Cattle with more reactive temperaments have lower resting muscle glycogen

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Abstract— **The objective of this experiment was to evaluate the effect of differences in animal temperament on muscle glycogen and lactate concentration. The temperament of 81 Angus steers was assessed seven times between weaning and yearling age using flight speed (m/s) and crush score (1 to 5) measurements. Muscle biopsy samples were collected four times from the *semimembranosus* (SM) and *semitendinosus* (ST) during the pasture finishing phase (around 18 to 24 months) and immediately after slaughter from each steer. Each biopsy was analysed for glycogen and lactate, and glycogen depletion due to pre-slaughter stress was calculated as the difference between on-farm glycogen and muscle glycogen at slaughter. An increase in flight speed from 0.75 to 2.25 m/s was associated with a reduction in muscle glycogen concentration by 8.9% ($P<0.05$) and an increase in muscle lactate concentration by 17.3% ($P<0.01$) in both the SM and ST. An increase in crush score from 1.25 to 3 reduced muscle glycogen by 10.4% ($P<0.05$) in the SM and ST and increased lactate in the ST by 42% ($P<0.05$). Thus, animals with calm temperaments had higher resting glycogen concentration and mobilised less during stress compared to more excitable animals. However, the effect of temperament on glycogenolysis pre-slaughter or glycogen concentration at slaughter was not evident, most likely due to these animals becoming habituated to human contact. In conclusion it is likely that improved temperament will increase muscle glycogen concentration prior to slaughter and reduce the incidence of dark, firm, dry beef.**

Keywords— **Glycogen, dark cutting, temperament**

I. INTRODUCTION

Dark cutting in the muscle of beef carcasses severely affects meat quality due to reduced shelf life, bland flavour and variable tenderness [1], and the dark meat colour is not acceptable for consumers as graded by Meat Standards Australia [2]. For these reasons, dark cutting costs the beef industry billions of dollars per annum worldwide in discounted beef [3, 4]. Dark cutting is caused by low muscle glycogen levels at slaughter, which results in high ultimate pH of the carcass (pH>5.7) as there is insufficient glycogen to produce enough lactic acid *post-mortem*. Muscle glycogen concentrations at slaughter are a function of the pre-existing glycogen levels on farm, minus the amount of glycogen mobilised during mustering, transportation and lairage periods prior to slaughter. Producers and processors are unable to totally eliminate stressors during this pre-slaughter phase, hence factors known to affect either glycogen storage and/or the level of stress-induced glycogenolysis like animal temperament warrants investigation.

Pre-slaughter stress is a well documented cause of differences in incidence of dark cutting syndrome. Sympathetic arousal due to emotional stress prior to slaughter causes the release of catecholamines [5] which stimulate glycogenolysis in the muscle. Sympathetic arousal pre-slaughter may vary between animals due to differences in temperament, although this has proven difficult to verify experimentally [6]. Voisinet *et al.* [7] showed that *Bos indicus* cattle with more excitable temperaments measured using a crush/chute score technique had a higher incidence of dark, firm, dry carcasses at slaughter. However it is not known whether the lower muscle glycogen concentrations in these animals at slaughter is a result of lower resting muscle glycogen concentrations or the result of increased stress induced glycogenolysis in the pre-slaughter period. Therefore the objectives of this study were to compare the effect of temperament on resting muscle glycogen concentrations and on glycogen depletion prior to slaughter. We hypothesised that cattle with more excitable, reactive temperaments would store less muscle glycogen, mobilise more metabolites for energy production, and have an increased rate of glycogenolysis pre-slaughter.

II. MATERIALS AND METHODS

In this experiment, 5 muscle biopsy samples were taken from the *semimembranosus* (SM) and *semitendinosus* (ST) to determine muscle glycogen and lactate concentration in 81 Angus steers which had also been assessed for temperament. The experiment was approved by the I&I NSW Animal Care and Ethics Committee, Orange, NSW, (Permit number ORA 08/016), jointly with the Animal Ethics Committee at Murdoch University, Perth, Western Australia (Permit number: R2272/09).

A. Temperament Assessments

Temperament was assessed using flight speed [8] and crush score [9] around weaning and yearling ages. Crush score and flight speed were assessed 3 times over a period of 3 weeks around weaning, and 3 times over a period of 7 weeks around yearling age. The animals were also assessed for flight speed and crush score at the first biopsy. For analyses, the 7 measurements of flight speed and crush score for each animal were averaged as this has been shown to give the most accurate measure of temperament (Burrow and Dillon 1997).

Flight speed and crush score were recorded when the cattle were processed for data collection purposes. Cattle were confined for a period of a few seconds without head constraint in a single animal weighing crate before being released. Flight time(s) was measured over a distance of 1.7 to 2.2 m, and converted to flight speed (m/s) for analyses [8]. Crush score was assessed during the pause before release from the weighing crate, and flight speed recorded after the cattle were released. Crush score was assessed visually on a 5 point scale of agitation where: 1 = Calm, standing still, head mostly still, slow movements; 2 = Slightly restless, looking around more quickly, moving feet; 3 = Restless, moving backwards and forwards, shaking crate; 4 = Nervous, continuous vigorous movement backwards and forwards, snorting; and 5 = Very nervous, continuous violent movement, attempting to jump out. This scale is based on the scoring system described by Grandin [9] which was applied to cattle restrained in a squeeze chute and head bail. Modifications were made to this scale so that it was more suitable for loosely-restrained cattle, as a hydraulic squeeze crush was not available. All crush score assessments were made by the same experienced observer (L.M. Cafe).

B. Muscle glycogen sampling and analysis

Muscle glycogen and lactate concentration were analysed from five muscle biopsies from the SM and ST of each animal, one taken during each stage of nutrition during the finishing phase and one at slaughter. The first sample was taken when the steers were between 14 and 16 months of age and biopsy 4 was taken 7 days prior to slaughter. These four samples were used to provide resting glycogen concentrations or basal glycogen. Glycogen depletion during the pre-slaughter period was calculated as the difference between biopsy 4 and muscle glycogen at slaughter. These muscle samples were taken with a purpose-built, 12 V motorised biopsy drill (Murdoch University, Western Australia) as described in Gardner *et al*. [10]. Glycogen concentrations in each muscle sample were measured using the enzymatic method of Chan and Exton [11], modified by removing the filter paper step.

C. Statistical analyses

Muscle glycogen and lactate concentrations were analysed using a linear mixed effects model [12], with biopsy number and muscle type as fixed effects and animal within sire as the random term. The two measures of temperament, flight speed and crush score plus interactions with biopsy number and muscle type were included in separate analyses as covariates. Non-significant terms ($P > 0.05$) were sequentially deleted from the models.

III. RESULTS

Animal temperament measured using flight speed had an effect on the basal concentrations of muscle glycogen ($P < 0.05$). Across all biopsies and muscling genotypes, as flight speed increased from 0.75 to 2.25 m/s, average muscle glycogen concentration decreased by 8.9% (Fig. 1, $P < 0.05$) and muscle lactate concentration increased by about 17.3% (Fig. 1, $P < 0.01$). There was no significant effect of flight speed on pre-slaughter glycogenolysis or muscle glycogen concentration at slaughter.

Increasing crush score was associated with a decrease in average muscle glycogen and an increase in muscle lactate in the ST. As crush score increased from 1.25 to 3, average muscle glycogen concentration declined by 10.4% ($P < 0.05$, Fig. 2) and lactate in the ST increased by 42% ($P < 0.05$) across the range of crush scores, but did not increase in the SM muscle (Fig. 2).

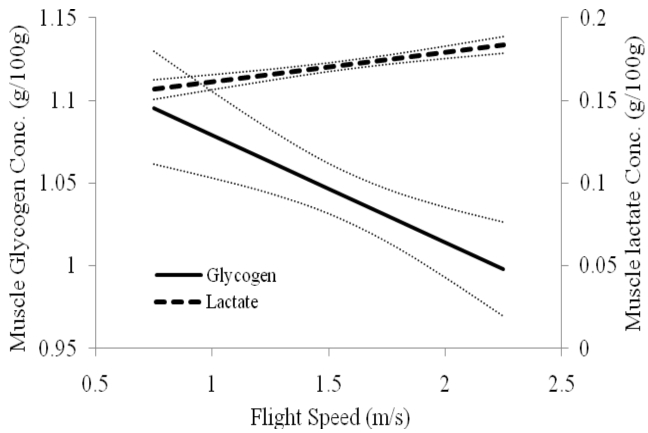

Fig. 1 The effect of flight speed on muscle glycogen concentration and muscle lactate concentration with predicted means for glycogen (solid line) and lactate (dotted line) ± s.e.m. (fine dotted lines) across all muscling genotypes.

IV. DISCUSSION

Animals with more excitable, reactive temperaments (higher flight speed and crush score) had lower resting muscle glycogen and higher muscle lactate. Plasma lactate concentrations taken immediately after the muscle biopsies were also higher in animals with more excitable temperaments, suggesting these animals had a faster rate of glycogenolysis. However, at slaughter, temperament had no effect on glycogen concentrations in the SM or ST, and also had no effect on the extent of glycogenolysis during the pre-slaughter process (ie the difference between on-farm and slaughter glycogen concentrations). Therefore these results partially support our initial hypothesis that cattle with more excitable, reactive temperaments store less glycogen and mobilise more glycogen under stress. These variable results may be due to the relatively calm temperament of these animals when compared with the flight speed results of earlier studies (Petherick *et al.* [13], thus weakening the association between temperament and stress responsiveness in these animals pre-slaughter.

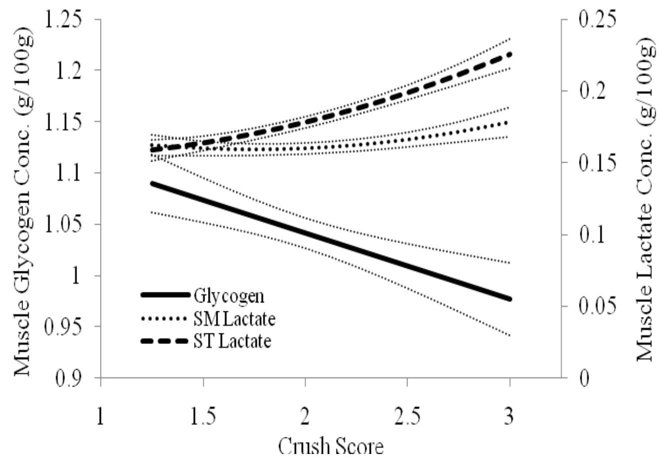

Fig. 2 The effect of flight speed on muscle glycogen concentration and muscle lactate concentration with predicted means for glycogen (solid line) and lactate (dotted line) ± s.e.m. (fine dotted lines) across all muscling genotypes.

This may have been compounded by the extensive human interaction throughout this study, habituating these animals to human contact by the time they were slaughtered. None-the-less, the greater stress responsiveness demonstrated during the earlier phases of this experiment highlights the association between temperament and glycogen mobilisation. Furthermore, this is likely to have impacted chronically, increasing glycogen turn-over and resulting in the lower muscle glycogen levels evidenced in these animals, even in a resting state on-farm. Therefore the combined effects of reduced muscle glycogen storage on-farm, as well as increased rates of glycogen mobilisation under stress are likely to increase the incidence of dark, firm, dry beef in non-habituated cattle. This supports the findings of Voisinet *et al.* [7] who showed that cattle with more excitable temperament ratings had a higher incidence of dark cutting syndrome. As such, it is likely that selecting cattle for calmer temperament will reduce the incidence of dark, firm, dry beef.

V. CONCLUSION

Despite the fact that the variation in temperament of the steers as assessed by flight speed and crush score was not large, temperament still had a substantial effect on resting muscle glycogen and lactate concentration. Animals with calm temperaments had higher resting glycogen concentration compared to more excitable animals. This is likely to result in increased glycogen prior to slaughter and a reduced incidence of dark, firm, dry beef.

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