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The impact of beef cattle temperament assessed using flight speed on muscle glycogen, muscle lactate and plasma lactate concentrations at slaughter

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Abstract

This study evaluated the effect of animal temperament measured using flight speed (FS) on plasma lactate, muscle glycogen and lactate concentrations at slaughter plus ultimate pH in 648 lot finished cattle of mixed breed and sex. Muscle samples were collected at slaughter from the *m. semimembranosus* (SM), *m. semitendinosus* (ST) and *m. longissimus thoracis* (LT) for analysis of glycogen and lactate concentration. Blood was collected after exsanguination and analysed for plasma lactate concentration and ultimate pH of the LT was measured. FS had no effect on muscle glycogen concentration in any muscle or ultimate pH of the LT ($P>0.05$). As FS increased from 1 to 5 m/s, plasma and muscle lactate concentration increased by 54% and 11.4% respectively ($P<0.01$). The mechanisms through which animal temperament contributes to variation in glycogen metabolism remain unclear.

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The risk of dark cutting was not impacted by temperament, indicating that other production and genetic factors have a greater impact on the incidence of dark cutting.

Keywords: Ultimate pH, glycogenolysis, muscle contraction, dark cutting, catecholamines, stress

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Keywords: Ultimate pH, glycogenolysis, muscle contraction, dark cutting, catecholamines, stress

1.0 Introduction

Dark cutting beef is one of the most prominent meat quality issues worldwide. Dark cutting beef has a reduced shelf life, bland flavour, and variable levels of tenderness, rendering it unacceptable for retailers, food service and consumers alike (Ferguson, et al., 2001). These negative impacts on meat quality have led to its exclusion from the Meat Standards Australia (MSA) grading system (Grunert, Bredahl, & Brunsø, 2004; Thompson, 2002; Watson, Polkinghorne, & Thompson, 2008).

Dark cutting beef is defined by MSA as any muscle tissue with an ultimate pH greater than 5.7 (Thompson, 2002) or an AUSmeat meat colour greater than 3 (AUS-MEAT, 2005; Watson, et al., 2008). The major determinant of ultimate pH is the concentration of muscle glycogen at slaughter, which in the anaerobic conditions of the muscle post-mortem, is metabolised through glycolysis to form lactate. The formation of lactate and the production of hydrogen ions lowers the intracellular pH of the muscle from a pH of around 7, which is standard in a living animal (Tarrant, 1989), down to a pH_u of around 5.4 to 5.7 during the first

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24 to 48 hours post mortem (Maltin, Balcerzak, Tilley, & Delday, 2003). However, if there is insufficient muscle glycogen concentration at slaughter, there will only be limited formation of lactate post mortem, and thus a pH_u of less than 5.7 will not be reached and dark cutting will result.

Muscle glycogen concentration at slaughter is a function of glycogen synthesised 'on-farm' through nutrition, minus the glycogen mobilised for muscle energy during the pre-slaughter period in response to stress or muscle contraction (McGilchrist, Alston, Gardner, Thomson, & Pethick, 2012). One factor which may impact both glycogen synthesis and mobilisation is temperament, a notion supported by Voisinet et al., (1997a). Cattle with flighty temperaments have been shown to have consistently lower feed intakes and growth rates relative to calm cattle (Busby, 2010; Cafe, et al., 2011; Fell, Colditz, Walker, & Watson, 1999; Petherick, Holroyd, Doogan, & Venus, 2002; Vann, Parish, & McKinley, 2008; Voisinet, Grandin, Tatum, O'Connor, & Struthers, 1997b). This indicates that flighty cattle may have a reduction in substrate available for glycogen synthesis, lowering their muscle glycogen concentrations prior to the pre-slaughter period.

In addition, it is well established that flighty cattle have higher basal concentrations of catecholamines and cortisol than calm cattle (Burdick, et al., 2010b; Curley, 2004; Vann, Burdick, Lyons, Welsh, & Randel, 2010). Catecholamines and cortisol are released in the event of stress and directly stimulate glycogen mobilisation. Furthermore, muscle contractions also stimulate glycogen mobilisation, and as cattle with 'excitable' or 'flighty' temperaments are more active than cattle with 'calm' temperaments during routine handling practices (Grandin, 1993) it is reasonable to expect that 'flighty' cattle will have increased muscle contractions during the pre-slaughter period and a greater mobilisation of glycogen during this period, thus less muscle glycogen at slaughter. Given the likely increase in stimulation of the catecholamine, cortisol and contraction linked mechanisms of glycogen mobilisation in flighty cattle, it can be assumed that they will also have elevated levels of muscle and plasma lactate, as lactate is the end product of mobilised glycogen under anaerobic conditions. Therefore, the combination of these factors is likely to result in reduced muscle glycogen at slaughter and a higher incidence of dark cutting in flighty cattle. However no study to date has measured the effect of temperament on glycogen concentration within muscle which maybe a more sensitive measure of potential risk to dark cutting.

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This study aims to identify whether a relationship exists between cattle temperament measured using flight speed and muscle glycogen concentration. We hypothesise that cattle with flighty temperaments and high flight speeds will have higher muscle and plasma lactate concentrations, higher ultimate pH and lower muscle glycogen concentrations at slaughter.

2.0 Material and Methods

2.1 Animals

Data were collected on cattle at two sites in Western Australia. Cattle at site A comprised of 547 feedlot 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. Cattle were sourced from sale yards and direct from producers. Cattle at site A ranged in age from 9 months (weaning age) through to 18 months at the time of induction into the feedlot. The source of each herd was recorded as the 'origin' of the cattle. At site A, the cattle came from 10 different origins, and there were between 1 and 4 breeds of cattle from each origin. Cattle were inducted into the commercial feedlot over a period of three months and were assigned to 'lots' in the feedlot according to their origin and/or weight at induction. Cattle from each origin were of similar age. Lots were formed by cattle from 1 or more origins. Lots were A1 to A8 for the cattle from site A. Upon induction into the feedlot, live weight (kg) and hip height (cm) measurements were recorded. All cattle were introduced to grain feeding using a 'step up' ration program where energy levels in the diet were increased over a 21 day period, after which time they were placed on the finisher ration. The finisher ration contained 12.6 MJ/kg dry matter of metabolisable energy and 11% crude protein on a dry matter basis and had a digestibility of 82.8%. Cattle were harvested based on liveweight (minimum 420 kg) after a minimum of 70 days in the feedlot, targeting a carcass weight of 220 to 320 kg.

Cattle at site B consisted of Angus steers (n= 101) which were inducted on a single date. Cattle were penned in groups of 10 or 11 and were not mixed with other cattle between induction and slaughter. These cattle were assigned to a single lot for analysis called B1. Live weight and hip height measurements were recorded monthly over the period of feeding. These cattle were also introduced to grain feeding using a 'step up' ration programme, with the finisher ration containing 10.6 MJ/kg dry matter of metabolisable energy and 19% crude

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protein on a dry matter basis and a digestibility of 73.2%. Cattle were harvested after 111 days on feed, when the average weight was 450 kg.

Cattle at site A were selected for slaughter 0 to 48 hours before departure from the feedlot, and cattle from different lots were transported in separate pens on the trucks to fill a consignment of cattle which met market specifications. Cattle were sent for slaughter on a weekly basis to either processor 'H' or processor 'W' which were 2 commercial export licensed processing plants. Each consignment was referenced as a 'slaughter group' (H1 to H10 and W1 to W3), to identify cattle by the date and location of slaughter.

The use of cattle at site A was approved by the Murdoch University Animal Ethics Committee (Permit No. O2391/11). The use cattle at site B was approved by the Department of Agriculture and Food Western Australia Animal Research Committee (Permit No. 6-10-44).

2.2 Temperament assessment

Temperament was assessed using flight speed (FS) (Burrow et al., 1988). FS measures the speed at which cattle exit the crush, with high flight speeds indicative of poor or 'flighty' temperaments (Burrow & Corbet, 2000). At site A, FS measurement was taken during induction into the feedlot on all cattle and 46% of cattle at site A had FS measured again 3 weeks later when the cattle were re-weighed for assessment of performance. For the 46% of cattle with 2 FS measurements, average FS of cattle at Site A was calculated for each animal. At site B, FS measurements were taken once at weaning time (7 months) and twice at the beginning of their time in the feedlot (9 months of age). Average FS for cattle at site B was the average of the 3 FS measurements.

Burrow and Corbet (2000) stated that the use of an average flight speed score substantially increased heritability of the trait (0.50) when compared with use of a single flight speed score, which is why an average was used for all cattle at site B and 46% of cattle at site A. Burrow and Corbet (2000) also noted that the heritability of single flight speed scores was in the moderate range (0.29 to 0.39), with heritability decreasing with age from weaning to 18 months of age, hence why at site A, FS was recorded at the time of induction. Cafe et al. (2011) states that the correlations between 8 different FS measures were moderate to high and all significant in Brahman cattle and 90% of correlations were significant in Angus cattle

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when measured at the same location, which is the case with cattle at sites A and B for this experiment.

Cattle were individually confined in a weighing chute before being released. Upon release from the weighing chute the cattle entered into a wide straight race and flight speed was measured over a distance of 1.7 to 2.2 m at both sites using dual laser beams. The time required to travel the measured distance was divided by the distance to calculate FS in metres per second (m/s) for analyses.

2.3 Plasma and muscle sampling

At slaughter, muscle samples from the *m. semimembranosus* (SM) and *m. semitendinosus* (ST) were taken on the slaughter floor immediately after the hide was peeled back from the hind leg (~10 minutes post slaughter), while the *m. longissimus thoracis* (LT) sample was taken after the carcasses had been split and was entering the chiller (~60 minutes post slaughter). The sample of LT 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. Immediately after each sample was 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 275 head only. Blood was collected post slaughter after exsanguination using K_3 EDTA vacutainers™ (Becton Dickinson, Franklin Lakes, NJ, Cat. No. 366457). Blood was collected from slaughter groups H6, H7, H8, H9 and H11. The blood tubes were placed on ice, centrifuged at 3000 x g for 15 minutes at 5ºC and the harvested plasma was frozen at -20°C for later laboratory determination of lactate.

2.4 Ultimate pH measurement

Ultimate pH is measured in the rib eye muscle (*longissimus thoracis)* of the chilled carcass at the quartering site approximately 20hrs post-mortem. The ultimate pH of all cattle slaughtered at processing plant H were measured. Temperature and pH were measured using an Meat Standards Australia approved TPS MC-80 or TPS WP-80M ph Meter (TPS Pty Ltd., Springwood, Brisbane, Qld, 4127, Australia). pH and temperature probes were calibrated to pH 4 and 7 at 25ºC. The probes were inserted into the muscle in close proximity to each other

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with enough time allowed for reading to be stabilised. Carcass grading could not commence if the loin temperature was above 12°C (AUS-MEAT, 2005).

2.5 Plasma and muscle analysis

Laboratory analyses of plasma lactate concentration was carried out using an enzymatic method (Barham & Trinder, 1972; Trinder, 1969). Analyses were automated using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Melville, New York) and the Olympus regent kit for lactate (Olympus Cat. No. OSR6193).

Muscle samples (approximately 2g from each muscle) were weighed and homogenized in a 30Mm HCl solution using a 10:1 ratio for 45 seconds at 27,000 rpm using a Bosch GGS 27C Professional. Laboratory analyses of lactate in the muscle homogenate was then carried out using the enzymatic methods for plasma lactate as described above.

Glycogen in the homogenate was hydrolised to glucose using a double enzyme method (Passonneau & Lauderdale, 1974). 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^{o}C. Laboratory analyses of digested homogenate was carried out using an enzymatic method for glucose (Barthelmai & Czok, 1962). Again analyses were automated using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Melville, New York) and Olympus regent kits for glucose (Olympus Cat. No. OSR6121). Total glycogen was calculated by halving the lactate value and adding it to the glucose value, expressed as grams of glycogen per 100 g wet muscle tissue. Total glycogen concentration was calculated to give a reflection of muscle glycogen at the time of slaughter, even though some of the lactate may have been in the muscle prior to the time of slaughter. The lactate in the muscle at the time of slaughter will still contribute to the ultimate pH of the muscle, hence why it is included.

2.6 Statistical analysis

The effect of production parameters on animal temperament measured using FS was analysed using a general linear model in SAS (2001). Gender (heifer or steer), feedlot (Site A or B), lot (A1 to A8 and B1) within feedlot, origin within feedlot and breed within origin by feedlot were included as fixed effects in the model. Hip height and entry weight were also included as covariates in the model in both the linear and curvilinear forms, plus interacted with the

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fixed effects. Terms in the model were deleted in a step-wise manner if non-significant (P> 0.05). Significant terms were interacted with FS for the analysis of muscle glycogen and lactate concentrations outlined below.

Muscle glycogen and lactate concentrations were analysed using linear mixed effects models (SAS, 2001). Animals with missing or incomplete data were excluded from the analysis. The initial model included fixed effects and their interactions for gender (heifer or steer), feedlot (Site A or B), lot number (A1 to A8 and B1) within feedlot, origin within feedlot, breed within origin by feedlot, slaughter group within feedlot and muscle (SM, ST and LT). Individual animal ID was used as a random term to account for multiple muscle samples taken from the same animal. Terms in both 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 model. This model 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. Muscle lactate data for the LT was removed from the analysis as it was sampled at an inconsistent time post slaughter between animals.

Plasma lactate concentrations and ultimate pH were analysed using a general linear model (SAS, 2001). The initial model included fixed effects and their interactions for gender (heifer or steer), feedlot (Site A or B), lot (A1 to A8 and B1) within feedlot, origin within feedlot, breed within origin by feedlot and slaughter group within feedlot. Terms 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 model. This model 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.

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3.0 Results

Suggested position of Table 1

3.1 Effect of flight speed on muscle glycogen, ultimate pH and lactate concentration

Flight speed had no significant effect on muscle glycogen concentration in the SM, ST or LT muscles ($P>0.05$, Table 2). FS also had no significant impact on ultimate pH ($P>0.05$, Table 2).

As FS increased from 1 to 5 m/s, muscle lactate concentration increased by 11.4% ($P< 0.01$) from 0.605 g/100g to 0.674 g/100g (Figure 1). The interaction of muscle and FS was not significant, indicating that the impact of FS on muscle lactate in the SM and ST muscles is the same. When FS was included in the statistical model, the effect of lot became non-significant demonstrating that the significance of Lot was due to animals within different lots having different temperaments or FS.

Suggested position of Figure 1 and Table 2

3.2 Effect of flight speed on plasma lactate

Flight speed had a significant effect on plasma lactate concentration ($P<0.01$, Table 2). As FS increased from 1 to 5 m/s, plasma lactate at slaughter increased by 54% from 8.83 mmol/L to 13.6 mmol/L (Figure 2).

Suggested position of Figure 2

3.3 Effect of muscle on glycogen

Muscle had a significant effect on glycogen ($P<0.001$, Table 2). The SM muscle had the highest concentration at 1.48 ± 0.024 g /100g (Figure 3) and was 8% higher than that of the LT (1.37 ± 0.024, $P<0.001$) and 16.3% higher than that of the ST (1.27 ± 0.024, $P<0.001$). The LTL muscle glycogen concentration was 7.6% higher than that of the ST ($P<0.001$). These differences between muscles did not change with the inclusion of FS in the statistical model.

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Suggested position of Figure 3

3.4 Effect of lot number on glycogen

Lot number had a significant effect on muscle glycogen ($P<0.05$, Table 2). Lot Number 5 had significantly lower muscle glycogen than all other lots at 1.23 ± 0.047 g/100g ($P<0.05$, Figure 4). A1, A2, A3, A4, A6, A7, A8 and B1 had 13.2%, 25.5%, 9.7%, 12.1%, 10.6%, 14.6%, 16.7% and 10.1% higher muscle glycogen than lot A5 (Figure 4). The effect of lot number on muscle glycogen concentration did not change with the inclusion of FS in to the model.

Suggested position of Figure 4

3.5 Effect of slaughter group on muscle glycogen

Slaughter group had a significant effect on muscle glycogen ($P<0.001$, Table 2). Muscle glycogen concentrations were lowest in slaughter group H7 at 1.26 ± 0.06 g/ 100g and highest in slaughter group W1 at 1.62 ± 0.06 g/ 100g (Figure 5). There was also a significant interaction between muscle and slaughter group on muscle glycogen concentration ($P<0.01$, Table 2). The interaction was similar to the effect of muscle on glycogen, where the SM muscle glycogen concentration was higher than that of the LT and ST in each slaughter group, and the LT muscle glycogen concentration was higher than that of the ST. These differences were generally consistent across all slaughter groups, with only a few instances where the ST or the LT demonstrated the highest muscle glycogen concentrations. The effect of slaughter group on muscle glycogen concentration did not change with the inclusion of FS in to the model.

Suggested position of Figure 5

Due to all the cattle being grain finished, there were very few (n=11) which were classified as dark cutters or non-compliant to MSA with a pH greater than 5.7 or a meat colour greater than 3 (Watson, et al., 2008). This is why muscle glycogen and not MSA compliance was analysed. Feedlot, origin within feedlot, breed within origin by feedlot, or gender did not have a significant impact on muscle glycogen ($P>0.05$, Table 2). Feedlot, origin within feedlot, breed within origin by feedlot, lot within feedlot or gender did not have a significant impact on muscle lactate or plasma lactate ($P>0.05$, Table 2).

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4.0 Discussion

4.1 Effect of flight speed on muscle glycogen concentration and ultimate pH

This study demonstrated that as FS increased or the animals became more flighty or reactive to human interaction, muscle glycogen concentration or ultimate pH did not change, contradicting our initial hypothesis. The muscle glycogen and ultimate pH results suggest cattle of all temperaments have an equal risk of dark cutting. The results of this study are supported by Gruber, et al. (2010) and Burnham, Purchas, and Morris (2005) which both showed that cattle with varying behavioural characteristics based on chute scores (unease on the scale, agitation, speed of exit) did not show any differences in ultimate pH of the LT or in the incidence of dark cutters. Further support for the results is presented by McGilchrist (2011) which showed that there was no association between temperament and muscle glycogen concentration in the SM or ST muscles of 80 Angus steers at slaughter. Interestingly, McGilchrist (2011) also reported that flighty cattle had significantly lower resting muscle glycogen than calm cattle during the finishing period on-farm in the SM and ST muscles, which contradicted the glycogen results at slaughter. McGilchrist (2011) suggested that the calm animals had mobilised significantly more glycogen during the pre-slaughter period, which could be an explanation why there was no effect again of temperament on muscle glycogen at slaughter in this study.

Based on the findings of Curley (2004), it can be extrapolated that flighty have significantly lower stress responses during the pre-slaughter period and thus lower glycogenolysis. Curley (2004) found that calm cattle had greater responses to exogenous ACTH and CRH challenges than flighty cattle, even though the flighty cattle had higher baseline serum cortisol levels. Work by Ebner et al., (2005) found that non-aggressive animals had increased plasma ACTH levels in response to social confrontation, whereas aggressive animals did not have a significant ACTH response at all, further supporting the aforementioned theory that temperament does indeed influence the stress responsiveness of animals. This highlights the prospect that cattle with higher flight speeds may have started with less glycogen as hypothesised but due to lower stress responsiveness to acute stressors during the pre-slaughter period, muscle glycogen concentration at slaughter is equal to that of calmer cattle.

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Even though the theory presented above is plausible, the results are not supported by Cafe et al. (2011) who reported that increasing flight speeds resulted in a higher ultimate pH and a darker meat colour and the study by Voisinet et al., (1997a) which showed that cattle with excitable temperaments are more likely to be borderline dark cutters, classified using meat colour only. Thus the effect of temperament differs between studies and its true impact on intermediary metabolism in particular glycolysis must be multifactorial. Further studies are required to determine the mechanism through which temperament acts on muscle glycogen concentration.

4.2 Effect of flight speed on plasma and muscle lactate

As FS increased, plasma and muscle lactate increased supporting the initial hypothesis that flighty cattle mobilise more glycogen in the period immediately prior to slaughter. The lactate produced is either shuttled into the blood as lactate or remains in the muscle cells and influences the quantity of lactate in the muscle immediately post-slaughter. These results are supported by those of Gruber, et al. (2010) which showed that cattle with adverse behavioural reactions to handling and chute restraint had higher plasma lactate at slaughter. King et al., (2006) also found that at 0.5 h post mortem, muscle samples from flighty cattle (temperament measured using FS) had lower muscle pH than calm cattle suggesting that the flighty cattle had higher levels of muscle lactate at the time of slaughter, causing an increased rate of pH decline in the first 30 minutes post slaughter. This is further supported by the work of Petherick et al., (2002) who found that cattle with flighty temperaments reached a pH of 6 faster than calm cattle, again possibly indicating a higher level of muscle lactate at slaughter. However, Cafe et al., (2011) found that as FS increased, the time taken to reach pH 6 increased, which contradicts the findings of this study and those of Petherick et al., (2002) and King et al., (2006). Considering temperament did impact muscle pH in the study by Cafe et al., (2011), then it can be assumed that the flighty cattle did have slower rates of glycogenolysis and glycolysis post mortem. Without taking numerous muscle samples at all times during the pre and post-slaughter period, it is impossible to tease apart the contributions of pre and post slaughter glycolysis to muscle lactate and therefore pH measurements.

Several studies have shown that calm cattle have lower basal levels of catecholamines and serum cortisol than flighty cattle (Burdick, et al., 2010a; Burdick, et al., 2010b; Burdick, Randel, Carroll, & Welsh, 2011; Vann, et al., 2010). Gruber, et al. (2010) also demonstrated that epinephrine levels at slaughter were lower in calm cattle. Seeing as though

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glycogenolysis and glycolysis are regulated by the concentration of catecholamines and cortisol, it makes sense that more reactive animals with higher concentrations of regulatory hormones have higher plasma and muscle lactate as seen in this study.

The results of this study do however indicate that the flighty cattle were mobilising glycogen at a greater rate than the calm cattle immediately before slaughter as measured by plasma lactate and lactate in the SM and ST muscles themselves. However, this concept completely challenges the muscle glycogen results of this study, in which FS did not impact on muscle glycogen concentrations. The reason why flighty cattle have higher glycogenolysis immediately pre-slaughter, yet no difference in muscle glycogen is unclear. One possible explanation is that calm cattle may have a higher level of glycogenolysis during the entire pre-slaughter period compared to flighty cattle due to upregulated stress responsiveness as discussed in section 4.1. This would result in cattle of all temperaments having equal muscle glycogen at slaughter. Thus, the susceptibility of calm cattle to stress may have had a greater influence on muscle glycogen over the entire pre-slaughter period than that experienced by the flighty cattle during the period immediately before slaughter. Therefore, it is reasonable to suggest that the effect of temperament on glycogenolysis varies depending on the intensity of stress, and the period of time the animals are exposed to stressor.

4.3 Effect of lot number and slaughter group on muscle glycogen

There was a significant variation in muscle glycogen concentration among cattle from different lot numbers and from different slaughter groups (cattle harvested on different days). The significant term for lot number suggests that cattle which are penned and finished together in a lot at a feedlot react similarly to each other in regards to nutrition, stress responsiveness and ultimately muscle glycogen concentrations.

The significant slaughter group effect suggests that there are factors unique to each date of slaughter, asides from lot number and animal temperament, that influence glycogen mobilisation. Such factors may include pre-slaughter drafting, trucking distance, time spent in lairage, weather and stockperson handling during mustering, transport, lairage and immediately prior to slaughter. This is supported by Johnston et al., (2001) who also found slaughter group (defined as animals run together from intake to slaughter) to be the second largest source of variation in LT shear force, after additive genetic variance.

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The commercial cattle were drafted between 24 to 48 hours before slaughter. The variation in time allowed for co-mingling may have impacted glycogen mobilisation in numerous ways. The cattle that were co-mingled up to 48 hours before slaughter may have had higher muscle glycogen depletion than those co-mingled for up to 24 hours, because of increased time available for antagonistic behaviour such as aggression and sexual activity to re-establish dominance (Ferguson, et al., 2001; Kenny & Tarrant, 1990). Alternatively, the increased time available may have allowed for a period of adaptation to the new social environment, preventing further glycogen depletion, whereas cattle co-mingled for only 24 hours or even less may still have been establishing the social structure up until and during lairage. McVeigh and Tarrant (1982) reported muscle glycogen levels of regrouped bulls to be significantly lower than those in the control group, remaining so until day 7 of the recovery period, whereas Warriss et al., (1984) found that the effect of regrouping cattle could be removed with a recovery period as little of 2 to 4 days.

Handling methods have been found to significantly impact glycogen depletion during the pre-slaughter period. Commercial handling methods, simulating stress, has been reported to increase plasma cortisol and plasma lactate, indicative of adrenaline release (Brown, Warriss, Nute, Edwards, & Knowles, 1998). Similarly, Gardner et al., (1999) found that lambs subjected to commercial handling methods had low muscle glycogen post mortem, high pHu and a significant loss of glycogen between the holding pen and slaughter.

The effect of weather has been included in several studies, but not specifically focussed on. Immonen et al., (2000) found that cattle mobilised more muscle glycogen when transported during hot weather in comparison to transportation during cool weather. This is supported by Scanga et al., (1998) who found that the incidence of dark cutters significantly increased when the temperature exceeded 35°C up to 2 days before slaughter. On the other hand the rate of dark cutting has been reported as been at its highest during very cold wet weather, and when there are large fluctuations in temperature over short periods of time (Grandin, 1992; Smith, Tatum, & Morgan, 1993). This suggests that cattle are at risk of extensive glycogen depletion during weather events which create conditions outside of their thermo-nuetral zone, as the glycogen is mobilised for physiological responses to maintain the core body temperature.

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5.0 Conclusion

The temperament of cattle, as characterised by FS, does not influence muscle glycogen concentration in the LT, SM or ST muscles or the ultimate pH of the LT. This suggests that cattle of all temperaments have an equal risk of dark cutting, all else being equal. However the plasma and muscle lactate results showed that animals with high FS do mobilise more glycogen during the period immediately before slaughter than calm cattle. Thus the duration and quantity of periods of high stress, close confinement and human interaction need to be minimised during the pre-slaughter period to ensure that flighty cattle do not become dark cutters due to excess glycogenolysis during these times. The muscle type and date of harvest had the largest impacts on muscle glycogen concentration. The lot number from which the cattle originated at the feedlot also affected muscle glycogen. As many of the stress events which occur during the pre-slaughter period can be minimised to some extent, these results highlight the opportunity for both producers and processors to reduce the risk of cattle becoming dark cutters. Therefore, it is recommended that best management handling practices should be used at all times during the pre-slaughter period to minimise the effect of acute stressors.

6.0 Acknowledgements

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7.0 References

AUS-MEAT. (2005). *Handbook of Australian Meat* (Vol. 7th Edition). Brisbane: AUS-MEAT Ltd.

Barham, D., & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst, 97*, 142 - 145.

ACCEPTED MANUSCRIPT

Barthelmai, W., & Czok, R. (1962). Enzymatic determinations of glucose in the blood, cerebrospinal fluid and urine. *Journal of Molecular Medicine, 40*(11), 585-589.

Brown, S. N., Warriss, P. D., Nute, G. R., Edwards, J. E., & Knowles, T. G. (1998). Meat quality in pigs subjected to minimal preslaughter stress. *Meat Science, 49*(3), 257-265.

Burdick, N. C., Carroll, J. A., Hulbert, L. E., Dailey, J. W., Ballou, M. A., Randel, R. D., Willard, S. T., Vann, R. C., & Welsh, T. H. (2010a). Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. *Innate Immunity, 17*, 355-364.

Burdick, N. C., Carroll, J. A., Hulbert, L. E., Dailey, J. W., Willard, S. T., Vann, R. C., Welsh, T. H., & Randel, R. D. (2010b). Relationships between temperament and transportation with rectal temperature and serum concentrations of cortisol and epinephrine in bulls. *Livestock Science, 129*(1), 166-172.

Burdick, N. C., Randel, R. D., Carroll, J. A., & Welsh, J. T. H. (2011). Interactions between Temperament, Stress, and Immune Function in Cattle. *International Journal of Zoology*, 1-9.

Burnham, D. L., Purchas, R. W., & Morris, S. T. (2005). Relationships between on-farm and pre-slaughter behaviour and growth and meat quality for bulls and steers. In *Proceedings of the New Zealand Society of Animal Production* (Vol. 65, pp. 261 - 265).

Burrow, H. M., & Corbet, N. J. (2000). Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pasture in the tropics. *Australian Journal of Agricultural Research, 51*(1), 155-162.

Busby, D. (2010). Disposition - Convenience Trait or Economically Important. In *2010 American Association of Bovine Practitioners Conference*. Albuquerque, New Mexico.

Cafe, L. M., Robinson, D. L., Ferguson, D. M., McIntyre, B. L., Geesink, G. H., & Greenwood, P. L. (2011). Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. *Journal of Animal Science, 89*(5), 1452-1465.

Curley, J. K. O. (2004). *Influence of temperament on bovine hypothalamic-pituitary-adrenal function.* Texas A&M University, College station,Texas.

Ebner, K., Wotjak, C. T., Landgraf, R., & Engelmann, M. (2005). Neuroendocrine and behavioral response to social confrontation: residents versus intruders, active versus passive coping styles. *Hormones and Behavior, 47*(1), 14-21.

Fell, L. R., Colditz, I. G., Walker, K. H., & Watson, D. L. (1999). Associations between temperament, performance and immune function in cattle entering a commercial feedlot. *Australian Journal of Experimental Agriculture, 39*(7), 795-802.

Ferguson, D. M., Bruce, H. L., Thompson, J. M., Egan, A. F., Perry, D., & Shorthose, W. R. (2001). Factors affecting beef palatability- farmgate to chilled carcass. *Australian Journal of Experimental Agriculture, 41*(7), 879-891.

Gardner, G. E., Kennedy, L., Milton, J. T. B., & Pethick, D. W. (1999). Glycogen metabolism and ultimate pH of muscle in Merino, first-cross, and second-cross wether

ACCEPTED MANUSCRIPT

lambs as affected by stress before slaughter. *Australian Journal of Agricultural Research, 50*(2), 175-182.

Grandin, T. (1992). Problems with bruises and dark cutters in harvest steers/heifers. In *Improving the Consistency and Competitiveness of Beef—A Blueprint for Total Quality Management in the Fed-Beef Industry—The Final Report of the National Beef Quality Audit—1991, Colorado State University, Fort Collins; Texas A&M University, College Station.*

Grandin, T. (1993). Behavioral agitation during handling of cattle is persistent over time. *Applied Animal Behavior Science, 36*, 1.

Gruber, S. L., Tatum, J. D., Engle, T. E., Chapman, P. L., Belk, K. E., & Smith, G. C. (2010). Relationships of behavioral and physiological symptoms of preslaughter stress to beef longissimus muscle tenderness. *Journal of Animal Science, 88*(3), 1148-1159.

Grunert, K. G., Bredahl, L., & Brunsø, K. (2004). Consumer perception of meat quality and implications for product development in the meat sector--a review. *Meat Science, 66*(2), 259-272.

Immonen, K., Ruusunen, M., Hissa, K., & Puolanne, E. (2000). Bovine muscle glycogen concentration in relation to finishing diet, slaughter and ultimate pH. *Meat Science, 55*(1), 25-31.

Johnston, D. J., Reverter, A., Robinson, D. L., & Ferguson, D. M. (2001). Sources of variation in mechanical shear force measures of tenderness in beef from tropically adapted genotypes, effects of data editing and their implications for genetic parameter estimation. *Australian Journal of Experimental Agriculture, 41*(7), 991-996.

Kenny, F. J., & Tarrant, P. V. (1990). The behavioural responses of beef cattle to short haul road transport. *Applied Animal Behaviour Science, 26*(3), 292-293.

King, D. A., Schuehle Pfeiffer, C. E., Randel, R. D., Welsh, J. T. H., Oliphint, R. A., Baird, B. E., Curley, J. K. O., Vann, R. C., Hale, D. S., & Savell, J. W. (2006). Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. *Meat Science, 74*(3), 546-556.

Maltin, C., Balcerzak, D., Tilley, R., & Delday, M. (2003). Determinants of meat quality: tenderness. *Proceedings of the Nutrition Society, 62*, 337- 347.

McGilchrist, P. (2011). *Selection for muscling effects carbohydrate and fatty acid metabolism in beef cattle.* Murdoch University, Perth, Australia.

McGilchrist, P., Alston, C. L., Gardner, G. E., Thomson, K. L., & Pethick, D. W. (2012). Beef carcasses with larger eye muscle areas, lower ossification scores and improved nutrition have a lower incidence of dark cutting. *Meat Science, 92*(4), 474-480.

McVeigh, J., & Tarrant, P. V. (1982). Behavioural stress and skeletal muscle glycogen metabolism in young bulls. *Journal of Animal Science, 54*, 790- 795.

MLA. (2010). MSA requirements for handling cattle. In. Sydney, Australia: Meat and Livestock Australia.

Passonneau, J. V., & Lauderdale, V. R. (1974). A comparison of three methods of glycogen measurement in tissues. *Analytical Biochemistry, 60*, 405-412.

Petherick, J. C., Holroyd, R. G., Doogan, V. J., & Venus, B. K. (2002). Productivity, carcass and meat quality of lot-fed *Bos indicus* cross steers grouped according to temperament. *Australian Journal of Experimental Agriculture, 42*(4), 389-398.

ACCEPTED MANUSCRIPT

SAS. (2001). Statistical analysis system. In (Version 8 ed. ed.). Cary, NC: SAS Institute Incorporated.

Scanga, J. A., Belk, K. E., Tatum, J. D., Grandin, T., & Smith, G. C. (1998). Factors contributing to the incidence of dark cutting beef. *Journal of Animal Science, 76*(8), 2040-2047.

Smith, G. C., Tatum, J. D., & Morgan, J. B. (1993). *Dark cutting beef: physiology, biochemistry and occurrence*: Colorado State University, Fort Collins.

Tarrant, P. V. (1989). Animal behaviour and environment in the dark-cutting condition. In S. U. Fabiansson, W. R. Shorthose & R. D. Warner (Eds.), *Dark cutting in sheep and cattle* (pp. 8 - 18). Sydney: Australian Meat and Livestock Research and Development Corporation.

Thompson, J. (2002). Managing meat tenderness. *Meat Science, 62*(3), 295-308.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry, 6*, 24 - 27.

Vann, R. C., Burdick, N. C., Lyons, J. G., Welsh, T. H., & Randel, R. D. (2010). Temperament influences stress hormone and IgG concentrations in Angus cross calves. In *2010 Animal and Dairy Sciences Annual Report* (pp. 36- 39).

Vann, R. C., Parish, J. A., & McKinley, W. B. (2008). Case Study: Mississippi Cattle Producers Gain Insight into Temperament Effects on Feedlot Performance and Subsequent Meat Quality. *The Professional Animal Scientist, 24*(6), 628-633.

Voisinet, B. D., Grandin, T., O'Connor, S. F., Tatum, J. D., & Deesing, M. J. (1997a). Bos indicus-cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. *Meat Science, 46*(4), 367-377.

Voisinet, B. D., Grandin, T., Tatum, J. D., O'Connor, S. F., & Struthers, J. J. (1997b). Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. *Journal of Animal Science, 75*(4), 892-896.

Warriss, P. D., Kestin, S. C., Brown, S. N., & Wilkins, L. J. (1984). The time required for recovery from mixing stress in young bulls and the prevention of dark cutting beef. *Meat Science, 10*(1), 53-68.

Watson, R., Polkinghorne, R., & Thompson, J. M. (2008). Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture, 48*(11), 1368-1379.

ACCEPTED MANUSCRIPT

Figure 1. Effect of flight speed on muscle lactate concentration in the *m. semimembranosus* and *m. semitendinosus* muscle approximately 10 minutes post slaughter. Line represents least square means while dashed lines denote standard error of least square means.

Figure 2. Effect of flight speed on plasma lactate concentration at exsanguination. Line represents least square means while dashed lines denote standard error of least square means. Muscles with different letters are different (P<0.001)

Figure 3. Effect of the *m. longissimus thoracis* (LT), *m. semimembranosus* (SM) and *m. semitendinosus* (ST) on muscle glycogen concentration. Bars denote standard error. Muscles with different letters are different (P<0.001)

Figure 4. The effect of Lot number on total muscle glycogen concentration. Lot numbers A1 to A8 are from site A and B1 is from site B. Bars denote standard error. Lots with different letters are different (P<0.05)

Figure 5. Effect of slaughter group on total muscle glycogen concentration. H1 to 10 represents slaughter groups at processor "H", whereas W1 to W3 represents slaughter groups at processor "W". Slaughter groups with different letters are different (P<0.05).

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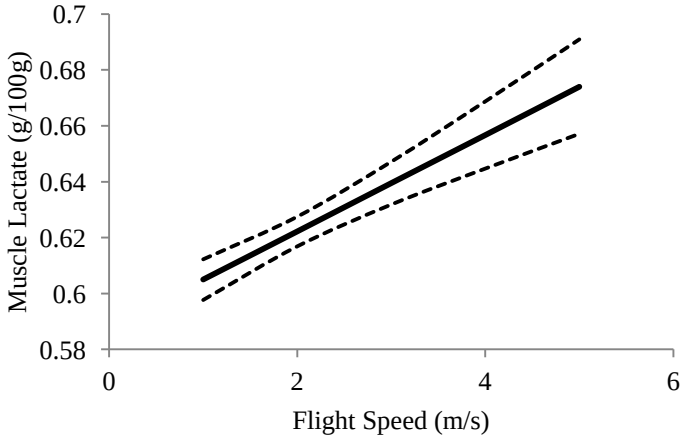
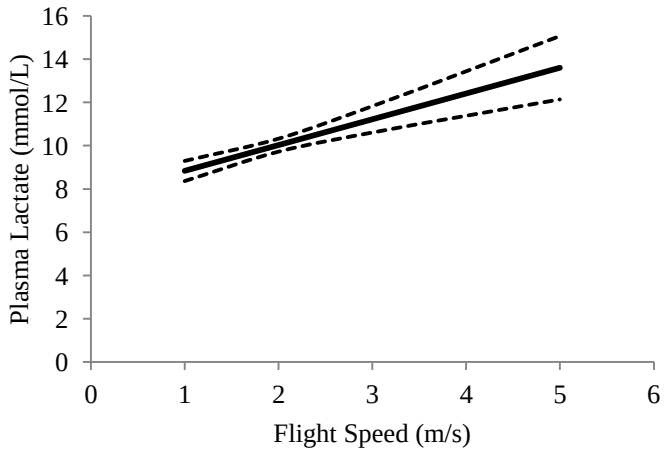


Figure 1



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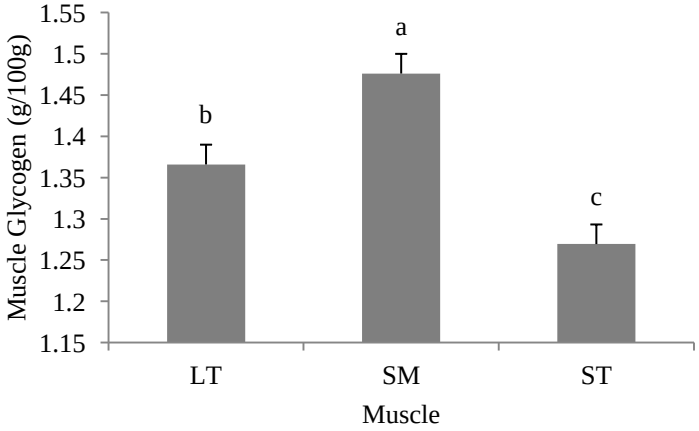

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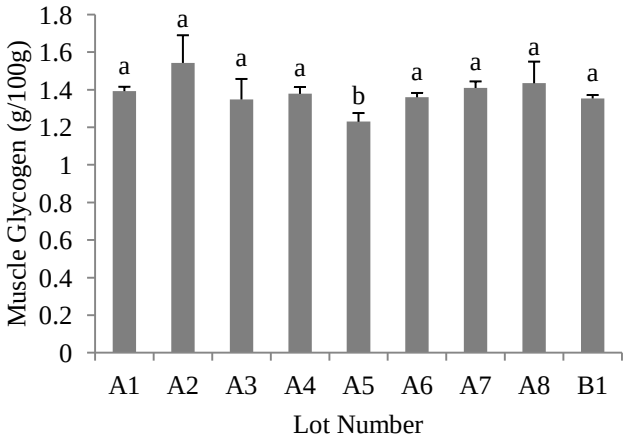

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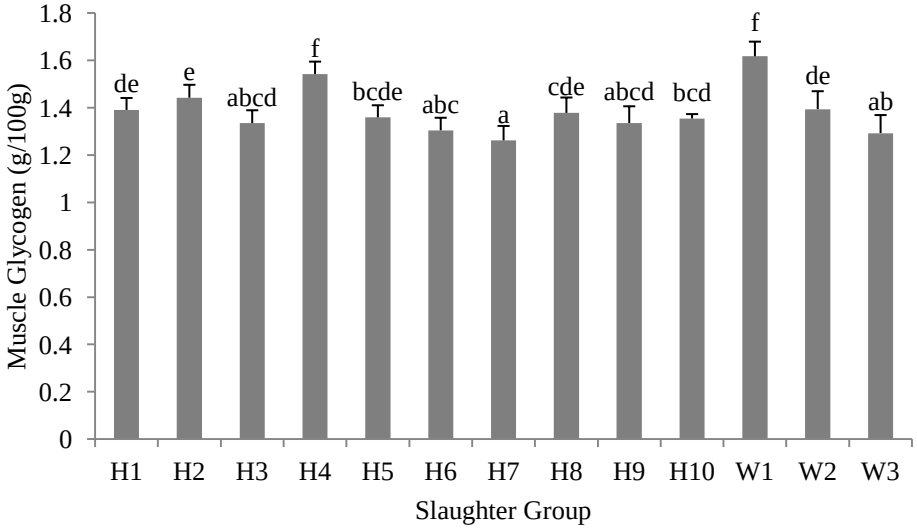

Figure 5

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Table 1. Descriptive statistics for number of samples (n), mean, standard error (Std Err), maximum and minimum values of hot standard carcass weight (HSCW) for cattle at Feedlots A and B, hip height (cm) of cattle at Feedlots A and B, total muscle glycogen concentration in the *m. semimembranosus* (SM), *m. semitendinosus* (ST) and *m. longissimus thoracis* (LT), muscle lactate concentration in the SM and ST, flight speed (FS), plasma lactate concentration and ultimate pH of the LT of cattle from Feedlots A and B.

Table 2. F values for the effects of feedlot, origin within feedlot, breed within origin by feedlot, lot within feedlot, slaughter group within feedlot, muscle, muscle by slaughter group within feedlot, flight speed (FS) and FS by muscle on muscle glycogen and lactate concentrations, plasma lactate concentration and ultimate pH from general linear models

Table 1

	n	Mean	Std Err	Minimum	Maximum
HSCW Feedlot A (kg)	544	239.5	0.8	202.7	308.5
HSCW Feedlot B (kg)	101	234	2.13	184.6	299.9
Hip Height Feedlot A (cm)	544	127.4	0.23	115	140
Hip Height Feedlot B (cm)	101	123.8	0.42	113	137
LTL Glycogen (g/100g)	599	1.36	0.27	0.39	2.18
SM Glycogen (g/100g)	611	1.46	0.3	0.64	2.48
ST Glycogen (g/100g)	611	1.25	0.26	0.48	2.19
SM Lactate (g/ 100g)	611	0.59	0.18	0.26	1.17
ST Lactate (g/ 100g)	611	0.63	0.16	0.21	1.49
FS (m/s)	612	1.98	0.81	0.3	4.69
Plasma Lactate (mmol/ L)	275	9.79	4.63	2.91	24.67
Ultimate pH Feedlot A	476	5.58	0.003	5.49	5.83
Ultimate pH Feedlot B	101	5.55	0.003	5.5	5.66

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Table 2

Effect	Glycogen Model		Muscle Lactate		Plasma Lactate		Ultimate pH	
	NDF, DDF	F Value	NDF, DDF	F Value	NDF, DDF	F Value	NDF, DDF	F Value
Feedlot							1, 519	14.33***
Origin(Feedlot)							9, 519	2.94**
Breed (Origin*Feedlot)							14, 519	2.3**
Lot(feedlot)	7, 1145	2.14*						
Slaughter Group(feedlot)	11, 1145	8.66***	12, 574	79.42***	4, 267	3.93**	8, 519	4.9***
Muscle	2, 1145	28.64***	1, 574	35.45***				
Muscle*Slaughter Group (feedlot)	24, 1145	2.17***	12, 574	6.7***				
FS	1, 1145	0.03	1, 574	10.81**	1, 267	6.99**	1, 519	0.06
FS*Muscle	2, 1145	2.52						

NDF, DDF – Numerator and Denominator degrees freedom *P<0.05, **P<0.01,***P<0.001

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