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**The effect of genetic selection for muscle yield on idiopathic myopathy in poultry:
implications for welfare?**

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SUMMARY

Plasma creatine kinase (CK) activity, an indicator of skeletal muscle damage was correlated with live weight (LW) and muscle yields in 37 different chicken lines representative of 3 categories; broiler (B), layer (L) and traditional (T). Measurements on these lines were made at 6 and 10 weeks of age. Significant increases in plasma CK activity associated with age were observed across all lines and were removed by regression on LW. Differences in CK activity between B lines and the other two categories were not removed by linear regression on LW. The results indicate that alterations in muscle membrane integrity have occurred in B lines that can not be explained by LW alone, and that there is a greater degree of muscle damage associated with the B lines than L and T lines ($P < 0.01$). It is proposed that genetic selection for improved growth rate and muscle yield in modern broiler lines has detrimental effects upon muscle function and membrane integrity and may be attributable to genetically induced alterations in muscle fibre status.

INTRODUCTION

Over the past 50 years genetic selection of poultry for economically important production traits has been extremely successful. Intensive commercial selection programs in broilers chickens aimed at improving live weight (LW) gain and higher meat yields have resulted in birds which grow up to 4-times faster than "layer" and control lines, and which exhibit 8-fold increases in breast muscle growth rate (Griffin and Goddard, 1994; Havenstein, et al., 1994). However, while these selection programs have led to major improvements in these and other production characteristics it has long been recognised that such selection practices are associated with a number of undesirable consequences (Savory 1995). Such problems include reduced immune function (Dunnington et al., 1987) and major musculo-skeletal (Siller, 1985; Thorp, 1994) and metabolic disorders (Scheele, 1997). It has been proposed by Scheele, (1997) that the increase in metabolic disorders in broiler chickens may be due to an imbalance in the production and supply of energy for maintenance requirements. The resulting homeostatic dysregulation then leads to cellular, tissue and organ dysfunction and a range of reported metabolic pathologies in rapidly growing stock.

Recent studies in our laboratories have demonstrated that genetic selection for increased muscle mass in poultry is associated with an increased incidence of spontaneously occurring skeletal muscle abnormalities (idiopathic myopathy). 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 internalized nuclei have also been observed (Mahon, 1999). In addition, intensively selected poultry lines exhibit increases in the plasma activity of the muscle enzyme creatine kinase (CK), which is released into the circulation as a consequence of muscle damage (Wilson et al., 1990; Mitchell and Sandercock 1995). 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). Complimentary 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).

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 (Sandercock and Mitchell 1994; Soike and Bergmann, 1998; Remignon, et al., 1996). The prevalence and the extent of genetic variation for this condition in chicken lines are not known. 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 (Taylor, 1976a,b). This approach has been previously used to assess the extent of genetic variation for economically important production traits in poultry (Hocking et al., 1985).

The objective of this study was 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, layer and traditional).

ANIMALS AND METHODS

Over 900 one-day old male chicks were obtained from 37 lines representing 3 categories: 12-broiler (B), 12-layer (L) and 13 traditional (T). B and L-lines were obtained from commercial breeders and were the progeny of four unrelated sires. T-lines were obtained from non-commercial rare-breeds certificated pedigree producers and were sired by two males. The birds were brooded in floor pens and randomly allocated to 4 large multi-breed pens (146 m²) at 2-3 weeks of age. The birds were reared on wood shaving litter and provided with *ad libitum* access to a broiler starter diet and water. House temperatures of 18-20°C were maintained by controlled ventilation and heating. The photoperiod was 14h light: 10h dark throughout, at a light intensity of 2 lux.

At 6 and 10 weeks of age a blood sample was taken from one progeny of each sire to determine plasma CK activity, a tissue specific indicator of skeletal muscle damage (Hamburg et al., 1991). Approximately 3 ml of blood was obtained via the brachial vein and transferred into a pre-heparinised (50 IU/ ml) blood collection tube. Plasma samples for CK determination were obtained by centrifugation of whole blood at 1500g for 5 minutes, frozen and stored at -20°C pending analysis. The activity of CK was assessed using a commercial kit modified for use with a multi-well plate spectrophotometer as previously described by (Mitchell et al., 1992).

Following blood collection, birds were subjected to an overnight fast after which, they were weighed and transferred to a local slaughter and processing facility and killed by a combination of electrical stunning and exsanguination. The processed carcasses (defeathered and eviscerated) were chilled overnight at 4°C. After this period, the carcasses were portioned and breast, thigh and drumstick muscles weights were obtained.

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 analyzed by multiple regression analysis of natural logarithms (ln) of the variables. Transformations to ln were necessary to normalize residual errors. The maximal model included effects for age, category, live weight and breast, thigh and drumstick muscle.

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

RESULTS

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. B-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 layer and traditional 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 ($P<0.01$).

The smallest residual mean squares for plasma CK activity was obtained by fitting category, age and the regression of CK on live weight within category: inclusion of any other term led to an increase in the residual mean squares. The category means and standard errors for plasma CK activity (back transformed means) for B, L and T lines respectively were 6.39 ± 0.05 (593), 5.59 ± 0.03 (268), 5.59 ± 0.03 (268) ($P<0.001$) ln CK; the regression coefficients were 0.52, 0.32 and 0.29 (se 0.054; $P<0.01$) ln CK/g live weight.



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 “traditional” types of chickens

DISCUSSION

The aim of this study was to correlate measurements of muscle damage (plasma CK activity) with LW, total muscle weight (MW) and the weights of breast, thigh and drumstick muscle in divergently selected chicken lines representing broiler “meat-type”, layer and unselected traditional lines. The production data and corresponding plasma CK activities reported in this study were consistent with those described in previous studies (Mitchell and Sandercock, 1994; Sandercock et al., 2001), with all lines examined exhibiting age dependent increases in plasma CK. At both ages, B-lines were on average 4-times heavier than the L and T lines and exhibited greater total muscle (6-fold), breast (8-fold), thigh (6-fold) and drumstick (5-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 ($P < 0.001$). Introducing total muscle weight and the various muscle components to the regression equation led to an increase in the residual mean squares. This results suggests 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 3 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 the growth of the muscle fibre may have out paced the supporting capillary supply and connective tissue leading to an increase in myodegenerative features associated with focal ischaemia. The observed increases in myopathological features in turkey muscle have been linked to disturbances in intracellular calcium regulation associated with defects in muscle mitochondria and sarcoplasmic reticulum function (Sosnicki et al., 1988). In broiler chickens, the mechanism of muscle membrane damage has been shown to involve the activation of phospholipase A₂ as consequence of raised intracellular calcium concentration (Sandercock, 1997).

In conclusion, the findings of this study suggest that muscle damage associated with increased LW is much greater in lines selected for high muscle yield and growth rates. It is also proposed that increase in extent of the muscle damage occurring in the more rapidly growing, myopathy susceptible lines may have important implications for bird welfare in terms of locomotory ability and possible muscle pain.

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