks			
		22	38	51	65
Thermo-neutral	Na	6.40 ^a	5.61 ^c	3.44 ^b	3.36 ^a
	LW	6.58 ^a	6.94 ^a	3.51 ^b	2.91 ^b
	NH	6.54 ^a	6.23 ^b	4.27 ^a	3.57 ^a
	Na × LW	6.69 ^a	5.65 ^c	4.44 ^a	3.32 ^a
	Na × NH	5.76 ^b	6.15 ^b	3.77 ^b	3.32 ^a
Heat stress	Na	4.20 ^b	4.66 ^a	1.95 ^b	1.82 ^c
	LW	4.58 ^b	4.91 ^a	2.76 ^a	2.31 ^b
	NH	5.57 ^a	4.12 ^b	2.86 ^a	2.87 ^a
	Na × LW	4.70 ^b	4.96 ^a	2.19 ^b	2.03 ^{bc}
	Na × NH	4.73 ^b	4.19 ^b	2.67 ^a	2.32 ^b
Pooled SEM		0.15	0.10	0.12	0.11
<i>ANOVA significance level P (F)</i>					
	T	***	***	***	***
	G	*	***	***	***
	T × G	***	***	***	***
Overall means					
Genetic group	Na	LW	NH	Na × LW	Na × NH
nmol/l	3.92	4.36	4.39	4.25	4.12
Temperatures	Thermo-neutral	Heat stress			
nmol/l	4.88	3.52			

^{a-c} means between genotypes within each ambient temperature and age having the different letters are significantly ($P < 0.05$) different; Na - Naked-neck (from Ethiopia); LW - Lohmann White; NH - New Hampshire; Na × LW - F_1 crosses of Na males with LW females; Na × NH - F_1 crosses of Na males with NH females; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS - not significant; SEM - standard error of mean

stress on T_3 level was significant ($P<0.001$) and consistent across the genetic groups resulting in a general depression of about 28% compared with those control groups. Moreover, significant differences ($P<0.01$) in plasma T_3 were observed between heat stressed genotypes at various ages. At 22, 51 and 65 week of age, the highest and lowest T_3 levels were observed in NH and Na genotypes, respectively. At 38 week of age however, the highest T_3 levels were found in LW and Na \times LW while the lowest in NH and Na \times NH genotypes. In general, the T_3 level was inconsistent among heat stressed genotypes with increasing age of birds.

Between 22 and 38 weeks of age, the T_3 level slightly increased in heat exposed Na, Na \times LW and LW genotypes, whereas it somewhat decreased in NH and Na \times NH genotypes. At 51 week of age, the T_3 level notably declined and remained constant thereafter across all genotypes, indicating reduced function of thyroid gland at the later ages.

Correlation analysis

The correlation analysis between feed consumption and CS showed a negative relationship while that of T_3 a positive correlation (Table 6). However, the correlation analysis between T_3 and performance traits showed a positive relationship in layer hens, being significant for feed consumption and body weight. A significant ($P<0.01$) and negative correlation was observed between CS and feed consumption, egg weight and total egg mass production. Moreover, the positive correlation between T_3 and feed consumption was significant ($P<0.01$).

Table 6. Correlation coefficients of performance traits with physiological parameters at heat stress (above broken line) and thermo-neutral (below broken line) ambient temperatures

Enzymes/hormones	Hen-housed egg production	Egg weight	Total egg mass production	Body weight ¹	Feed consumption
Triiodothyronine	0.17 ^{ns}	0.26*	0.18 ^{ns}	0.30*	0.37**
Corticosterone	-0.32*	-0.44**	-0.39**	-0.28*	-0.41**
Triiodothyronine	0.22 ^{ns}	0.10 ^{ns}	0.20 ^{ns}	-0.12 ^{ns}	0.13 ^{ns}
Corticosterone	-0.09 ^{ns}	-0.13 ^{ns}	-0.11 ^{ns}	-0.12 ^{ns}	-0.03 ^{ns}

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; ^{ns} $P>0.05$; ¹ average body weight of both ages

DISCUSSION

Performance traits. Decreased body weight in the current study may be due to a reduction in feed consumption and feed conversion ratio and is in good agreement with the reports of Mashaly et al. (2004). The decline in egg production traits in experimental groups agree with those of Franco-Jimenez et al. (2007).

The decrease in egg production traits in the present and previous results might be most likely due to the decrease in feed consumption, reducing the available nutrients for egg production. Heat stress not only reduces feed intake but has been reported to also reduce digestibility of different components of the diet (Bonnet et al., 1997). Furthermore, it has been reported that exposure to high temperature decreased plasma protein concentration (Zhou et al., 1998) and plasma calcium concentration (Mahmoud et al., 1996), both of which are required for egg formation. Furthermore, Zhou et al. (1998) reported that exposure to high temperature decreased plasma protein concentration, which is an essential nutrient for egg formation.

The reduction in feed consumption in response to heat stress in the present study is consistent with the findings of Mashaly et al. (2004) and Lu et al. (2007). A reduction in feed consumption is the birds' effort to reduce energy intake in response to the increase in the heat energy from the environment, thereby reducing the energy needed from the feed. The reduced feed consumption and subsequent loss of needed nutrients quickly affect the productivity of the flock.

Plasma corticosterone (CS). The increased level of CS in heat exposed chickens in the current study is consistent with previous findings (Davis et al., 2000; Piestun et al., 2008). Changes in hormonal status, particularly CS, may have a considerable influence on responses to heat exposure. In the literature, the effect of heat stress on CS concentration has not been consistent. McFarlane and Curtis (1989) reported that exposing 7-d-old chickens to environmental heat stress for 7 d did not increase CS levels. On the contrary, Ben-Nathan et al. (1976) found an increased level of CS in chickens exposed to constant chronic heat stress (32.2°C) compared with control (21°C). These contradictory observations might be associated with the age or genetic background of chickens involved in the study.

The level of CS in the current results was affected by birds' age, which was related to the egg production cycle. Plasma CS considerably increased during peak egg production between 22 and 38 week of age and declined thereafter. The higher CS level during peak egg production phase might be interpreted as period of physiological stress. As CS is a gluconeogenic hormone to produce glucose from endogenous sources, usually from protein (Davis et al., 2000), and elevated CS is correlated to its metabolic effects to provide glucose and energy for peak egg production. The decline in CS level with increasing age is in accordance with Gould and Siegel (1985). With advancing age, the difference between experimental and control groups in CS level became narrower, suggesting possible acclimation of chickens to chronic heat exposure over time.

Correlation analysis between feed consumption and CS showed a highly significant negative relationship. This suggests that high levels of CS could be

associated with lower rate of feed consumption, which might have contributed to a reduced performance of heat stressed chickens indicating a severe disturbance in the normal physiological process of the birds' body. There is very little information in the literature dealing with the correlation between physiological parameters and performance traits in layer hens.

Plasma triiodothyronine (T_3). The general reduction in T_3 level in all heat stressed genotypes is consistent with previous reports (Silva, 2003; Gharib et al., 2008). Geraert et al. (1996) found a sharp decline of plasma T_3 in broiler chickens held at 32°C environmental temperatures.

The lowest T_3 concentration observed in Na and Na × LW genotypes suggests improved adaptability to long-term heat exposure due to reduced feather coverage and relative body size. Reduced feather coverage should improve and enhance heat dissipation and consequently alleviate the effects of heat on chickens reared in hot climates (Ajang et al., 1993). Moreover, it has been documented that genotypes with small body size demonstrated better heat-tolerance to stressful environments (Zeman et al., 1996). This may further suggest that the thyroid gland in small body sized chickens produces little T_3 , which is beneficial for better adaptability in hot environments by reducing the metabolic body heat output.

On the other hand, both LW and NH genotypes were less effective in reducing plasma T_3 , which might explain their greater difficulties to adapt to the long-term heat challenges, as reflected by their significant reduction in performance traits and increased mortality rate (data not shown). A decreased heat-tolerance observed in high performing hens may be attributable to a reduced ability to lose heat (MacLeod and Hocking, 1993) or an inappropriately increased heat production during exposure to high thermal loads (Sandercock et al., 1995). In agreement with the findings of Davis et al. (2000), the levels of circulating T_3 in the current study varied with respect to the age and egg production cycle of the hens. Plasma T_3 increased to its highest level during peak and mid egg production periods and then, declined until the end of the experiment (68 weeks of age). Lien and Siopes (1993) observed a similar response in turkeys when T_3 peaked during the early onset of lay and then steadily declined during the remaining egg production cycle. Thus, increase in T_3 during the first phase of egg production in laying hens are most likely related to the adaptation to changes in metabolic demands caused by physiological stress. It could be thus speculated that basal metabolic rate might have been augmented to meet the increased demand for high egg production during this phase.

As discussed above, the highly productive layer hens in the current study were very similar in their endocrine profiles but differed markedly (not significantly) from the Na chickens. The Na chickens were characterized by lower plasma T_3 across all bleeding age points (except at 65 week of age) compared with LW and

NH hens. Contrary to the present finding, Gonzales et al. (1999) reported a high T_3 levels in Naked-neck male broiler chickens at similar age.

In the current result, a significant ($r=0.37$; $P<0.01$) positive correlation was observed between feed consumption and T_3 in heat stressed chickens which is in agreement with the findings of Yahav et al. (1996), who found a significant correlation between these in broiler chickens. This may suggest that feed consumption depression was associated with reduced thyroid activity resulting in reduced metabolic heat production, which is an essential physiological mechanism for adaptive responses of birds to long-term heat exposure. Surprisingly, no relationship was obtained between feed consumption and plasma T_3 in hens held at thermo-neutral temperature.

CONCLUSIONS

These results suggest that there are major differences in thermoregulatory responses to heat stress in all five genotypes, possibly due to differences in their overall genetic background attributable to differing efficiencies of heat loss mechanisms. Accordingly, the Naked-neck (Na) chickens and their F_1 crosses with Lohmann White (LW) and New Hampshire (NH) hens were more heat tolerant than high performing LW line and NH breed. Although the corticosterone levels increased in all heat stressed hens, the pattern was inconsistent among genotypes. The Na and Na \times LW genotypes demonstrated the lowest T_3 levels with improved performance, suggesting physiologically stable adaptability to long-term heat stress. Since responses of plasma T_3 levels were consistent in all heat stressed genotypes, this hormone might be considered as reliable indicator of long-term heat stress in laying hens.

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