Spontaneous and stress induced myopathies in modern meat birds: a cause for quality and welfare concerns

Mitchell, MA; Sandercock, DA

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SPONTANEOUS AND STRESS INDUCED MYOPATHIES IN MODERN MEAT BIRDS: A CAUSE FOR QUALITY AND WELFARE CONCERNS

M.A. MITCHELL and D.A. SANDERCOCK

Summary

Modern, rapidly growing strains of meat poultry exhibit an elevated incidence of spontaneous or idiopathic myopathy and an increased susceptibility to stress induced myopathy. These pathologies are attributable to alterations in intracellular calcium homeostasis and consequent changes in sarcolemmal integrity and may result from excessive myofibre hypertrophy and inadequate development of support tissues and vascular supply. These myopathies may have, in turn, a range of implications for both product quality and bird welfare.

I. INTRODUCTION

Artificial genetic selection for improved body weight gains and food conversion has resulted in rapid muscle growth in commercial broiler chickens and turkeys. Current commercial strains of meat type poultry appear to exhibit an increased incidence of idiopathic and stress induced myopathies compared to their slower growing counterparts or genetic predecessors (Mitchell, 1999). The mechanisms of these pathologies have not been fully elucidated but understanding the patho-physiological basis of this muscle damage is important in relation to both bird productivity and welfare. Thus derangements of ante-mortem muscle cell metabolism and alterations in sarcolemmal integrity and tissue structure associated with the presence of myopathy may have profound implications for meat quality and the incidence of specific conditions such as Pale, Soft Exudative (PSE)-like meat. Also it may be suggested that muscle dysfunction may lead to problems of altered locomotor capability and therefore behavioural changes and reduced welfare. This situation may be further compounded if the observed myopathies are accompanied by muscle discomfort or pain. The present review addresses these issues and examines current knowledge of the causes and mechanisms of myopathy in poultry and the consequences for productivity.

(a) Muscle abnormalities in poultry

Muscle abnormalities including inherited muscular dystrophy, deep pectoral myopathy, dietary deficiency myopathies and toxic myopathies have long been recognised and described in poultry. Reports of an increased incidence of Pale, Soft Exudative meat (PSE) in modern, commercial turkeys and broilers (Barbut, 1997ab) and the recognition of muscle damage associated with thermal stress and catching and transport in broilers (Mitchell, 1999) have focused attention upon the possible link between rapid growth rates, stress induced myopathy and product quality in poultry. The patho-physiological mechanisms of such myopathies and other muscle abnormalities have been the subject of an extensive review (Mitchell, 1999). A common feature of all myopathic and dystrophic conditions is the leakage of the intracellular muscle enzyme creatine kinase (CK) into the circulation. Thus increased plasma activity of CK is a useful diagnostic indicator of muscle pathology and altered sarcolemmal integrity.

Roslin Institute, Roslin, Midlothian, EH25 9PS, United Kingdom

(b) Growth rate and muscle damage

In both broiler chickens and turkeys, plasma CK is elevated as body size increases (Mitchell, 1999) and it is proposed that this is indicative of a growth-associated myopathy. It is hypothesised that this increased occurrence of muscle abnormalities may be attributable to tissue growth ultimately exceeding metabolic, physiological or anatomical limits (Mitchell, 1999), although such mechanisms have yet to be fully characterised. Selection for growth rate has resulted in more and larger muscle fibres in slow, tonic and fast twitch muscles of the broiler chicken without any effect on fibre typing (Rémignon *et al.*, 1996ab). It is apparent that, in order to support the increased growth rate of the "demand organs" such as muscle, bone, fat, skin and feathers, appropriate adaptations must occur in the "supply organs" including the cardiovascular and respiratory systems, intestine and liver. It may be postulated that failure of the supply systems to meet the demands of a disproportionately large growth rate may underlie many of the current pathologies and welfare problems encountered in commercial broiler production. Whilst divergent selection for breast meat yield in broiler chickens was without effect upon histological character and meat quality attributes of breast and leg muscles in the studies of Rémignon *et al.* (1996ab), in another study a comparison of fast growing broiler lines with laying lines with a slower growth rate has revealed "significant changes of structural, metabolic and functional parameters in skeletal muscle" in meat type birds (Soike and Bergmann, 1998). Disseminated muscle fibre degeneration and hypercontraction were particularly prevalent in the breast muscle of these broiler chickens.

In addition it may be suggested that the apparent increased susceptibility to "stress" of the modern broiler fowl may be attributable to the exploitation of the genetic potential for growth in these birds in the absence of compensatory development of the corresponding homeostatic and regulatory mechanisms (Mitchell, 1999). Such a phenomenon may occur during the rapid skeletal muscle growth in the current commercial broiler chicken, resulting in alterations in tissue characteristics.

(c) Genetic origins of idiopathic myopathy

Recent studies have examined the effects of genetic selection for rapid growth rate upon muscle membrane integrity or "leakiness", using plasma CK activity as an index of the extent of pathology. Two lines of broiler chickens were compared. These lines were designated "selected" and "relaxed". The selected line represents a current commercial broiler strain, which has been continuously selected for growth rate and high feed conversion efficiency. The slower growing birds were selected for growth rate until 1978, when selection was discontinued or "relaxed", and thus represent a control line. The findings demonstrated that muscle enzyme (CK) loss increased with age in both lines of broilers but was always much greater, and thus muscle damage more extensive, in the highly selected, rapidly growing birds than in their slower growing counterparts. This was true even when actual body weight was the same in birds from the two lines (at 70 days of age in the relaxed line and 42 days in the selected birds). No differences in muscle enzyme content were found which could explain this disparity. It thus appears that the elevated enzyme efflux and muscle damage in the selected line broilers are associated with increased growth rate and not body size (Mitchell, 1999). Similar findings have been reported in comparisons of slow growing traditional line turkeys and a more rapidly growing commercial male line (Mills *et al.*, 1999). Other studies have also demonstrated that rapidly growing broiler lines are more susceptible to stress-induced myopathy than genetically slower growing ones (Sandercock *et al.*, 2001). Myopathy as evidenced by elevated plasma CK, is associated with demonstrable histo-pathological changes in muscle tissue. The condition is characterised by histological changes

indicative of muscle degeneration including hyaline (hypercontracted) fibres, fatty infiltration, fragmentation of the sarcoplasm, mononucleocyte infiltration and focal necrosis. Indicators of tissue regeneration such as basophilic fibres and internalised nuclei have also been observed (Mahon, 1999). The onset of pathological changes appears to correlate with the attainment of a specific fibre diameter regardless of age or body weight suggesting a limit for fibre hypertrophy beyond which muscle function may be compromised (Mills *et al.*, 2000).

Complementary studies have examined the development of idiopathic myopathy in selected and control line birds beyond normal slaughter age and through to mature body weight. The relative body weights of the selected and relaxed lines of broilers increased over the experimental period up to sexual maturity with the selected line being significantly heavier throughout. Consistent with the earlier studies, plasma activities of CK were found to increase throughout development in both lines but with a much more rapid rate of rise in the selected commercial broilers, plasma CK being four-fold higher at mature body weight in the fast growing birds (Mitchell and Sandercock, 1996). It was concluded that this reflected cumulative muscle damage associated with the continuing high body weight gains up to maturity. Indeed the final body weight of the selected broilers was about 6.0 kg as opposed to less than 3.5 kg in their relaxed counterparts.

Such investigations of the effect of genetic selection on idiopathic myopathy in poultry have been confined to a relatively small number of studies comparing small numbers of genetically divergent lines (e.g. Rémignon, *et al.*, 1996ab; Soike and Bergmann, 1998). The prevalence and the extent of genetic variation for this condition in chicken lines have only been addressed recently. Estimates of genetic variation in skeletal muscle status can be achieved using a multi-breed approach employing a large number of pure-lines but testing only a small number of individuals per line. This approach has been previously used to assess the extent of genetic variation for economically important production traits in poultry (Hocking *et al.*, 1985).

A study was undertaken in the authors' laboratory to correlate the extent of idiopathic muscle damage (as determined by plasma CK activity) with measurements of live weight (LW), total skeletal muscle weight (MW) and the weight of breast, thigh and drumstick muscle in 37 different chicken lines representative of 3 line categories (broiler [B], layer [L] and traditional [T]). CK activity, live weight and total muscle weights were assessed by analysis of variance. The relationship between CK activity and live weight and muscle composition was analysed by multiple regression analysis of natural logarithms (ln) of the variables. Transformations to ln were necessary to normalise residual errors. The maximal model included effects for age, category, live weight and breast, thigh and drumstick muscle.

Combined category means (6 and 10 weeks of age) for live weight, muscle weights and plasma creatine kinase (CK) activity are presented in Table 1. Broiler lines were significantly ($P<0.001$) larger, contained proportionally more breast muscle as a fraction of total muscle weight and exhibited higher plasma CK activities than the L and T lines. Weights and plasma CK activities increased from 6 to 10 weeks ($P<0.001$) whereas the relative muscle proportions did not change with age. Plots of log (ln) transformed live weight versus CK activity at 6 and 10 weeks of age in B, L and T-lines are shown in Figure 1. Plasma CK activity was higher at the same live weight in B-lines compared with L and T lines ($P<0.01$). Elevations in CK activity with increased live weight were also greater in B-lines than L and T lines ($P<0.01$).

Table 1. Category means at 6 and 10 weeks of age for creatine kinase (CK) activity, live weight, total muscle weight and breast, thigh and drumstick muscle as a proportion of total muscle.

Variable	Broiler	Layer	Traditional	Sed
Live weight (g)	3559	852	933	84.6
Total muscle (g)	1333	203	235	39.6
Breast, g/kg total muscle	532	431	426	4.3
Thigh, g/kg total muscle	295	333	334	3.2
Drumstick, g/kg total muscle	173	236	240	3.5
Creatine kinase (IU/l)	1017	237	247	20.6

The production data and corresponding plasma CK activities reported in this study were consistent with those described in previous studies (Mitchell, 1999; Sandercock *et al.*, 2001), with all lines examined exhibiting age dependent increases in plasma CK. At both ages, B-lines were on average four times heavier than the L and T lines and exhibited greater total muscle (six-fold), breast (eight-fold), thigh (six-fold) and drumstick (five-fold) yields. Multiple regression analysis showed the strongest correlation (smallest residual mean squares) for plasma CK activity was obtained by fitting category and the regression of CK on LW within category ($P<0.001$). These results suggest that increases in plasma CK activity were positively associated with increases in muscle mass and were not affected by changes in the relative proportion of breast meat. Comparisons of the differences in slopes and intercepts of the three line categories suggested that the observed differences in plasma CK activity between B and the L and T lines could not be explained by changes in LW alone. In addition, increases in plasma CK activity with LW were markedly higher in the B-lines compared with the L- and T-lines. This suggests that detrimental alterations in muscle function and membrane integrity exist in the B-lines that may be attributable to genetically induced changes in muscle fibre status. Sosnicki and Wilson (1991) have postulated that, in turkeys, selection for rapid growth and meat yield may have resulted in the growth of the muscle fibres outpacing the supporting capillary supply and connective tissue, leading to an increase in myo-degenerative features associated with focal ischaemia.

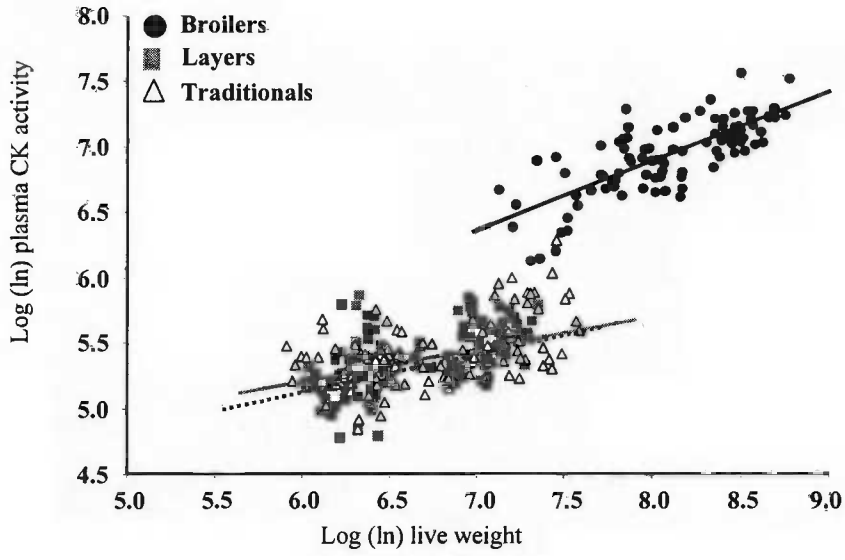

Figure 1. Regression analysis of log (ln) live weight versus log CK activity at 6 and 10 weeks of age in meat, layer and unselected

(d) Mechanisms of myopathy in poultry

Whilst it seems likely that the fundamental mechanisms mediating muscle cell damage and characterised in mammals, will pertain in birds, until recently there has been little work examining these processes in relation to genetic selection programmes and modern poultry production practices and problems. *In vitro* preparations of isolated skeletal muscle from broilers have been employed to elucidate the mechanisms of both stress induced myopathy and monensin myo-toxicity (Mitchell, 1999; Sandercock and Mitchell, 2003ab). These studies have examined both the uptake of radio-isotopic calcium (Ca^{45}) and the efflux of CK. The results demonstrate that elevating intracellular calcium either by increased entry of external calcium (by calcium specific ionophores) or release from sarcoplasmic stores, results in altered membrane integrity and efflux of enzymes particularly CK. Both sodium and calcium overload will induce enzyme efflux and membrane disruption. The mechanism of membrane damage involves activation of phospholipase A_2 (PLA_2) probably as a direct consequence of the raised intracellular calcium as described for mammalian muscle. CK efflux, following raised intracellular calcium, can be reduced by inhibitors of PLA_2 (Mitchell, 1999; Sandercock and Mitchell, 2003a). In response to monensin treatment increased entry of sodium, by sodium-proton exchange (Sandercock and Mitchell, 2003b) into muscle cells promotes calcium entry by the sodium-calcium exchange mechanisms in the sarcolemma in addition to releasing calcium into the myoplasm via the sarcoplasmic reticulum calcium channel or ryanodine receptor (Mitchell and Sandercock, 1997; Mitchell, 1999). Monensin induced CK efflux from isolated muscle can be inhibited by dantrolene, an agent which specifically blocks the ryanodine receptor, confirming the role of this channel in the process. These findings offer a complete explanation for the myotoxic effects of monensin in broilers. Increased entry of extracellular calcium or release of calcium from intracellular stores such as the sarcoplasmic reticulum, initiate the cascade of events which culminates in changes in sarcolemmal or muscle membrane integrity and the associated increase in enzyme efflux. On the basis of additional experiments in which acute heat stress induced increases in plasma CK

in broilers could be inhibited by dantrolene it has been proposed that similar mechanisms involving altered ion balance and calcium release through the sarcoplasmic reticulum, ryanodine sensitive, calcium release channel (SR-RSCRC) may mediate the myopathy induced by stress and thermal challenge (Mitchell, 1999). This mechanism may also underlie the progressive muscle damage seen in rapidly growing birds and the concomitant increased sensitivity to heat stress-induced myopathy.

It thus appears that the disturbances in ion balance contributing to the aetiology of various myopathies in poultry are consistent with the mechanisms proposed in mammals. The suggested mechanism would have parallels with the well-recognised and characterised Porcine Stress Syndrome (PSS) and the incidence of PSE in pigs.

(e) Stress, pathophysiological mechanisms, myopathies and meat quality

It has been proposed that artificial genetic selection for growth rate and the existence of overt muscle pathology result in changes in muscle cell structure and function resulting in alterations in histological characteristics which may have important implications for meat quality (Rémignon *et al.*, 1996ab; Soike and Bergmann, 1998). In addition some of the commercial procedures and environmental challenges known to induce muscle damage or myopathy in poultry have been linked with alterations in meat quality (McKee and Sams, 1997). Susceptibility to pre-slaughter stressors may be genetically determined and lines of quail selected for high and low fear exhibit differences in adrenocortical response (plasma corticosterone), muscle damage (plasma CK activity), drip loss and pH of breast meat following exposure to acute stress in a crush cage (Rémignon, 1998). Genetically determined changes in muscle function may predispose the tissue to stress related damage and further effects upon meat quality. Thus, pre-slaughter heat stress negatively affects meat shrink loss, colour and toughness in broilers. Acute pre-slaughter heat stress accelerates the rate of pH decline in turkey meat and increases the likelihood of PSE and may thus explain the higher incidence of this condition during the summer (McKee and Sams, 1997). Extended transportation of broilers can induce a number of disturbances in physiological variables and in some lines this may be associated with a tendency towards reduced meat quality and a PSE like condition. It has been suggested that PSE or a condition closely approximating that encountered in mammalian livestock is becoming more common in turkeys and broilers (Barbut, 1997ab). Broiler breast and leg meat colour and other meat quality variables may be adversely affected by pre-slaughter holding conditions and transportation (Kannan *et al.*, 1997), although in this latter study overall meat quality was unaffected. The exact physiological mechanistic basis of these problems and the relationship between muscle cell function, myopathic change and meat quality are still, however, poorly understood. Thus, whilst numerous instances of altered meat quality in poultry have been described, any patho-physiological characteristics or responses in live muscle directly responsible for changes in the post-mortem attributes of the tissue have not been identified. For example, in the case of PSE, the high rate post-mortem glycolysis induced by acute ante-mortem stress and leading to a rapid fall in tissue pH is responsible for the meat quality problem (Barbut, 1997ab; McKee and Sams, 1997). However, it has not been previously established if alterations in ante-mortem cell calcium homeostasis, PLA_2 activity, lipid peroxidation and sarcolemmal permeability predispose to this condition as is recognised in pigs (Klont *et al.*, 1994). Recently, however, Soares *et al.* (2003) have reported that mitochondrial PLA_2 activity in chicken muscle increases with age and is correlated with elevated sarcoplasmic calcium content and the incidence of PSE. Current studies in the present authors' laboratory are examining the possible relationships between ante-mortem physiological stress and post-mortem meat quality attributes in broiler chickens. Preliminary findings indicate that marked

pre-slaughter hyperthermia (body temperature increased by 2.2°C) during three hours of simulated transport is associated with disturbances in acid-base balance (hypocapnic alkalosis), elevated plasma creatine kinase activity (+40%) and a significant reduction in pH, a significant increase in drip loss, an elevated haemorrhage score and a paler appearance of the breast fillet (Sandercock *et al.*, 2001). The role of sarcoplasmic reticulum calcium release and other aspects of myoplasmic calcium regulation are to be characterised in parallel investigations in lines of broilers differing in susceptibility to physiological stress and growth associated myopathy.

II. FUTURE DEVELOPMENTS

It may be proposed that selection for improved growth rate and feed efficiency in meat birds has resulted in changes in cellular regulation of free calcium distribution and concentration. The consequences of these derangements include changes in intracellular enzyme activity, hydrolysis of membrane lipids, peroxidation and altered sarcolemmal permeability. These biochemical responses may underlie detrimental effects upon *in vivo* muscle cell function and peri-mortem metabolism and meat quality parameters. It is thus suggested that identification of the genes associated with idiopathic myopathy, calcium homeostasis and the control of muscle cell regeneration and repair constitute important routes for progress in the alleviation and prevention of growth associated and stress induced myopathies in commercial poultry.

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