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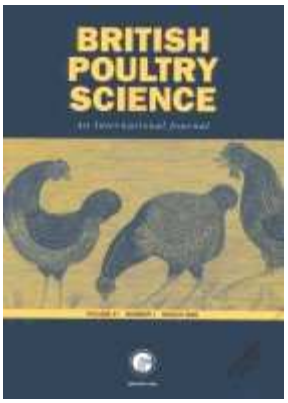

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Thermoregulatory capacity and muscle membrane integrity are compromised in broilers compared with layers at the same age or body weight

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Abstract 1. The effects of acute heat stress (2 h at 32°C and 75% RH) on body temperature and indices of respiratory thermoregulation and skeletal muscle function were examined in two divergently selected male grandparent lines of broiler and layer-type chickens at two ages (35 and 63 d), or at a similar body weight (~2.2 kg).

2. The two chicken lines exhibited markedly different base-line blood acid-base and skeletal muscle characteristics. At the same age or liveweight, birds from the broiler line had significantly higher venous blood carbon dioxide tensions associated with lower blood pH. Plasma creatine kinase (CK) activities reflecting muscle membrane damage were also greatly elevated in the broiler line.

3. Exposure to acute heat stress caused an increase in deep body temperature, panting-induced acid-base disturbances and elevated plasma CK activity in both lines of chicken, an effect that increased with age. The extent of disturbances in acid/base regulation and heat stress-induced myopathy were more pronounced in the broiler than the layer line at the same age or similar liveweights.

4. It is suggested that genetic selection for high muscle growth in broiler lines has compromised their capacity to respond to an acute thermal challenge, leading to detrimental consequences for muscle function. This reduction in heat tolerance may have important implications for bird welfare and subsequent meat quality.

INTRODUCTION

Programmes of genetic selection in poultry for economically important production traits such as high growth rate, food conversion efficiency and muscle yield have been extremely successful. Intensive commercial selection in broiler chickens aimed at improving weight gain and meat yields have resulted in birds that grow up to 4 times faster than layer strains and exhibit 8-fold

increases in breast muscle growth rate (Griffin and Goddard, 1994). Whilst genetic selection in broiler chickens has made major advances in the development of production traits, it is increasingly recognised that these may also be associated with a number of undesirable patho-physiological metabolic derangements (Scheele, 1997). These include respiratory and cardio-pulmonary disorders (Julian, 1993, 1996) and skeletal disease (Thorp, 1994). Selection for high meat yields coupled with rapid growth rates in chickens have also led to an increase in the incidence of pathological features observed in skeletal muscle. Degenerative changes include focal fibre necrosis, hypercontraction, mononuclear cell infiltration, alterations in cel1 membrane integrity and the loss of myo-cellular constituents (Siller, 1985; Mitchell and Sandercock, 1996; Soike and Bergmann, 1998; Mahon 1999; Cooke *et al*., 2003). Sandercock and Mitchell (2003, 2004) published evidence that such growth-associated or stress-induced myopathies may result from disruption of intracellular calcium homeostasis in muscles of rapidly growing broiler birds exhibiting high metabolic rates (Mitchell, 1999). It has been proposed that the increased incidence of such metabolic disorders in broiler chickens is due to an imbalance between the production and supply of energy and metabolites for maintenance requirements (Savory, 1995; Scheele, 1997). The resulting homeostatic dysregulation then leads to cellular, tissue and organ dysfunction.

During the course of production, poultry may be exposed to stressful environmental challenges. Chronic thermal stress (high or low environmental temperatures) has been shown to have a detrimental effect upon production efficiency and mortality (Washburn, 1985). Episodes of acute heat or cold stress in broilers may be experienced under commercial production and transport conditions (Webster *et al*., 1993; Mitchell *et al*., 1992, 1997; Mitchell and Kettlewell 1998). In the case of heat stress, elevated deep body temperature is accompanied by panting in order to increase respiratory evaporative heat loss (Hillman *et al.,* 1985). Panting induces a

hypocapnic alkalosis due the increased elimination of carbon dioxide resulting from elevated respiratory minute volume (Wideman *et al*., 2003; Borges *et al*., 2004). Acute hyperthermia and respiratory alkalosis are in turn associated with a heat-stress-induced myopathy, a pathology that may underlie subsequent problems with meat quality (Sandercock *et al*., 2001). Such skeletal muscle damage "in transit" associated with hyperthermia, and reflected in measurement of plasma creatine kinase (CK) activities, has been described in broiler chickens exposed to acute heat stress under both actual and simulated commercial transport conditions (Mitchell *et al.,* 1992; Mitchell and Sandercock, 1995 a, b; Sandercock *et al*., 2001).

Tolerance of short or long term elevated thermal loads is greater in more traditional and less selected chicken breeds than commercial intensively selected broiler lines (Arad and Marder, 1982 a, b, c; Berrong and Washburn, 1998; Zulkifli *et al.*, 1999). A reduced resistance to heat stress in broiler birds 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). Comparative studies that characterise the responses to thermal challenge in broiler chickens and in lines not selected for enhanced growth and feed conversion efficiency at the same age may be confounded by disparities in body size and geometry (Monteith, 1973). To overcome some of the difficulties this presents in interpretation, comparisons can be made at “matched” liveweights. While it is acknowledged that this approach can also present problems in interpretation due to age-dependent developmental differences, it does provide a more acceptable indication of the severity of a thermal challenge between different lines (Zulkifli *et al.,* 1999).

Elevated plasma CK activity is recognised as a reliable diagnostic marker of muscle damage (Hamburg *et al.,* 1991; Mitchell and Sandercock 1995 a, b). It is indicative of disruptions in muscle cell membrane (sarcolemma) function and permeability (Mitchell 1999). Previous

studies have demonstrated a strong relationship between the extent of histologically demonstrable muscle fibre pathology and plasma CK activity (Soike and Bergmann 1998; Cooke *et al.* 2003). Thus, baseline absolute CK activity reflects the extent of any idiopathic myopathy present whereas changes following an environmental challenge indicate the degree of stress induced muscle damage.

The objectives of the present study were to characterise the effects of acute heat stress (HS) on physiological indices of thermoregulatory capacity and muscle membrane integrity in divergently selected pure male lines of chickens (meat and layer type) at the same age and very different body weights or at two different ages but the same body weight. The birds were subjected to a thermal load whose duration and magnitude was representative of that known to induce symptoms of heat stress in chickens during commercial transportation (Mitchell and Kettlewell, 1998). Blood pCO_2 and pH were measured to assess the extent of respiratory thermoregulatory effort and plasma CK activity was used as a tissue specific indicator of skeletal muscle damage.

MATERIALS AND METHODS

Animals and husbandry

The birds used in the study were pedigreed male chicks from a male great grandparent broiler (B), and White Leghorn layer (L) line obtained from two commercial breeding companies. Ninety-six birds of both lines were reared together from 1-d-old in 12 pens (3.6 m^2) on wood shaving litter (8 birds per line per pen). Room temperatures of 18-20°C were maintained by controlled ventilation and heating. Birds were provided with *ad libitum* access to a commercial broiler diet and water and were subjected to a 14h light: 10h dark photoperiod for the duration of the experiment. All birds were beak trimmed at 14 d of age and light intensities reduced to < 5

lux to minimise possible outbreaks of bird-to-bird pecking (Kjaer and Vestergaard, 1999). A second batch of 1-d-old broiler chicks (n = 48) was obtained 10 weeks after the arrival of the initial consignment of White Leghorn birds to allow for the comparison of the two lines at the same liveweight. These birds were reared as previously described and comparisons of the birds' responses to acute HS at approximately the same liveweight were carried out in the B- and L-line respectively at 35 and 105 d of age. All experiments were performed with authorisation of the UK Home Office and with the approval of the Roslin Institute Ethical Committee.

Heat stress protocol

At 35 and 63 d of age (same age) and at 35 (B) and 105 (L) d (same liveweight), 24 birds from both lines were transferred into 4 or 6 commercial transport containers (0.7 × 0.5 × 0.3 m). Half the containers were placed in a controlled climate chamber at 32°C/75% relative humidity (RH) for a period of 2 h. The remaining containers were placed in an adjacent identical climate chamber at 21°C/50% RH (thermoneutral) for the same duration. The experiment was repeated with 12 L at 105 d of age when they were of comparable body weight to the second batch of 12 B at 35d. Feed was withdrawn from the birds 4 prior to the heat stress experiments

Rectal temperatures and blood samples were taken from all birds immediately before (T_0) and after (T_1) the heat stress exposure period using a thermistor probe (Model 612-849; RS Components Ltd., Northants, UK) inserted 5 cm into the bird's rectum. Blood samples were obtained at the same time as rectal temperature by superficial venepuncture (brachial vein) and transferred into 5-ml blood collection tubes containing Li-heparin anti-coagulant (50 IU ml^{-1}) and immediately chilled on ice. Blood pCO_2 and pH were measured within 2 minutes of sample collection using an automated blood gas analyser (Model 238 pH/Blood Gas Analyser, CIBA-Corning, Halstead, UK) with body temperature compensation. Plasma samples for CK

determination were obtained by centrifugation of whole blood at 1500 *g* for 5 minutes and stored at –20°C pending enzyme analysis. The activity of CK was assessed using a commercial kit (Biotrol CK Monoreactif, Alpha Laboratories, Hants, UK) modified for use with a multi-well plate spectrophotometer (MR 5000, Dynatech Laboratories, West Sussex UK) as previously described (Mitchell *et al.,* 1999).

Statistical analyses

The first experiment (comparing two lines at two ages) was a 2^4 factorial design (Line × Treatment × Time × Age). Blocking factors were pen within crate and bird as a split-plot effect. The second experiment (comparing two lines at the same body weight) was a 2^3 factorial design (Line × Treatment × Time) with the same blocking factors. Standard analysis of variance methods were used to assess the treatment effects using Genstat (http://www.vsn-intl.com/genstat/). For analysis at the same age, p_vCO_2 and plasma CK were transformed by taking natural logarithms to obtain residual errors that were normally distributed.

RESULTS

The thermal conditions experienced by birds of the two lines were similar at the two ages for both control (C) and acute heat stress (HS) treatments. Mean ambient temperatures (°C) and RH respectively were 22.5 °C/29.1 % RH at 35 d; 21.3 °C/30.5 % RH at 63 d for C and 32.5 °C/67.1 % RH at 35 d; 32.1 °C/75.5 % RH at 63 d for HS. For birds compared at the same liveweight, mean values for C and HS treatments respectively were 21.9°C/29% RH and 32.3°C/71.3% RH.

Comparisons at the same age

Differences in liveweight were observed between the two lines at the two ages (P<0.001). Mean body weights of B at 35 and 63 d, respectively, were 2.27 and 5.58 kg compared with 0.41 and 0.98 kg in L (sed 0.027). Both lines exhibited significant (P<0.001) increases in liveweight with

age of 2.5 and a 2.4-fold respectively from 35 to 63 d of age in the B- and L-line. There were no differences in liveweight between the control and HS groups in any of the treatment comparisons.

Mean trait values before and after HS in control and treated birds of both lines at the two ages are presented in Table 1.

Table 1 near here

Rectal temperature

The change in rectal temperature in response to HS was significantly greater in the B-line compared to the L-line leading to a Line × Treatment × Time interaction (P<0.001). In the B-line, mean HS-induced changes in body temperature were +1.7 °C greater than in controls at the same age, at both ages compared with 0.5 and 0.2 °C respectively at 35 and 63 d in the L-line (Table 1). Average rectal temperature was greater at 63 than 35 d (P<0.001).

Venous blood carbon dioxide (p_vCO_2)

The 4-way interaction of Line × Age × Treatment × Time was highly significant (P<0.001) for pvCO2. Basal p_vCO_2 was greater (P<0.001) in the B-line compared to the L-line at 35and 63 d. Changes in p_vCO_2 in response to HS challenge in the B-line at both ages, and in L-line at 63 d were significantly (P<0.001) lower than in the controls (Table 1).

Venous blood pH (pH_v)

The 4-way interaction of Line × Age × Treatment × Time was significant (P<0.01) for pH_v. Baseline pHv was similar in B- and L-lines at 63 and 35 d of age. The increase in pH_v following exposure to HS was greater (P<0.001) in the B- compared with L-line birds at both ages and in the L-line birds at 63 (P<0.001) compared with 35 d (not significant).

Plasma creatine kinase (CK) activity

Plasma CK activities were significantly higher in B- compared with L-line before and after HS at both ages (Line × Age × Time interaction $P<0.01$). Exposure to HS at 35 and 63 d respectively was associated with increases of 28 and 25% in CK in B-line and 12% and 24% in L-line birds.

Comparisons at the same liveweight

Body weights of B were slightly greater than L (2.17 *vs.* 1.93, sed 0.036 kg; $P<0.001$) at the time of the experiment when they were respectively 35 and 105 d old. Responses to HS in the two lines are presented in Table 2.

Table 2 near here

Rectal temperature

Acute heat stress increased rectal temperatures in both lines ($P<0.001$). The extent of the change was significantly greater in B-line than in the L-line birds, reflecting a mean increase in rectal temperature compared with controls of +1.55 ºC in the B-line and +1.16 ºC in the L-line (Treatment × Line × Time interaction $P<0.05$).

Venous blood carbon dioxide (p_vCO_2)

The Treatment × Line × Time interaction for p_vCO_2 was significant ($P<0.001$). Baseline p_vCO_2 measurements were greater in B-line and increased in the control whereas p_vCO_2 declined in L-line control and in both lines subjected to HS. Exposure to acute heat stress reduced blood p_vCO_2 in both lines and the extent of the reduction was much greater in the B-line compared to the L-line.

Venous blood pH (pH_v)

Heat stress induced a greater increase in pH_v in the broilers than in the layers (Treatment × Line interaction $P<0.05$) and the change was greater under HS than control conditions (Treatment x Time $P<0.001$).

Plasma creatine kinase (CK) activity

Plasma CK activities were significantly (P<0.001) different between the two lines at the same live weight with the B-line birds exhibiting an average 1.8-fold greater plasma CK activity than the L-line. Hyperthermia-associated CK activity was 19 and 18 % higher respectively in the B-line and L-line compared with the controls (Treatment × Time interaction P<0.001)

DISCUSSION

Exposure to acute heat stress of the magnitude and duration used in this study produced a significant hyperthermia in both lines at both ages. The extent of the hyperthermia was much greater in the more rapidly growing B-line than the L-line, irrespective of age or liveweight. The disparities in extent of the increase in deep body temperature at the same age are most likely to be attributable to differences in body size and geometry and their effects upon heat exchange (Monteith, 1973; Hillman *et al*., 1985). These findings are consistent with a previous report in which slower growing "dwarf line" broilers exhibited greater heat stress resistance than more rapidly growing lines, a characteristic that was attributed to differences in body size (Deeb and Cahaner 2001). In the present study in birds of the same liveweight, exposure to HS induced a greater degree of hyperthermia in the B-line compared to the L-line. The differences in the degree of induced hyperthermia may reflect variations in thermotolerance in the two lines, possibly attributable to differing efficiencies of heat loss mechanisms (Sandercock *et al.,* 1995). Deep body temperature represents the balance between heat production and heat loss (Monteith, 1973; Hillman *et al*., 1985) and changes when one exceeds the other, such as during acute heat stress (Lin *et al*., 2004). Relative differences in hyperthermia or "heat storage" may result from a reduced capacity for heat loss, an increase in heat production, or a combination of both. Temperature regulation by panting carries a high metabolic cost, which has been shown to

increase heat production and may have an influence upon thermoregulatory efficiency in birds (Whittow, 1976). It has previously been demonstrated that modern broiler birds have a relatively high body-weight corrected heat production associated with hyperthermia and panting compared to their genetic predecessors (Sandercock *et al*., 1995).

It has long been established that profound respiratory alkalosis is induced in broiler chickens by heat stress (Hillman *et al*., 1985; Teeter *et al*., 1985). Examination of the effects of HS on these indices revealed differences in the extent of panting-induced hypocapnic alkalosis that were negatively correlated with differential rectal temperatures in the two lines at the same age. These findings may be explained, at least in part, by the influence of body size and geometry upon heat transfer and therefore core body temperature disturbances and the resultant thermoregulatory demands. In the body-weight matched birds there was a marked difference in the extent of the HS-induced acid-base and blood gas disturbances. These results indicate that the thermoregulatory respiratory effort was much greater in the broiler line, resulting in elevations in total respiratory ventilation rates. From the measurements made in this study it is not possible to determine precisely the contributions of higher respiratory frequencies and greater relative tidal volumes in the B-line. The key finding is that the broiler line experiences a greater degree of hyperthermia despite an apparently increased thermoregulatory effort in terms of panting when compared to the layer line even at the same body weight. We hypothesise that the heat loss mechanism is less efficient in the broiler birds than in the layers, or, is associated with a higher heat production that leads to increased heat storage, acute hyperthermia and more marked heat stress.

Interestingly, the two lines used in this study also exhibited markedly different baseline blood p_vCO_2. The different magnitude of the decline in p_vCO_2 between the lines may be attributable to differences in body weight specific metabolic rate. This would be consistent with

the findings of Korte *et al.,* (1999), who reported that selection for rapid growth and a high metabolic rate in broilers results in a high basal p_vCO_2. Alternatively broilers may have an inadequate control of CO_2 elimination and regulation of blood gas and acid-base status. Olkowski *et al.,* (1999) reported that p_vCO_2 was higher in broiler birds than age matched (35 d) White Leghorn chickens, that p_vCO_2 increased with age in the broilers and was accompanied by a tendency towards increasing acidosis. Scheele *et al.,* (2005) have recently described elevated p_vCO_2 and lower pH_v in native breeds compared to modern selected lines and found a positive correlation between a predisposition to hypercapnic acidosis and the incidence of ascites syndrome precursors in young birds. Examination of baseline pH_v values in the different lines in the current study revealed similar mean values for pH_v (7.38±0.03) in the L-line at 35, 63 and 105 d and 35 d in B-line, despite line disparities in baseline p_vCO_2. The generally higher blood pCO_2 and a trend towards lower pH in rapidly growing broilers may predispose the birds to a number of other pathologies including the ascites syndrome (Olkowski *et al.,* 1999; Scheele *et al*., 2003; Wideman *et al*., 2003).

Differences in plasma CK activities resulting from efflux of the enzyme from muscle in birds of different sizes or lines are unlikely to be attributable to different volumes of distribution as similar mean body-weight specific plasma volumes have been reported in both broiler and layer-type chickens (at ages used in this study), constituting approximately 5-6% liveweight (Sturkie, 1976; Yahav *et al.,* 1997).

At matched ages and body weights the B-line exhibited much more extensive idiopathic myopathy than the L-line, the baseline CK activities averaging 2.4 and 3.4 fold higher in the broilers respectively at 35 and 63 d of age and 1.8 fold greater at similar weights. It has been proposed that this pathology is a direct consequence of selection for high growth rate from comparisons of fast and slow growing lines of broilers and turkeys (Mitchell and Sandercock

1994, 1995b; Hocking *et al*., 1998; Sandercock *et al*., 2001). Soike and Bergmann (1998) have suggested also that selection for high growth rate and muscle yield have adverse effects upon structural, metabolic and functional parameters in poultry skeletal muscle. In the current age-matched comparisons the proportional increase in plasma CK activity following HS was higher in the B-line than in the L-line at 35 d and was similar at 63 d of age. The post-stress absolute activities, however, at 35 and 63 d respectively were 3.6 and 2.7 fold greater compared with pre-stress values in the B- than in the L-line. In the body weight matched groups the heat stress induced proportional increase in plasma CK activity was similar in the two lines whereas the absolute values were about 2-fold greater in the B- compared with the L-line. It is concluded that acute heat stress induces further muscle damage in rapidly growing broiler chickens, which already exhibit a substantial degree of spontaneous or idiopathic myopathy. An increased susceptibility to heat stress-induced myopathy in rapidly growing broiler lines has been proposed previously (Mitchell and Sandercock, 1995b).

In summary, we have shown that there are major differences in thermoregulatory and respiratory responses to heat stress in lines of domestic fowl selected for either greater reproductive or meat traits. The two lines differ in their ability to cope with exposure to an acute thermal challenge. The present study showed that the fast-growing commercial broiler lines were less able to withstand an acute heat stress challenge than a slower growing line at the same liveweight. The broilers developed a more profound hypocapnic alkalosis during thermal stress despite exhibiting higher resting p_vCO_2 and a lower blood pH_v than the L-lines at thermoneutral temperatures. Base-line variation in plasma CK activities suggests that disruption of muscle sarcolemmal integrity is much greater in the broiler line and that their skeletal muscle exhibits an enhanced sensitivity to acute thermal challenge associated with a greater degree of hyperthermia and disturbances in acid-base and blood gas status. It is proposed that genetic selection for

improved growth and muscle yields in broiler chickens has had a detrimental effect upon thermotolerance and deleterious consequences for muscle function. This may have implications both for meat quality and bird welfare by limiting the capacity of broiler chickens to respond to an acute thermal challenge.

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Table 1. *Mean rectal temperature, venous blood pCO_2 and pH ,and plasma creatine kinase activity (CK) in divergently selected male-line broiler (B) and White Leghorn layer (L) lines of chickens under control (C) and acute heat stress (HS) conditions at two different ages (35 and 63 d). T0 is pre-treatment sample and T1 post-treatment sample.*

Trait	Treatment	35 d				63 d				Sed[1]	Sed[2]
		Broiler		Layer		Broiler		Layer			
		T0	T1	T0	T1	T0	T1	T0	T1		
Rectal temperature (°C)	C	41.3	41.9	41.2	41.5	41.3	42.1	41.5	41.9	0.15	0.16
	HS	41.3	43.6	41.3	42.0	41.3	43.8	41.4	42.1		
Plasma p_vCO_2 (*ln* mm Hg)	C	3.79	3.83	3.58	3.49	3.89	3.50	3.52	3.43	0.050	0.052
		44.2[3]	*45.9*	*35.8*	*32.6*	*48.9*	*33.1*	*33.8*	*30.8*		
	HS	3.77	3.23	3.51	3.43	3.77	3.01	3.51	3.00		
		43.5[3]	*25.3*	*33.5*	*30.7*	*43.5*	*20.2*	*33.4*	*20.1*		
Plasma pH_v	C	7.39	7.38	7.38	7.36	7.30	7.45	7.37	7.43	0.018	0.019
	HS	7.32	7.51	7.38	7.39	7.35	7.56	7.39	7.51		
Plasma CK (*ln* IU l^{-1})	C	6.34	6.76	5.47	5.61	6.84	6.98	5.63	5.99	0.056	0.057
		567[3]	*860*	*236*	*274*	*932*	*1073*	*278*	*399*		
	HS	6.65	7.00	5.45	5.73	6.88	7.20	5.64	6.21		
		774[3]	*1099*	*232*	*307*	*972*	*1339*	*280*	*496*		

[1] Standard error of a difference between means across rows (Line, Time and Age).

[2] Standard error of a difference between two means within a column (C vs. HS).

[3] Back transformed values in italics.

Table 2. *Mean rectal temperature, venous blood pCO_2 and pH, and plasma creatine kinase activity (CK) in a divergently selected male broiler and layer lines under control (C) and acute heat stress (HS) conditions at the same approximate live weight (~2.2 kg). T0 is pre-treatment sample and T1 post-treatment sample*

Trait	Treatment	Broiler (35 d)		Layer (105 d)		Sed[1]	Sed[2]
		T0	T1	T0	T1		
Rectal temperature (°C)	C	41.5	41.7	41.5	41.8	0.11	0.10
	HS	41.3	43.2	41.4	42.9		
Plasma p_vCO_2 (mm Hg)	C	40.4	44.8	37.6	33.1	1.47	1.49
	HS	44.1	27.9	37.0	27.9		
Plasma pH_v	C	7.37	7.41	7.39	7.41	0.016	0.017
	HS	7.35	7.51	7.38	7.46		
Plasma CK (IU l^{-1})	C	782	878	428	503	45.2	32.6
	HS	798	1046	437	597		

[1] Standard error of a difference between means across rows (Line vs. Time).

[2] Standard error of a difference between two means within a column (C vs. HS).