Effect of Chronic Heat Stress on Performance and Oxidative Damage in Different Strains of Chickens

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Heat stress (HS) causes poor growth, immunosupression and high mortality, through physiological dysfunction. We have reported that ‘acute’ HS increases mitochondrial reactive oxygen species (ROS) and oxidative damage in the skeletal muscle of broiler chickens (Cobb), but that this is not for the case for males of the laying-type white leghorn (WLH) strain of chickens (Mujahid et al., 2005b). We have now studied the effects of ‘chronic’ HS on performance and oxidative damage to skeletal muscle in different strains of chickens. Meat-type male chickens (Ross and Cobb) and male laying-type chickens (WLH) of 14 d of age were arranged according to a factorial design (strains x conditions): control (24°C), cyclic (32–24–32°C: 32°C for 8 h/d, 32–24–32 HS), constant (32 and 34°C, 32 HS and 34 HS, respectively) for 14 d. Feed consumption and body weight gain were measured. Thiobarbituric acid reactive substances (TBARS) content in skeletal muscle was measured colorimetrically with BHT (butylated hydroxytoluene) and expressed as malondialdehyde (MDA) equivalent. Body weight gain and feed consumption for the strains (Ross, Cobb and WLH) were decreased by HS and 32 HS, but not by 32 HS. The decrease in weight gain of WLH chickens for the HS and 32 HS conditions was smaller than for broiler chickens (Ross and Cobb). The 32 HS and 34 HS groups showed slightly enhanced MDA levels (P<0.05) in the skeletal muscle of all 3 strains, but not for the 32 24–32 HS group. The results suggest that depression in growth performance due to chronic heat stress is more evident in meat-type chickens (Ross and Cobb) than male laying-type chickens (WLH), and that reduction in the performance are associated with enhancement of oxidative damage to skeletal muscle.

Key words: chicken strains, chronic heat stress, oxidative damage, performance


Introduction

High ambient temperature, whether ‘acute’ or ‘chronic’ in nature, significantly hinders growth of animals. Reduced growth has been considered a problem of reduced feed intake (Hurwitz et al., 1980; Howlider and Rose, 1987). Feed intake is inversely related to environmental temperature in chickens (McDonald et al., 1981; Suk and Washburn, 1995). Furthermore, reduced efficiency is a common phenomenon in hot conditions. Howlider and Rose (1987) found that reduced growth does not exactly parallel the reduction in feed intake, and suggested, therefore, that factors other than feed intake and digestibility are involved.

‘Acute’ heat stress in broiler chickens increases mitochondrial ROS production via increased ‘substrate oxidation’, which is linked to mitochondrial electron transport chain, and downregulation of the avian form of mitochondrial uncoupling protein (avUCP), resulting in higher oxidative damage to mitochondrial proteins and lipids (Mujahid et al., 2007a). The increase in mitochondrial ROS production in acute heat-stressed birds may lead to decreased growth (Mujahid et al., 2009). This speculation was supported by a recent study that cyclic high temperature (28–34°C) slightly increased mitochondrial ROS generation and MDA content in breast muscle and suppressed growth performance because both increments were not found for the case in pair-fed groups kept at 24°C of broiler chickens (Feng et al., 2008). Heat-induced ROS formation could therefore be responsible for growth retardation in chickens.

Reports about the effects of ‘chronic’ heat stress on broilers have mainly focused on physiological (Donkoh, 1989), biochemical indices (Geraert et al., 1996), and pathological damage to tissue such as heart, liver and kidney (Aengwanich and Simaraks, 2004). There were
few reports on the comparative effects of chronic heat stress on oxidative stress in different strains of chickens, although Mujahid et al. (2005) showed that ‘acute’ heat stress increased mitochondrial ROS production and oxidative damage in the skeletal muscle of broiler chickens (Cobb), but that this was not the case for males of the laying-type white leghorn (WLH) strain of chickens at their same age.

Comparative studies on growth and malondialdehyde (MDA) content were therefore performed on different strains of chickens (meat- and laying-type) under ‘chronic’ heat stress conditions. Feed consumption and body weight gain for the clarification of performance parameters were measured, and MDA content in skeletal muscle as marker for lipid peroxidation was also measured.

Materials and Methods

Animals and Experimental Design

Meat-type male chickens (Gallus gallus, Ross and Cobb strain) and male laying-type chickens (Gallus gallus, White Leghorn, WLH strain) obtained from a commercial hatchery (Economic Federation of Agricultural Cooperatives hatchery, Iwate, Japan) at 1 d of age were housed in electrically-heated batteries under continuous light for 11 days. They were provided with access to water ad libitum and commercial starter meat-type chick diet (crude protein, 23%; metabolizable energy content, 3150 kcal/kg). The CP and ME values are slightly high for test.

Growth Performance

Table 1 shows body weight gain of control and heat-stressed birds of each strain with similar BW (Ross, 471 ±7 g; Cobb, 419 ±5 g; WLH, 159 ±1 g) were transferred to 4 temperature-controlled chambers, where they were allocated equally to 4 treatments in 3 genotypes with 6 chickens in each: control (24°C), cyclic (32–24–32°C: 32°C for 8 h/d, 32–24–32 HS), and constant (32 and 34°C, 32 HS and 34 HS) treatments. Cycling temperature was 32°C for 8 h (from 9.00 a.m to 17.00 p.m) and 24°C for 12 h, whereas 2 h for heating up was taken over for shifting from 24°C to 32°C (2°C/40 min), and same manner was applied for 2 h-cooling down. For 32 and 34°C treatments on the initial day the chamber temperatures were increased gradually by 1°C/60 min from 24°C to 32 and 34°C, respectively. The relative humidity was kept as close to 55%. Birds were provided with ad libitum access to water and diet during 2 weeks of treatments, killed by decapitation, and portions of the pectoralis superficialis muscle rapidly excised. Tissues were immediately frozen in liquid nitrogen and powdered. The samples were stored at −80°C until required for further analysis. Weights of individual birds at the start and end of the experiments were measured. Feed consumption during the heat treatments was recorded. Body weight gain for each chick was calculated. The Animal Care and Use Committee of the Graduate School of Agricultural Science, Tohoku University approved all procedures, and efforts were made to minimize pain or discomfort of the animals.

Determination of Skeletal Muscle MDA

Pectoralis superficialis muscle was used for MDA measurements. Tissues were homogenized in buffer (100 mM KCl, 50 mM Tris-HCl, and 2 mM EGTA, pH 7.4), briefly centrifuged at 700 g, and the supernatants collected. Lipid peroxidation was assayed colorimetrically as a 2-thiobarbituric acid reactive substance (TBARS; see Mujahid et al., 2007b). In brief, 400 µL tissue homogenate were mixed with 100 μL 8.1% SDS, 0.75 mL of 20% acetic acid (pH 3.5), 25 μL 0.8% butyrylated hydroxyl toluene (BHT), and 0.75 mL 0.8% 2-thiobarbituric acid (TBA). After vortexing, samples were incubated on ice for 60 min and heated at 95°C for 60 min in a water-bath. After cooling, 2.5 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added and the samples were mixed by vortexing. After centrifugation at 1000 g for 10 min, the organic layer was extracted and read spectrophotometrically at 532 nm. The TBARS content was expressed as nmol of malondialdehyde (MDA) per equivalent g wet tissue. The samples were analyzed within 1 week of storage at −80°C.

Statistical Analysis

Data were analyzed using the statistical analysis system (SAS, 1985). Data were first analyzed by a general linear model analysis of variance procedure. The means were compared using Duncan’s least significance multiple-range test. All data are expressed as mean ± standard error (SE, n = 6). Differences were considered significant for values of P<0.05.

Results

Growth Performance

Figure 1A shows body weight gain of control and heat-stressed Ross, Cobb and WLH chickens. The average body weight gains of Ross, Cobb and WLH control birds were 1189 ± 25 g, 1122 ± 24 g, and 222 ± 5 g, respectively. In heat-stressed birds, body weight gain in the Ross strain was decreased to 18, 44 and 66% by cyclic (32–24–32 HS), 32 HS and 34 HS treatments, respectively. Body weight gain in Cobb strain was not changed by cyclic heat treatment, but was reduced to 28 and 56% in constant 32 HS and 34 HS groups, respectively. WLH chickens showed only 12 and 14% of reduction in body weight gain in the 32 HS and 34 HS conditions, respectively. Thus, reduction in body weight gain was more evident in meat-type chickens (Ross and Cobb) than in male laying-type chickens (WLH).

Figure 1B shows feed consumption of control and heat-stressed Ross, Cobb and WLH chickens. In the controls, the average feed consumption of Ross, Cobb and WLH chickens were 1668 ± 11 g, 1626 ± 32 g, and 614 ± 23 g, respectively. In heat-stressed birds, feed consumption in Ross strain was reduced to 17, 33 and 50% in cyclic (32–24–32 HS), 32 HS and 34 HS treatment, respectively. Feed consumption in Cobb strain was not changed by cyclic heat treatment, but was reduced to 27 and 43% in
Fig. 1  Body weight gain (A), feed consumption (B) and feed efficiency (C) of meat-type chickens (Ross and Cobb) and laying-type male chickens (WLH) exposed to different heat treatments. The temperature protocol was control (24°C), cyclic (32-24-32°C; 32°C for 8 h/d, 32-24-32HS), and constant (32°C and 34°C, 32HS and 34HS, respectively). Control values in body weight gain: 1189 ± 29 g (Ross); 1122 ± 24 g (Cobb); 222 ± 5 g (WLH). Control values in feed consumption: 1668 ± 11 g (Ross); 1626 ± 32 g (Cobb); 614 ± 23 g (WLH). Control values in feed efficiency: 0.71 ± 0.01 (Ross); 0.69 ± 0.01 (Cobb); 0.37 ± 0.02 (WLH). Values represent the mean ± SE for 6 chickens in each treatment. * Bars with different letters are significantly different (p < 0.05).

constant 32 HS and 34 HS treatment, respectively. WLH chickens also showed 32 and 24% reduction in feed consumption in 32 HS and 34 HS conditions, respectively.

Figure 1C shows efficiency of control and heat-exposed Ross, Cobb and WLH chickens. In controls, the average efficiency of Ross, Cobb and WLH chickens were 0.71 ± 0.01, 0.69 ± 0.01, and 0.37 ± 0.02, respectively. In heat-stressed birds, feed efficiency in Ross strain was reduced to 17% and 32% in 32 HS and 34 HS treatments, respectively. Feed efficiency in Cobb strain was not changed by
Malondialdehyde (MDA) levels in pectoralis muscle of meat-type chickens (Ross and Cobb) and laying-type male chickens (WLH) exposed to different heat treatments. The temperature protocol was control (24°C), cyclic (32–24–32°C: 32°C for 8h/d, 32–24–32HS), and constant (32°C and 34°C, 32HS and 34HS, respectively). Control values in MDA content: 60.7±4.0 nmol/g wet tissue (Ross); 61.1±7.0 nmol/g wet tissue (Cobb); 77.2±2.2 nmol/g wet tissue (WLH). Values represent the mean ± SE for 6 chickens in each treatment. **Bars with different letters are significantly different (p < 0.05).**

Oxidative Damage to Skeletal Muscle

Malondialdehyde (MDA) is the end-product of lipid peroxidation and can serve as index of oxidative damage. Figure 2 shows skeletal muscle MDA levels of control and heat-exposed Ross, Cobb and WLH chickens. In controls, the MDA levels of Ross, Cobb and WLH chickens were 60.7±4.0 nmol/g, 61.08±7.04 nmol/g, and 77.24±2.24 nmol/g wet tissue, respectively. In heat-stressed birds, MDA levels in Ross strain were increased to 25 and 46% with 32 HS and 34 HS treatments, respectively. There were no differences in MDA levels between control and cyclic HS, in the Cobb strain, but were increased to 31 and 45% under constant 32 HS and 34 HS treatment. WLH chickens also showed 19%, 22% of increment in MDA levels by 32 HS and 34 HS treatments, respectively. Importantly, the increase in MDA content of WLH chickens for the 32 HS and 34 HS conditions was less than in meat-type chickens (Ross and Cobb).

Discussion

The consequences of chronic heat exposure generally involve a reduction in feed intake, growth rate, efficiency, and meat yield and quality. These negative effects were found to be greater in broilers with a higher genetic potential for growth rate than in broilers with lower growth rates (Cahaner and Leenstra, 1992). The present results confirm some parts of their findings: feed consumption for the 3 strains were decreased by 32 HS and 34 HS (Ross, 33% and 50%; Cobb, 27% and 43%; WLH, 32% and 24%, respectively). Furthermore, constant heat exposure decreased body weight gain to a larger extent in the meat-type chickens. The reduction in growth for the meat-type chickens exposed to chronic heat stress was similar to results obtained by Lu et al. (2007). The growth in WLH chicken was not very much affected by constant heat exposure (32 HS and 34 HS; 12% and 14%, respectively). If anything, WLH chicks showed higher feed efficiency which might be due to only reduced feed consumption; they have higher resistance to high ambient temperature. This may be due to the fact that heat production to keep body temperature must be relatively more for WLH than broiler chickens. On the other hand, meat-type chicks exposed to 34 HS showed severe growth depression compared to 32 HS conditions, implying existence of the critical level of high ambient temperature. Negative effects of ‘cyclic’ high temperature (32–24–32 HS) on performance parameters were seen in Ross strain (the meat-type chickens, 18% of weight loss and 17% of less feed consumption), but were not evident for either Cobb (meat-type chickens) or male WLH (laying-type) chickens. The former findings support the results of Tabiri et al. (2002) who reported that cycling high temperature (33–27–33°C, 14 days) slightly reduced body weight gain of Ross strain. However, Kutlu and Forbes (1993) found that cyclic high temperatures (36–24–36°C, 36°C for 6 h/d, 14 days) considerably depressed weight gain, the degree of the depression being 91% due to reduced feed intake. The reason for the discrepancy between our results and theirs is not yet understood as regards the response of body weight gain to heat stress despite using a similar cyclic heat pro-
Lipid peroxidation level basically gives a good indication of cellular oxidative damage, even though 8-oxodG in mtDNA, protein carbonyls (and other more specific markers as MDA-lys or carboxymethyl lysine) and lipid peroxidation products (4-hydroxy-2-nonenal) could be used as marker of oxidative damage. Acute heat-stressed birds showed higher MDA content in skeletal muscle (Mujahid et al., 2009; Wang et al., 2009) than chronic stressed birds (Feng et al., 2008). Moreover, Altan et al. (2003) reported that, upon exposure to acute heat stress (38°C for 3h), 5-week-old Ross broilers had a higher plasma MDA content than Cobb broilers. In this experiment for chronic heat stress, the 32 HS and 34 HS groups, the Ross and Cobb broilers had relatively similar MDA content values. This difference may be due to the conditions between ‘acute’ and ‘chronic’ treatment as well as age of the birds. In our study, we applied constant heat stress treatment for 2 week to 2-week-old birds. Importantly, WLH chickens showed relatively lower changes in MDA content in skeletal muscle with heat exposure than meat-type chickens. These results are in agreement with Mujahid et al. (2005) who found that acute heat-stressed WLH chickens showed no increase in oxidative damage to skeletal muscle compared to meat-type chickens (Cobb). Low efficient broilers had higher oxidative damage to skeletal muscle than high efficient broilers (Iqbal et al., 2004), implying that oxidative damage may be responsible for performance. This supports our observation: the higher the oxidative damage, the lower the feed efficiency.

In conclusion, our results suggest that the depression of growth due to ‘chronic’ heat stress is more evident in meat-type (Ross and Cobb) than male laying-type chickens (WLH), and that reduction in performance was associated with a relative enhancement of oxidative damage to skeletal muscle. Among the heat-stress conditions and strains of chickens examined, 34°C and Ross strain were best for studying effects of chronic heat stress.

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References