CATTLE WITH FLIGHTY TEMPERAMENTS HAVE INCREASED MUSCLE GLYCOGEN IN THE *LONGISSIMUS THORACIS ET LUMBORUM* AT SLAUGHTER COMPARED TO CALM CATTLE

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Abstract – This study measured the effect of cattle temperament on plasma lactate, muscle glycogen and muscle lactate at slaughter in commercial (n=547) and research (n=101) lot fed cattle. Temperament was measured using flight speed (FS) at induction into the feedlot for the commercial cattle, and at weaning for the research cattle. Muscle samples taken at slaughter from the *semimembranosus* (SM), *semitendinosus* (ST) and the *longissimus thoracis et lumborum* (LTL) were analysed for total muscle glycogen and lactate concentration. Blood was collected after exsanguination and analyzed for plasma lactate. As FS increased from 1 to 5 m/s, plasma and muscle lactate concentration increased by 44 and 11% respectively (*P<0.05*). On the contrary, as FS increased from 1 to 5 m/s, muscle glycogen concentration increased by 19% in the LTL muscle (*P<0.05*), but there was no effect in the SM or ST. The higher plasma and muscle lactate concentrations of the flighty cattle suggests they mobilize more muscle glycogen immediately before slaughter, but this did not influence total muscle glycogen concentration at slaughter. Thus in this study, it is apparent that flighty cattle are at lower risk of dark cutting, although the mechanisms through which animal temperament delivers variation in glycogen metabolism remain unclear.

Key Words – Dark cutting, glycolysis, lactic acid.

I. INTRODUCTION

Dark cutting syndrome has a negative impact on meat quality and the profitability of the beef industry worldwide. Dark cutting is caused by low muscle glycogen at the time of slaughter, limiting the production of lactic acid post-mortem. Muscle glycogen concentration at slaughter is a function of glycogen synthesized 'on-farm' through nutrition, minus the glycogen mobilized for muscle energy during the pre-slaughter period in response to stress or muscle contraction. One factor which may impact both glycogen synthesis and mobilization is temperament, a motion supported by Voisinet *et al*., [1]. Cattle with flighty temperaments have been shown to have consistently lower feed intakes and growth rates relative to calm cattle [2-4]. This indicates that flighty cattle may have a reduction in substrate available for glycogen synthesis, making their muscle glycogen concentrations lower prior to the pre-slaughter period.

In addition, flighty cattle have higher basal concentrations of catecholamines and cortisol than calm cattle [5-7]. Furthermore, flighty cattle are more active than cattle with 'calm' temperaments during routine handling practices [8] increasing the quantity of muscle contractions and glycogen mobilization. Muscle contractions, catecholamine and cortisol all stimulate glycogen mobilization. Thus we hypothesize that cattle with flighty temperaments will have higher muscle and plasma lactate concentrations at slaughter in comparison to cattle with calm temperaments, and that cattle with flighty temperaments will have lower muscle glycogen concentrations at slaughter.

II. MATERIALS AND METHODS

Data were collected on commercial and research cattle at two sites in Western Australia. The commercial group comprised of 547 lot fed steers (n=313) and heifers (n=234) of both *Bos taurus* (Angus, Murray Grey, Limousin, Charolais and Simmental) and *Bos indicus* (Brahman, Santa Gertrudis, Droughtmaster) descent. The research group consisted of Angus steers (n= 101). The commercial cattle were slaughtered in 11 different harvest groups over an 8 week period at 2 different abattoirs (H and W), while the research steers were harvested in 1 group at abattoir H. The use of animals in this experiment were approved by the Murdoch University Animal Ethics Committee (Permit No. O2391/11) and by the Department of Agriculture and Food Western Australia Animal Research Committee (Permit No. 6-10-44).

A. *Temperament assessment*

Temperament was assessed using flight speed (FS)[9]. FS measures the speed at which cattle exit the crush, with high flight speeds indicative of poor or 'flighty' temperaments [10]. A single FS measurement was taken during induction into the feedlot for the commercial cattle, while 3 FS measurements were taken on the research cattle at weaning time (7 months). Cattle were individually confined in a weighing chute before being released into a wide straight race and flight time (s) recorded over a distance of 1.7- 2.2 m at both sites using dual laser beams. The time recorded was converted to FS (m/s) for analyses.

B. *Plasma and muscle sampling*

At slaughter, muscle samples from the SM and ST were taken at 10 minutes post slaughter, while the LTL sample was taken ~60 minutes post slaughter. The sample of LTL was obtained from the superficial (dorsal) region of the muscle, adjacent and caudal to the 12th rib. Samples of SM and ST were obtained from dorsal, proximal regions of each muscle. Once samples were taken, visible fat was removed and samples were frozen in liquid nitrogen and later stored at –20°C for glycogen and lactate analysis.

Blood samples were collected from the jugular post slaughter after exsanguination using K_3 EDTA vacutainers™ (Becton Dickinson, Franklin Lakes, NJ, Cat. No. 366457). Blood was collected from harvest groups H6 to H9, which totaled 294 head. The blood tubes were placed on ice, centrifuged and the harvested plasma was frozen at -20°C for later laboratory determination of lactate.

C. *Plasma and muscle analysis*

Laboratory analyses of plasma lactate and muscle lactate concentration in the homogenate was carried out using an enzymatic method [11, 12]. Analyses were automated using the Olympus AU400 automated chemistry analyzer (Olympus Optical Co. Ltd, Melville, New York) and the Olympus regent kit for lactate (Olympus Cat. No. OSR6193).

Muscle samples were weighed and homogenized in a 30Mm HCl solution using a 10:1 ratio. Glycogen in the homogenate was hydrolyzed to glucose using a double enzyme method [13]. 125 µl of muscle homogenate was digested in 1ml of enzyme mixture (8mg amylase and 8mg amyloglucosidase in 50ml of 40mM sodium acetate buffer pH 4.8) for one hour in a water bath at 37°C. Laboratory analyses of digested homogenate was carried out using an enzymatic method for glucose [14]. Again analyses were automated using the Olympus AU400 automated chemistry analyzer (Olympus Optical Co. Ltd, Melville, New York) and Olympus regent kits for glucose (Olympus Cat. No. OSR6121). Total glycogen was calculated and expressed as grams of glycogen per 100 g wet muscle tissue.

D. *Statistical analysis*

Muscle glycogen, muscle lactate and plasma lactate concentrations were analyzed using a linear mixed effects models [15]. The initial model included fixed effects and their interactions for gender (heifer and steer), feedlot (commercial or research), lot (C1 to C8 and R1) within feedlot, origin within feedlot, breed within origin by feedlot and harvest group within abattoir (H1- H9, W1- W3) by feedlot. In the statistical models for muscle lactate and glycogen, muscle (SM, ST and LTL) was also included as a fixed effect and individual animal ID was used as a random term to account for multiple muscle samples taken from the same animal. Muscle lactate data for the LTL were removed from the analysis as they were sampled at an inconsistent time post slaughter between animals. Terms in all models were deleted in a step-wise manner if non-significant ($P>0.05$) in order to arrive at the base model. The linear and curvilinear forms of the covariate FS were then interacted with the significant terms in the base models. These models was regressed in a step-wise manner deleting terms which were non-significant ($P>0.05$) in order to arrive at a FS adjusted model.

III. RESULTS AND DISCUSSION

Flight speed had a significant effect on muscle glycogen concentration, but only in the LTL muscle ($P<0.05$). As FS increased from 1 to 5 m/s, muscle glycogen also increased by 19% from 1.31 ± 0.04 to 1.50 ± 0.007 g/100g (Figure 1), but there was no significant effect of temperament on muscle glycogen concentration in the SM or ST.

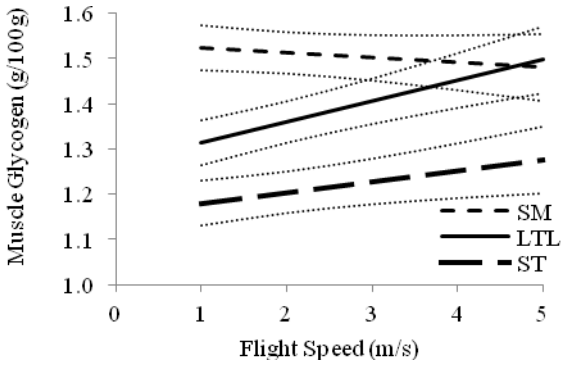

Figure 1. Effect of flight speed on muscle glycogen concentration (g/100g). Dashed lines denote standard errors.

As FS increased from 1 to 5 m/s, plasma lactate concentration increased by 44% (*P<0.05*) from 9.67 mmol/L to 14.0 mmol/L. Likewise, as FS increased from 1 to 5 m/s, muscle lactate also increased by 11% (*P<0.05*) from 0.63 g/100g to 0.70 g/100g in both the SM and ST. Abattoir had no effect on muscle glycogen or plasma lactate.

Muscle had a significant effect on glycogen concentration (*P<0.05*). The SM muscle had the highest average concentration at 1.51 ± 0.045 g /100g (Figure 1) which was 16% higher than that of the LTL (1.35 ± 0.046, *P<0.05*) and 31% higher than that of the ST (1.20 ± 0.045, *P<0.05*). The LTL muscle glycogen concentration was 15% higher than that of the ST (*P<0.05*). These differences between muscles did not change with the inclusion of FS in the model. Harvest group had the most significant effect on muscle glycogen (*P<0.01*). Average muscle glycogen concentrations across slaughter groups ranged from 1.19 ± 0.06 g/ 100g to 1.56 ± 0.06 g/ 100g. Lot within harvest group also had a significant effect on muscle glycogen (*P<0.05*), while the origin of the cattle or gender did not have an effect.

This study demonstrated that as FS increased, glycogen concentration in the LTL also increased, contradicting our initial hypothesis. Conversely there was no effect of FS on glycogen concentration in the SM or ST, which is also in contrast to our initial hypothesis. These results suggest that there is no impact of temperament on dark cutting, and imply that flighty animals are at a lower risk of dark cutting syndrome in this study. This contradicts the findings of Cafe *et al*., [2] and Voisinet *et al*., [1].

A possible explanation is that the higher basal levels of catecholamines and cortisol in flighty cattle [5-7] may dampen their acute stress responsiveness during the pre-slaughter period. Curley [5] found that calm cattle had greater responses to exogenous adrenocorticotropic hormone (ACTH) and Corticotropin-releasing hormone challenges (indicative of emotional stress) than flighty cattle, even though the flighty cattle had higher baseline serum cortisol levels. Work by Ebner *et al*., [16] also found that non-aggressive rodents had increased plasma ACTH levels in response to social confrontation, whereas aggressive animals did not have a significant ACTH response at all. Thus it is reasonable to suggest that the magnitude of the emotional stress responses of the cattle with 'calm' temperaments may have resulted in increased glycogenolysis during the pre- slaughter period (a period of acute stress). Consequently, the LTL, a postural (and not locomotive) muscle which is prone to glycogen depletion during periods of emotional stress [17], had significantly lower muscle glycogen concentration in the calmer cattle than that of cattle with flighty temperaments. Thus it is suggested that the emotional state of the animal will have a greater influence on glycogen depletion than physical activity in the LTL of calm cattle.

On the contrary, as FS increased, plasma and muscle lactate increased supporting the initial hypothesis. These results suggest that flighty cattle do mobilize more glycogen during stressful events like that experienced in the forcing yards and race immediately before slaughter. It is evident that some lactate has shuttled into the blood but it appears the majority remains in the muscle cells and influences the quantity of lactate in the muscle immediately post-slaughter and is additive to the quantity of total glucose. Therefore, it can be suggested that the effect of temperament varies during different levels of stress and the response in the muscle is impacted by muscle type.

IV. CONCLUSION

Temperament, as measured by FS, does influence muscle glycogen in the LTL only, with more flighty cattle having higher glycogen concentrations at slaughter. The biological mechanism which delivers the difference in muscle glycogen at slaughter is very complex

and unclear, but based on the current literature, it can be reasoned that calm cattle are more susceptible to the acute stressors during the pre-slaughter period. It is important to recognize that whilst the flighty cattle may appear chronically stressed and do actually mobilize more glycogen immediately pre-slaughter, it is the potential stress response of calm cattle to acute stressors, which may significantly decrease muscle glycogen at slaughter, increasing their risk of dark cutting. Therefore, it is recommended that calm cattle be treated with greater care during the pre-slaughter period to reduce their risk of dark cutting.

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