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Full Length Research Paper

Effect of long-term heat stress on key enzyme activities and T_3 levels in commercial layer hens

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High environmental temperatures are the most important inhibiting factors to poultry production in hot regions. The objective was to test adaptive responses of different chicken genotypes to long- term high temperature and identify suitable indicators of physiological parameters. Forty eight female chickens from each genotype of Lohmann Brown (LB), Lohmann White (LW), New Hampshire (NH), White Leghorn selected for improved feed efficiency (WL-FE) and dwarf White Leghorn (WL-dw) were randomly assigned either to the control group (18 to 20°C) or to the experimental group (30 to 32°C). Blood samples were collected from randomly selected 12 birds of each genotype at three age points. Levels of glutamic- pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), creatine kinase (CK), lactate dehydrogenase (LDH) and 3,5,3′-triiodothyronine (T_3) were determined in plasma. The results indicated that compared to controls, the GPT activity in heat stressed chickens significantly increased by 29.2% in all genotypes. The CK activity in heat stressed chickens was only significantly higher at 22 weeks old. Activities of GOT and LDH were variable in all heat stressed chickens. The T_3 concentration significantly reduced by 41% in all heat stressed chickens and the WL-dw had the lowest value. We concluded that T_3 could be considered as reliable indicator of long-term heat stress. Moreover, LW and WL-dw genotypes demonstrated better heat tolerant.

Key words: Layer hens, long-term heat stress, enzyme activities, T_3 concentration.

INTRODUCTION

Stress occurs when an animal experiences changes in the environment that stimulate body responses aimed at re-establishing homeostatic conditions (Mumma et al., 2006). Environmental stress include abiotic factors (for example, climate, temperature and chemical components) and biotic factors (for example, competition, nutrition and various forms of infectious diseases), which can act independently, but often act synergistically (Bijlsma and Loeschcke, 2005). According to Leeson (1986) and Balnave (1996) high environmental temperatures are the most important inhibiting factors to poultry production in hot regions, apparently because chickens cannot dissipate fast enough the heat produced following meals, which subsequently leads to reduced feed intake and lower weight gain or egg production (Cahaner and Leenstra, 1992). Heat stress causes serious losses in poultry production because it increases mortality and reduces performance in broilers and laying hens (Teeter et al., 1985). Stress responses are considered to be essentially adaptive or protective and thus should prevent or minimize detrimental effects of the stressor that was imposed upon the animal. High environmental temperatures affect not only performance parameters, but require also various physiological (Koelkebeck and Odom, 1995; Maak et al., 2003; Tao et al., 2006; Star et al., 2008) and immunological (Mashaly et al., 2004; Star et al., 2009) adaptations of birds.

Ambient temperatures exceeding the thermoneutral zone lead to elevated core temperature and consequently initiate a number of responses leading to the neutralization

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Table 1. Genetic structure and size of experimental flocks.

Ambient temperatures	Commercial layers				
	LW	LB	NH	WL-dw	WL-FE
Normal (control group, 18-20°C)	24	24	24	24	24
High (experimental group, 30-32°C)	24	24	24	24	24
Total (n)			240		

LW = Lohmann White; LB = Lohmann Brown; NH = New Hampshire; WL-dw = dwarf White Leghorn; WL-FE = White Leghorn selected for improved feed efficiency.

of the heat induced metabolic changes (Gonzalez-Esquerra and Leeson, 2006). Among others, the plasma levels of different enzymes are affected (Bogin et al., 1996a). Activities of plasma enzymes, including CK, GOT and GPT, increase in humans after severe exercise (Kayashima et al., 1995; Mashiko et al., 2004), in rats after restraint with water immersion (Arakawa et al., 1997), in sheep after repeated restraint and isolation (Apple et al., 1993) and in Friesian calves after exposure in summer season (Marai et al., 1995). Animals behave and respond differently to heat stress and thus, their structural composition and genetic characteristics contribute to their ability to resist heat stress (Bogin et al., 1996a, b). These authors demonstrated that prolonged heat stress results in a dramatic physiological change in chicken organs. They suggested that these physiological changes might be used as indicator of heat stress in the selection of thermo-tolerant chicken lines. The importance of thyroid gland hormones in adaptation to heat stress is related to the central role that thyroid hormones play in the regulation of metabolic rate of birds (McNabb, 1988). This effect has been demonstrated by thyroid hormone administration, which stimulates heat production (Arieli and Berman, 1979) and by surgical or chemical thyroidectomy of chicken, which produces a decrease in metabolic rate and body temperature (Lam and Harvey, 1990).

The major hormone product of thyroid gland, thyroxine (T_4), is considered to be a prohormone of the more biologically active 3,5,3 -triiodothyronine (T_3) (He et al., 2000). Both T_3 and T_4 play important roles in regulating metabolism and thermogenesis in chickens (Tao et al., 2006). The selective peripheral conversion of T_4 to T_3 or reverse T_3 (r-T_3) is believed to play an important role in thermoregulation in domestic fowl (Rudas and Pethes, 1984). When chickens are exposed to warm temperatures, T_4 is inactivated by conversion into r -T_3, whereas during cold exposure T_4 is converted into T_3, which stimulates metabolic activity. While it is generally accepted that T_3 stimulates metabolic rate and that both T_3 and T_4 concentration depressed following heat stress, this pattern is not universally observed (Scheele et al., 1991; Etches et al., 1995). However, the differences in the blood concentrations were more expressive for T_3 than for T_4, emphasizing that T_3 variation is more accurate in studies of thermal stress (Lawrence and Fower, 1997).

The effects of hot environment has been found to vary in magnitude among different chicken breeds or stocks (Deeb and Cahaner, 2001; Mashaly et al., 2004). The aim of this study was thus to test the adaptive responses of different chicken genotypes to long-term high environmental temperatures and identify the suitable indicators of physiological parameters such as key enzyme activities and T_3 levels in plasma under long-term heat stress situations.

MATERIALS AND METHODS

Experimental animals

Forty-eight female chickens from each of the following genotypes were used to test their adaptability to long-term heat stress: Dwarf White Leghorn (WL-dw), White Leghorn selected for improved feed efficiency (WL-FE), New Hampshire (NH), Lohmann White (LW) and Lohmann Brown (LB). The chicks were hatched at the same time and randomly assigned either to the control group (18 to 20°C) or to the experimental group (30 to 32°C) as presented in Table 1. They were raised on floor pens in the respective temperatures up to 20 weeks of age and transferred into battery cages. The hens were then kept in temperature regulated conventional individual layer cages (1000 cm² per hen) from week 21 to the end of the experimental period (week 56). The chamber was ventilated using automatic fans and the lighting regimes was maintained under 12L:12D. The management practices in both groups were essentially the same. 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 growing chicks and pullets, respectively.

The matured hens were fed on commercial laying feed with 11.4 MJ/kg energy and 17% crude protein contents. They were fed in-group *ad libitum* (4 hens/feed pan) and supplied with water using individual nipple drinkers.

Blood sampling

Blood samples were collected from randomly selected 12 birds of each genotype at 22, 43 and 54 weeks age with 180 total samples (12 birds x five genotypes x 3 age points). Blood samples were taken by a qualified veterinarian from the wing vein of the bird using disposable syringes and directly collected into ethylene diamine tetra acetic acid (EDTA) coated test tubes. Blood was taken in the morning between 8.00 and 10.00 am, and the time needed between handling of each chicken and bleeding was in most cases less than

Table 2. Activities of GPT, GOT, CK and LDH enzymes in blood plasma of layer hens kept at high and normal environmental temperatures.

Enzyme (IU/L)	Ambient temperature	LW	LB	NH	WL-dw	WL-FE
GPT	Normal	8.42^{aA}	7.58^{aA}	7.01^{aA}	7.15^{aA}	7.87^{aA}
	High	6.85^{bAB}	6.34^{bB}	6.43^{aB}	7.23^{aA}	6.46^{bB}
GOT	Normal	54.8^{aA}	54.7^{aA}	55.9^{aA}	55.8^{aA}	52.5^{aA}
	High	59.7^{aA}	60.4^{aA}	56.6^{aAB}	60.7^{aA}	54.4^{aAB}
CK	Normal	342^{aC}	566^{aAB}	553^{aAB}	625^{aA}	410^{aBC}
	High	349^{aB}	575^{aA}	595^{aB}	657^{aA}	412^{aB}
LDH	Normal	182^{aA}	191^{aA}	178^{aA}	187^{bA}	176^{aA}
	High	197^{aB}	203^{aAB}	190^{aB}	214^{aA}	205^{aAB}

$^{A-E}$ Means between genotypes within a temperature with different uppercase superscript letters are significantly ($p<0.05$) different. ^{a,b} Means between temperatures within a genotype with different lowercase superscript letters are significantly ($p<0.05$) different; LW = Lohmann White; LB = Lohmann Brown; NH = New Hampshire; WL-dw = dwarf White Leghorn; WL-FE = White Leghorn selected for improved feed efficiency; GPT= glutamic-pyruvic transaminase; GOT = glutamic-oxaloacetic transaminase; CK = creatine kinase; LDH = lactate dehydrogenase.

1 min. After sampling, blood was centrifuged and plasma was stored at −20°C until further processing.

Determining the activity of key enzymes

The activities of creatine kinase (CK) and lactate dehydrogenase (LDH) were determined photometrically at 340 nm wavelength absorbance with commercially available test kits (Sigma, Berlin). The activities of glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) were assayed colorimetrically with Sigma test kits. These enzymes catalyse the transfer of α-amino groups from specific amino acids to α-ketoglutaric acid to yield glutamic acid and oxalacetic acid or pyruvic acid. These keto acids are then determined colorimetrically after their reaction with 2,4-dinitrophenyl -hydrazine. The absorbance of the resulting colour was measured at a wavelength of 505 nm to take advantage of the great difference in the absorption, which exists between hydrazone of α-ketoglutaric acid and the hydrazones of oxalacetic acid or pyruvic acid (Kachmar, 1970). The enzyme activities in plasma were determined either within one week (CK and GPT) or two weeks (GOT) after collecting the blood sample to avoid the deterioration of enzyme activities through extended storage time. Since the LDH activity progressively declines with storage time, the assaying procedure was completed within three days of blood collection for optimum result.

All determinations of enzyme activities were carried out in duplicates and some analyses were repeated when unreliable results were obtained.

Measuring plasma levels of T_3 (3,5,3′-triiodothyronine)

Total plasma T_3 was determined by commercially available Milenia total T_3 endpoint enzyme immunoassay kit (EIA-Test-kit; DPC, L.A). In this procedure, a micro plate reader at 450 nm wavelength was employed. Moreover, appropriate software package was used for facilitating data generation, analysis, reporting and quality control. Each sample was prepared in duplicate to enhance precision. Assaying was performed within four weeks of blood collection. The mean intra-assay (within-run) and inter-assay (run-to-run) variation for T_3 was 4.93 and 7.17%, respectively. The antiserum used in the Milenia total T_3 test-kit is highly specific for T_3, with a relatively low cross activity to other substances present in samples. The assay's detection limit, defined as the concentration two standard deviations below the response at zero doses, is 6.7 ng/dl. All analyses (enzyme activities and T_3 levels) were performed essentially as described in the manufacturer's manuals.

Statistical analysis

All statistical analysis was done with General Linear Models Procedure of Statistical Analysis System (SAS, 2001). All parameters were analysed in a complete 2 x 5 factorial design (2 normal and high ambient temperatures; 5 genotypes). When significance differences in ANOVA were detected, comparisons of multiple means were made by using Tukey's HSD test. All statements of statistical differences were based on $p<0.05$ unless noted otherwise.

RESULTS

Enzyme activities

As shown in Table 2, no significant differences were found between genotypes in control group for GPT, GOT and LDH activities. However, significant differences ($p<0.05$) between genotypes in warm environment were noted for all enzymes investigated. Accordingly, the WL-dw had the highest LDH activity ($p<0.05$) compared to LW, LB and NH genotypes. Moreover, the WL-dw had higher ($p<0.05$) CK activity than LW, NH and WL-FE genotypes. Similarly, the WL-dw had the largest GPT activity ($p<0.05$) compared to LB, NH and WL-FE genotypes. A comparison of the enzyme levels between

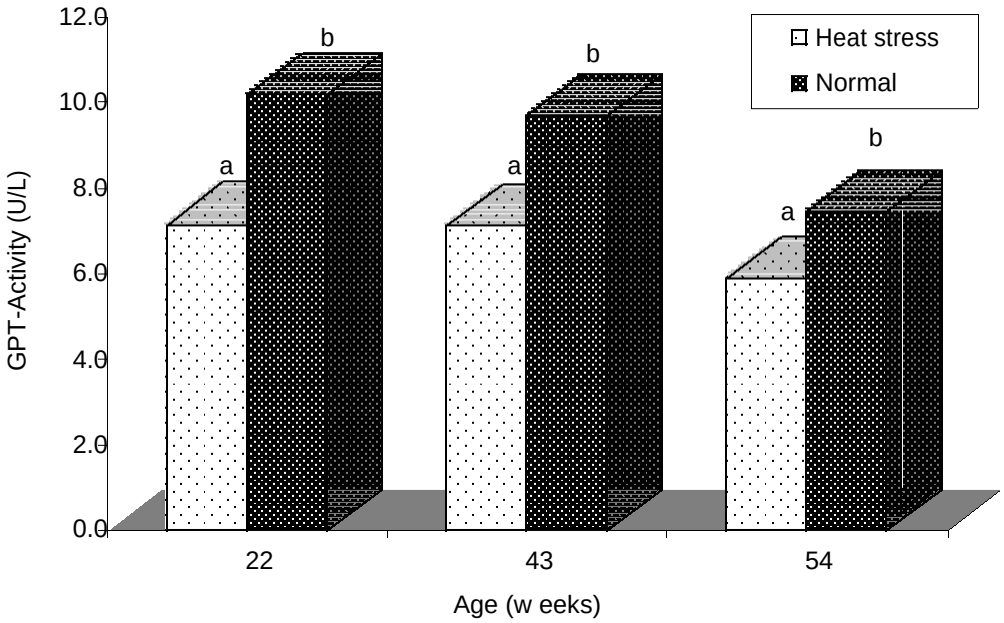

Figure 1. Physiological response of layer chickens to long-term heat stress as measured by GPT activity.

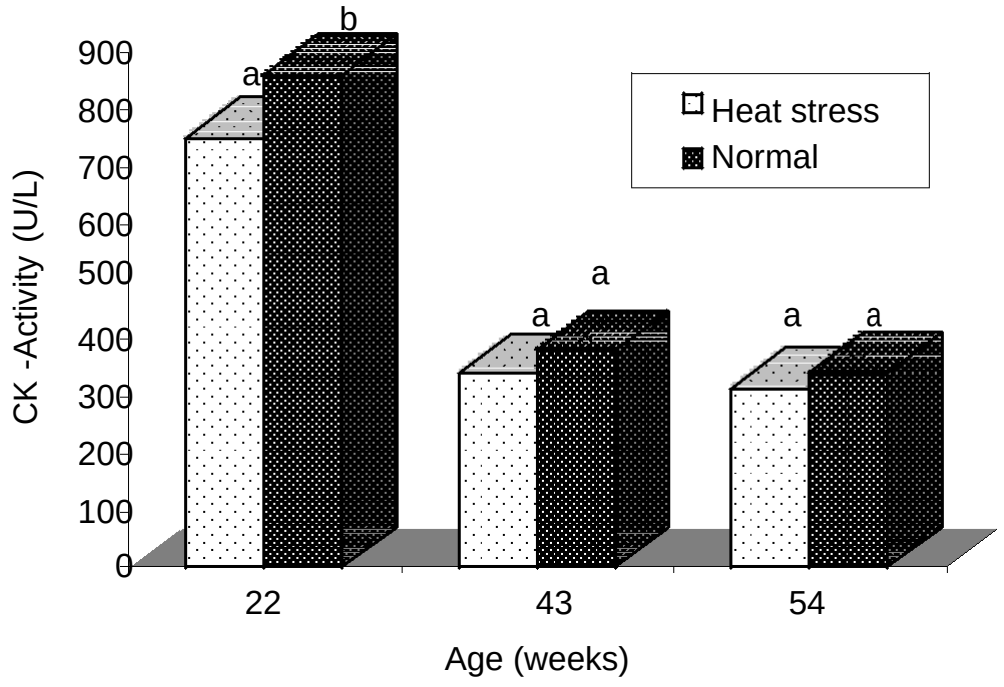

Figure 2. Plasma CK activity in different layer hens kept at high environmental temperature.

layers reared at normal and high ambient temperatures revealed significant differences between the treatment groups for LDH, GOT and GPT (Figure 1) but only at 22 weeks of age for CK (Figure 2). A constant decline in all investigated enzymes was observed with advancing age of the hens. It must be noted that the levels of LDH and GOT are still within the respective physiological ranges (LDH: 215.8 IU/l; GOT: 82.28 IU/l) in the control as well as in the experimental hens. This may suggest that the hens of all investigated genotypes were able to adapt to

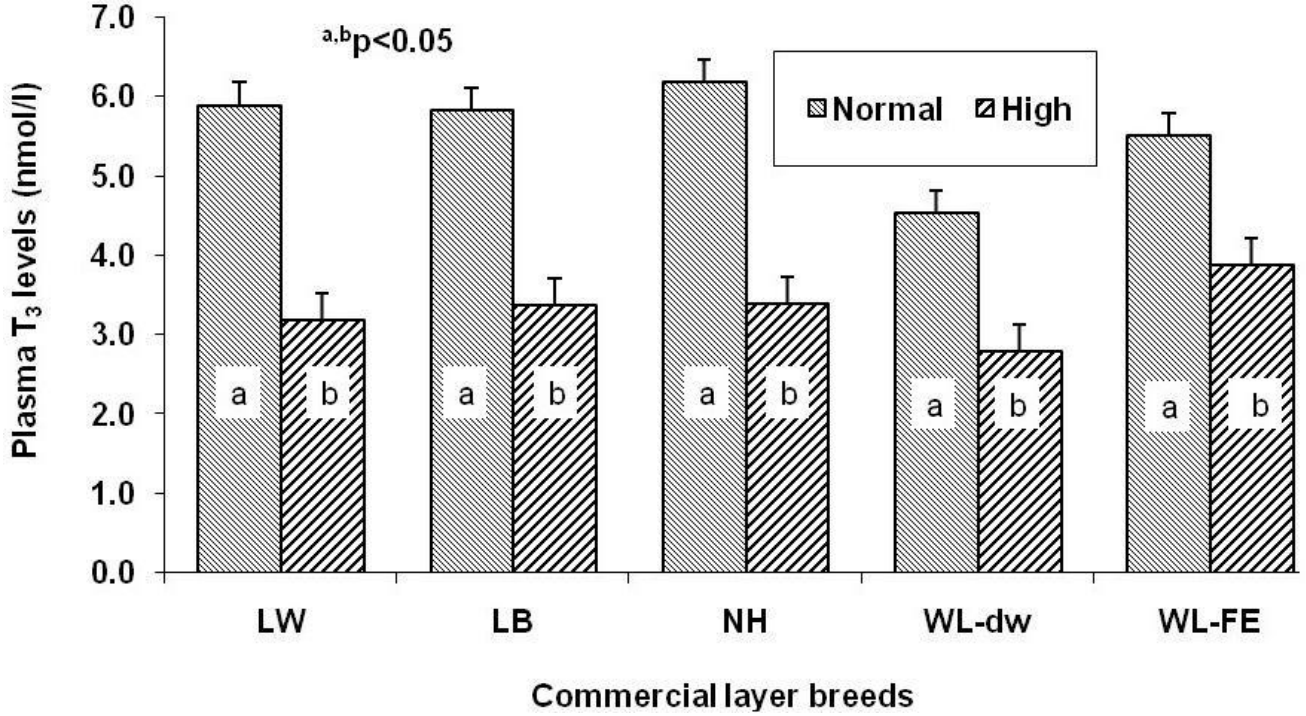

Figure 3. Levels of plasma T_3 in layer genotypes kept at normal and high ambient temperatures. Each bar on the graph represents genotype mean ±SE.

Table 3. Levels of plasma T_3 (nmol/l) in layer chickens at 22, 43 and 54 weeks of age kept at normal and high ambient temperatures (mean ± SD; n = 60/age).

Age (week)	Ambient temperature	LW	LB	NH	WL-dw	WL-FE
22	Normal	5.48^{a}±0.93	6.45^{a}±1.59	5.93^{a}±1.39	4.05^{a}±0.67	5.72^{a}±1.25
	High	4.33^{b}±0.66	4.73^{b}±1.01	4.60^{a}±1.10	3.72^{a}±0.50	5.58^{a}±0.86
43	Normal	6.62^{a}±1.01	6.33^{a}±0.46	7.07^{a}±0.42	5.47^{a}±1.38	6.15^{a}±1.02
	High	2.63^{b}±0.71	2.83^{b}±1.04	2.87^{b}±0.30	2.45^{b}±0.65	2.65^{b}±0.69
54	Normal	5.53^{a}±0.67	4.63^{a}±0.54	5.50^{a}±0.34	4.05^{a}±0.56	4.78^{a}±1.14
	High	2.55^{b}±0.58	2.52^{b}±0.72	2.68^{b}±0.55	2.20^{b}±0.75	2.37^{b}±0.64

^{a,b} Means between temperatures within a genotype with different superscript letters are significantly ($p<0.05$) different. LW = Lohmann White; LB = Lohmann Brown; NH = New Hampshire; WL-dw = dwarf White Leghorn; WL-FE = White Leghorn selected for improved feed efficiency.

30°C ambient temperature without a major physiological change in the plasma enzyme levels.

T_3 levels in blood plasma

As presented in Figure 3, the level of T_3 in blood plasma was reduced considerably by 41% ($p<0.05$) in all heat stressed genotypes. The average reduction was 16.1, 57.5 and 45.2% at the ages of 22, 43 and 54 weeks, respectively. In general, the WL-dw had the lowest and WL-FE the highest T_3 concentration, whereas the values for NH, LB and LW genotypes fall in between (Table 3). Among heat stressed genotypes, WL-dw had the lowest T_3 levels followed by LW, LB, NH and WL-FE with an increasing order. The difference in T_3 levels between experimental and control groups were lowest at sexual maturity, but increased considerably (58%) when the hens were 43 weeks old. The trend, however, became smaller at the end of the experiment. Levels of plasma T_3 consistently declined in all heat stressed genotypes with increasing age (Table 3), indicating a depressed function

of thyroid gland at the later ages.

DISCUSSION

Change in key enzyme activities

A number of enzymes are used in the clinical biochemistry as tools for differential diagnosis, such as CK, GPT, GOT and LDH. Since the bulk of each is located in different tissues, their abnormal appearance in the blood plasma can give a hint to specific muscle or organ damages (Pech-Waffenschmidt, 1992) . By evaluating the effects of long-term hyperthermia on organ tissues, Bogin et al. (1996b) showed that adaptation to heat stress took place, as evidenced by enhanced enzyme expression. In broiler chickens, CK is released into the circulation following changes in the permeability of the sarcolemma in response to various pathologies and exposure to environmental stressors (Mitchell et al., 1992; Mitchell and Sandercock, 1995). Publications about the effect of high environmental temperatures on the CK activity are not consistent, possibly due to the large variability of the enzymes (Melesse et al., 1998; Melesse, 2000). The effect of heat stress on the activity of investigated enzymes was found to be inconsistent in relation to age and genotypes. According to the results obtained by Pech-Waffenschmidt (1995), heat exposure did not significantly change the enzyme activities in the chicken's serum. This was also supported by previous findings of Ward and Peterson (1973), who reported that the activities of CK and GOT were not influenced even by acute heat exposure. This suggests that virtually no cellular damage, resulting in leakage of intracellular enzymes into the blood, took place.

In a recent study, Sandercock (2001) observed a significant increase in CK activity of broilers exposed to acute type of heat stress reflecting heat stress-induced myopathy. Similarly, Yalçin et al. (2009) reported an increase in plasma CK activity on broilers exposed to daily cyclic heat treatment. On the other hand, feed restriction, as a stress factor, caused a decreased activity of plasma CK in turkeys (Hocking et al., 1998). Overt muscle damage in birds is associated with an increase in the plasma activity of the intracellular muscle isoenzyme CK (Siller et al., 1978; Lumeij et al., 1988). According to Ward and Peterson (1973), the increase in enzyme activity may be partly attributed to cellular damage as a direct consequence of heat stress. Under hyperthermic conditions, the activities of CK, GOT, GPT and LDH were generally higher for the WL- dw line. The WL-dw genotype is normally characterised by small body size. This comprehensive phenomenon along with increased activities of enzymes at high temperature may suggest improved heat tolerance of the WL-dw genotype at the expense of excessive tissue damage. This adaptability to prolonged heat exposure could be thus a genetic characteristic. Therefore, those plasma enzyme activities that are highly correlated with the ability to withstand heat stress may serve as genetic markers for the selection and development of a chicken more resistant to heat stress. Mitchell and Sanderock (1996) and Hocking et al. (1998) reported that the onset of sexual maturity in female turkeys and broilers was associated with a decreased plasma CK activity. This is not in agreement with our result, in which the CK activity was considerably higher just after sexual maturity for all genotypes. In earlier studies, however, Berschneider and Wilsdorf (1975) reported that a maximum CK activity in pig serum was observed during the main growing phase of the investigated animals. This might be caused by a physiological stress due to high metabolic activities taking place during reproduction period.

The high level of plasma CK activity at the onset of sexual maturity may further suggest that the degree of heat stress during growing and reproduction periods could be more severe on the tissues (muscle) of a chicken than at the later ages. The decline in plasma CK activity with increasing age is consistent with the results of Pech-Waffenschmidt (1992), which might be associated with the recovery of tissues over time suggesting adaptability of the investigated chicken genotypes to long-term exposure to high environmental temperature. On the contrary, Mitchell and Sanderock (1994) and Hocking et al. (1998) and Sandercock et al. (2006) reported an increased level of plasma CK with increasing age in turkeys. The increased activity of plasma GOT with age agrees with previous reports by Pech-Waffenschmidt (1992).

Response of plasma T3-levels

The importance of thyroid gland hormones in adaptation to heat stress is related to the central role that thyroid hormones play a key role in the regulation of metabolic rate of birds during growth and egg production (McNabb, 1988; McNabb and King, 1993; Renden et al., 1994). Chronic heat stress markedly depressed the activity of the thyrotrophic axis in layer hen as reflected by reduced plasma T_3 concentration (Williamson et al., 1985; Decuypere and Kuhn, 1988) resulting in functional hypothyroidism (Mitchell and Carlisle, 1992). As a result, heat tolerance improves, as thyroid function is reduced (Hahn et al., 1966; Bowen and Washburn, 1985). In some instances, however, acute exposure of growing chickens to elevated temperature (35 to 41°C) does not appear consistently to affect serum T_3 or T_4 concentrations (Bowen and Washburn, 1985; May et al., 1986). In Japanese quail and pigeons, for example, plasma T_3 and T_4 concentrations have been reported to increase, decrease or remain unchanged following heat stress (Bowen and Washburn, 1985) . The level of plasma T_3 was greatly depressed in heat exposed layer genotypes during the course of this experiment indicating a reduced thyroid function, which suggests a gradual acclimation to

long-term heat exposure. The overall heat stress depression in T_3 is in agreement with other authors, who reported a decreased thyroid activity in chickens exposed either to acute or chronic type of heat stresses (Brigmon et al., 1991; Yahav and Plavnik, 1999; Maak et al., 2003; Tao et al., 2006). Mitchell and Carlisle (1992) and Geraert et al. (1996) found a dramatic decline of plasma T_3 in broiler chickens reared at 35 and 32°C environmental temperatures, respectively.

It has been reported that thyroid hormone administration stimulates heat production with increased metabolic rate resulting in reduced thermo-tolerance (Bowen and Washburn, 1985) and in increased mortality. In the present study, the WL-FE, LW, LB and NH genotypes were less effective in reducing plasma T_3, which might explain their greater difficulties to cope up with chronic type of heat stress challenges, as indicated by increased mortality (with the exception of the WL-FE). Plasma T_3 plays a major role in feed intake, which is demonstrated by the existence of a linear correlation between the level of plasma T_3 and feed intake in broiler chickens (Williamson et al., 1985; Yahav et al., 1996). The main consequence of the heat stress on animal productivity is thus related to a decrease of feed intake. The feed intake in all genotypes was significantly ($p<0.05$) reduced by 26%, the individual decrease being 28.8, 26.5, 23.6, 25.5 and 25.3% for LW, LB, NH, WL-dw and WL-FE genotypes, respectively (Melesse et al., unpublished data). In another study, Melesse et al. (2005) reported a general decline of 20.4% feed intake in commercial layer hens due to chronic heat stress, which is slightly lower than the average value observed for the five genotypes used in present study. The WL-dw genotype consumed about 26.4, 27.6 and 22.6% less feed compared to LW, LB and NH genotypes, respectively. This physiological response is necessary to reduce the metabolic heat output during heat stress situations, as the process of acclimatisation is mainly associated with low basal metabolic rate at high ambient temperatures. This change could be associated with changes in sizes of the thyroid gland and rate of thyroid secretion.

The decline in body weight due to heat stress is also associated with reduced feed consumption, which induces a decreased metabolic activity by minimising excessive heat production, which is in turn very essential for the maintenance of body temperature during heat exposure (Loehle et al., 1987). The lower feed intake together with a decrease in blood circulating thyroid hormone levels determine lower metabolic and thermogenic rates, which explain the decrease of animal productivity during exposures to chronic stressful heat conditions. Therefore, thyroid hormones are of utmost importance in the heat adaptation process, allowing the adjustment of the metabolic rates in favour of the body heat balance. Age related changes in plasma T_3 concentration in our studies are in accordance with earlier findings. The gradual decline in thyroid activity in all heat stressed genotypes with increasing age is consistent with the results reported by Grandhi et al. (1975), May and Marks (1983), Buyse et al. (1991) and Williams and Njoya (1998). The latter authors found higher plasma T_3 concentrations at 14 weeks than at adult stage maintained at the same environment. This confirms the previous findings (Heninger et al., 1960; Huston and Carmon, 1962) that the thyroid gland of birds decreases in size and activity when the birds become acclimated to high environmental temperature.

In a recent study, Tona et al. (2004) reported decreased levels of T_3 with increasing age of broilers carrying the sex -linked dwarfing “dw” gene, which is in good agreement with the present findings. They reported that at each age these levels were similar between lines, which are consistent to the current work observed in heat stressed genotypes. The increase in T_3 concentration during the peak egg production is consistent with the results of Lien and Siopes (1993), who noted a maximum T_3 levels in turkeys during the early onset of lay, which then gradually declined with age. This phenomenon might be related to the adaptation of birds to changes in metabolic demands caused by physiological stress and increased metabolic activity for more egg production. The difference between experimental and control groups in T_3 concentration became narrower as the hens advance in age, suggesting development of tolerance to long- term heat exposure over time. Similar trend in T_3 levels has been observed in F_1 crosses between Ethiopian indigenous naked neck and commercial layer hens exposed to long- term high environmental temperatures (Melesse et al., unpublished data) . The basal heat production varies with the size of the animal. In general, as the size of animal increases, basal heat production per unit of body weight decreases (Austic and Nesheim, 1990).

The lowest T_3 concentration observed in dwarf WL chicken genotype may suggest improved adaptability to long-term heat exposure due to their small body size, which virtually contributes to the low basal heat production compared to other genotypes. It has been documented that genotypes with small body size demonstrated better heat-tolerance to stressful environments as reported earlier (Bowen and Washburn, 1985; Tixier-Boichard et al., 1990; Zeman et al., 1996). This may further suggest that the thyroid gland of chickens with small body size produces little T_3, which confirms the pronounced hypothyroidic state in the Leghorn dwarf line observed previously by Brown et al. (1972), Tixier-Boichard et al. (1990) and Zeman et al. (1996). On the other hand, the elevated level of T_3 observed in other normal sized chicken lines in the current study may be attributable to a decreased ability to lose heat (MacLeod and Hocking, 1993) or an inappropriately increased heat production during exposure to high thermal loads (Sandercock et al., 1995). It has been

established that tolerance of short- or long-term elevated thermal loads is greater in more traditional and less selected chicken breeds than commercial intensively selected broiler or layers lines (Berrong and Washburn, 1998).

Conclusions

i) Both WL-dw and LW genotypes proved to be the best heat tolerant chickens while the WL-EF the least.
ii) Keeping layer chickens at higher environmental temperature increased the plasma activities of some enzymes while it reduced the T_3 levels considerably.
iii) The T_3 was found to be a suitable indicator to long-term heat stress in the investigated layer chickens. Thus, T_3 level in blood plasma could be used as a criterion to select thermo-tolerant commercial chicken breeds in hot climates.

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