# final report

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# MSA Long Distance Transport Research

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## Abstract

Proposed indicators of pre-slaughter stress due to time from dispatch to slaughter and transport distance were measured in 294 yearling cattle and 130 export cows harvested in 5 and 4 separate lots respectively. Plasma lactate, urine specific gravity (Usg), D-3-hydroxybutyrate (BHB), carcass weight, meat colour and pHu measurements were taken on all animals with muscle glycogen, muscle lactate and all MSA measurements also recorded in all yearlings. There was a strong effect of kill group on plasma lactate, BHB and Usg but none of these measures had a strong linear relationship (positive or negative) with distance travelled or time from despatch to slaughter. The variation between and across killgroups was also large for all parameters. This indicates that blood lactate or BHB and Usg can't be accurately used as a predictor of long term stress or time in transit. Increased "flightiness" in the yearling cattle was associated with increased muscle and plasma lactate concentration, but more glycogen in the *longissimus thoracis et lumborum*. It is concluded that cattle with increased flight speed have a stronger acute stress response immediately preslaughter but that this response is so short term that it does not affect the final ultimate pH at grading.

## Executive Summary

The aim of this project was to:

- To bench mark stress measures for acute stress, feed deprivation and dehydration in slaughter cattle.
- Relate the stress measures to extended transport. Additionally link this project to an Animal Science honours project which investigated the relationship between acute measures of stress and cattle temperament and MSA grading.

Proposed indicators of pre-slaughter stress due to transport distance were measured in 294 yearling cattle and 130 export cows harvested in 5 and 4 separate lots respectively. Plasma lactate, urine specific gravity (Usg), D-3-hydroxybutyrate (BHB), carcass weight, meat colour and pHu measurements were taken on all animals at the abattoir with muscle glycogen and muscle lactate also measured in all yearlings which were also MSA graded. There was a strong effect of kill group on plasma lactate, BHB and Usg but none of these measures had a strong linear relationship (positive or negative) with distance travelled or time from despatch to slaughter. This indicates that blood lactate or BHB and Usg can't be accurately used as a predictor of long term stress or time in transit.

Ultimate pH and meat colour had the strongest association between transport distance, however the project design was confounded since this relationship was driven by the older cows versus the yearling cattle contrasts and these age groups were not balanced for feed type and transport distance. Regardless of the validity of this result the ultimate pH and meat colour outcomes are already captured by MSA grading and so any potential effects are accounted for within the current MSA grading system.

Increased "flightiness" in the yearling cattle was associated with increased muscle and plasma lactate concentration, but more glycogen in the *longissimus thoracis et lumborum*. It is concluded that cattle with increased flight speed have a stronger acute stress response immediately

preslaughter but that this response is so short term that it does not affect the final ultimate pH at grading.

## Contents

# 1 Project Objectives

- To bench mark stress measures for acute stress, feed deprivation and dehydration in slaughter cattle.

- Relate the stress measures to extended transport. Additionally link this project to an Animal Science honours project which investigated the relationship between acute measures of stress and cattle temperament and MSA grading.

# 2 Background

Stress is a variable concept and is known to influence eating quality of beef. In this project 3 aspects of stress have been measured:

(i) Plasma lactate immediately post stunning – this measure represents the mobilisation of muscle glycogen as lactate and subsequent release into the blood. Blood lactate has a short half life (minutes) and so the level represents acute stress close to the time of sampling, in this case immediately before stunning. A modifying factor will be the level of glycogen in muscle at the time of slaughter with lower levels likely to lead to lower lactate release into the blood.

(ii) Plasma D-3-hydroxybutyrate (BHB) immediately post stunning – this is a measure of feed deprivation as the resulting release of non esterified long chain fatty acids are metabolised in part via BHB.

(iii) Urine specific gravity (Usg) measured on urine extracted from the bladder 30mins post slaughter – this is a measure of hydration status in the 48 hours leading up to slaughter.

# 3 Hypotheses

- Acute (plasma lactate) and chronic (plasma BHB, Usg) measures of stress will relate to the distance cattle travelled from consignment location to slaughter.

- Cattle with flighty temperaments will have higher plasma and muscle concentration of lactate (indicating a greater susceptibility to acute stress pre-slaughter).

- Cattle with flighty temperaments will have lower muscle glycogen concentration at the time of slaughter and a higher pHu at MSA grading.

# 4 Methods and materials

The cattle measured as part of the long distance transport study in WA included two principal subsets of data. The first group (kill groups 1 to 5) were 294 yearling cattle finished in feedlots at two locations. Feedlot 1 was a commercial feedlot at Tammin (3 hrs east of Perth) where the cattle were mostly fed for between 60 to 80 days and the other feedlot was at the DAFWA Vasse Research Station. All 294 mixed sex yearlings had flight speed measurements taken at weaning or induction, travelled either 2hrs (Vasse Research Station) or 5 hrs (Tammin) to the processor at Harvey, and all were MSA graded. The other 130 samples were collected from cows and bullocks which had spent between 6 hrs and 9 days in transit. The cattle which travelled extreme distances were from the Kimberley region in northern WA. During the 9 days transit, almost 6 days was spent in yards with access to water and roughage. The specifics of the cattle in each kill group including their origin, type, sex, distance travelled to abattoir and the time from property of origin to slaughter and slaughter date is shown in Table 1.

Plasma lactate and BHB, Usg, AUSmeat colour, ultimate pH ($pH_u$) and carcass weight were measured on all 424 animals. All yearling cattle had flight speed measurements taken at weaning (DAFWA research steers) or at induction (Tammin Feedlot), plus at slaughter muscle biopsies were taken for muscle lactate and glycogen analysis. All yearling cattle were MSA graded. Blood was collected after sticking within 5mins of stunning, placed on ice and then plasma collected after centrifugation. Plasma lactate and BHB were determined enzymatically using an Olympus AU 400 autoanalyser. Urine was extracted from the bladder on the slaughter floor 30 minutes post stunning and specific gravity measured using a hand held refractometer.

Muscle biopsies were collected from the yearling cattle on the slaughter floor from the *m. semimembranosis* (topside), *m. semitendinosis* (eye round), *m. longissimus thoracis et lumborum* (striploin) on the slaughter floor 15mins post slaughter.

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

Glycogen and lactate in the muscle samples was measured after the muscle was homogenised (250 mg muscle added to 30mM HCl in a ratio of 1 part muscle to 10 parts acid). Lactate analysis on the homogenate was done on an Olympus AU 400 autoanalyser using an enzymic method. Glycogen in the homogenate was hydrolysed to glucose using a double enzyme method and glucose was measured on the Olympus AU 400. The total glycogen was calculated by halving the lactate value and adding it to the glucose value and expressing it as grams of glycogen per 100g muscle (see appendix 1 for full description of assay methods).

Statistical analysis was undertaken using a linear mixed effects models (SAS, 2001) – see appendix 1 for more detail.

| Kill Group | Origin | Cattle Type | Sex | n | Distance Travelled (km) | Time dispatch to slaughter (hrs) | Slaughter Date |
|---|---|---|---|---|---|---|---|
| 1YGTam | Tammin | Feedlot Yearlings | Mixed | 49 | 320 | 20 | 9/05/2011 |
| 2YGTam | Tammin | Feedlot Yearlings | Mixed | 66 | 320 | 20 | 16/05/2011 |
| 3YGTam | Tammin | Feedlot Yearlings | Mixed | 34 | 320 | 20 | 30/05/2011 |
| 4YGTam | Tammin | Feedlot Yearlings | Mixed | 44 | 320 | 20 | 20/06/2011 |
| 5YGVas | Vasse | Feedlot Yearlings | Steers | 101 | 114 | 19 | 28/06/2011 |
| 6CMar | Marble Bar | Export Cows | Cows | 30 | 1629 | 96 | 23/06/2011 |
| 7CHals | Halls Creek | Export Cows | Cows | 30 | 2876 | 216 | 23/06/2011 |
| 8CAlb | Albany | Export Cows | Cows | 30 | 369 | 24 | 27/07/2011 |
| 9CMar | Marble Bar | Export Cows | Cows | 30 | 1629 | 96 | 27/07/2011 |
| 10SSDer | Derby | Export Steers | Bullocks | 10 | 2574 | 168 | 27/07/2011 |

**Table 1:** The specifics of the cattle in each kill group including their origin, type, sex, distance travelled to abattoir and the time from property of origin to slaughter and slaughter date

# 5 Results and Discussion

*Urine Specific Gravity*

Kill group had the largest significant effect on Usg but there was no effect of distance travelled or time in lairage on Usg. There was a large variation in the percentage of cattle with urine in their bladders (Table 2), but there was no effect of sex, carcass weight or P8 fat on Usg.

Previous research at Murdoch University (McLennan, 2005) has shown that 48 hours of water withdrawal in yearling Bos tauris cattle results in a Usg of 1.032 (Table 3). Clearly in most consignment groups there were individual cattle at our above the 48 hours water withdrawal threshold for Usg but on average only 2 slaughter groups showed significant dehydration equivalent to 24-48 hours off water (slaughter groups 8CAlb and 9Cmar, Table 2). The reasons for these 2 slaughter groups having a higher than typical Usg are not clear from this study and further work would be required with a larger number of slaughter groups.

**Table2:** The mean, standard deviation, minimum and maximum measurements of urine specific gravity for each kill group and the percentage of cattle with a bladder containing urine.

| Kill Group | Mean | Std Dev | Minimum | Maximum | % with Urine |
|---|---|---|---|---|---|
| 1YGTam | 1.016 | 0.0124 | 1.002 | 1.04 | 87.8% |
| 2YGTam | 1.010 | 0.0090 | 1.003 | 1.037 | 98.5% |
| 3YGTam | 1.010 | 0.0065 | 1.003 | 1.027 | 82.4% |
| 4YGTam | 1.011 | 0.0094 | 1.003 | 1.033 | 77.3% |
| 5YGVas | 1.013 | 0.0079 | 1.004 | 1.039 | 97.0% |
| 6CMar | 1.011 | 0.0103 | 1.003 | 1.032 | 43.3% |

| | | | | | |
|---|---|---|---|---|---|
| 7CHals | 1.010 | 0.0078 | 1.002 | 1.025 | 33.3% |
| 8CAlb | 1.038 | 0.0077 | 1.013 | 1.048 | 100.0% |
| 9CMar | 1.025 | 0.0128 | 1.003 | 1.045 | 96.7% |
| 10SSDer | 1.007 | 0.0041 | 1.003 | 1.016 | 80.0% |

Table 3: Effect of water withdrawal pre-slaughter on urine specific gravity (McLennan, 2005)

| Water treatment pre-slaughter | Urine Specific Gravity |
|---|---|
| 0 hrs water withdrawal | 1.015 (±0.001) |
| 24 hrs water withdrawal | 1.022 (±0.003) |
| 36 hrs water withdrawal | 1.032 (±0.003) |

Usg did not correlate with BHB (Figure 1), plasma lactate, muscle glycogen or muscle lactate.

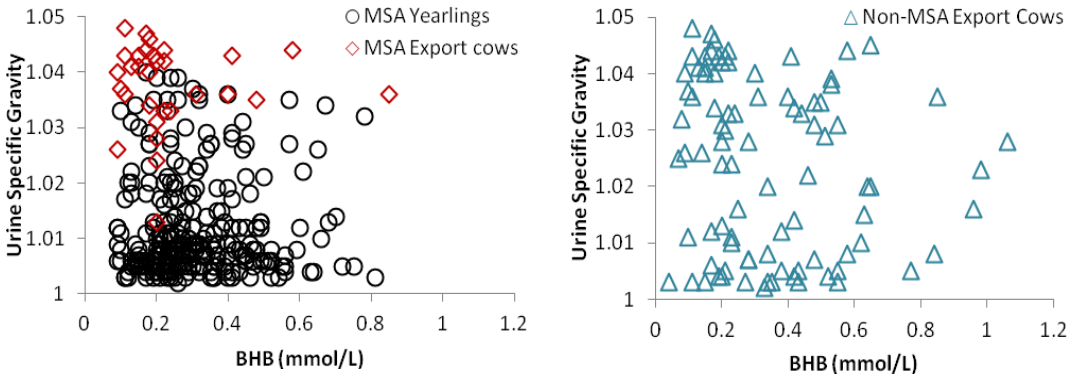


**Figure 1:** The relationship between urine specific gravity and D-3-hydroxybutyrate in MSA eligible and Non-MSA eligible cattle

*D-3-hydroxybutyrate*

Kill group had a large and significant effect on BHB (Table 4), but there was no consistent correlation between BHB and distance travelled or time from dispatch to slaughter. As P8 fat depth increased from 5 to 45mm, BHB concentration significantly increased by 50.2% from 0.27 to 0.40mmol/l, showing that fatter animals mobilised more fat and so produced more BHB. D-3-hydroxybutyrate was also positively correlated with $pH_u$. As BHB concentration increased from 0.1mmol/l to 0.9mmol/l, $pH_u$ increased from 5.59 to 5.67 indicating that as the animal mobilised more energy reserves there was a related reduction of muscle glycogen content at slaughter.

The causes of variation at the lower levels of BHB in plasma can be difficult to determine. This is because BHB is synthesised from butyrate produced during rumen fermentation and/or from non esterified long chain fatty acids mobilised as feed intake falls below maintenance. Thus the transition from full feeding to fasting can see plasma BHB levels fall and then rise again. The mean levels of plasma BHB attained in this study would suggest that on average all groups of cattle were at worst only under mild feed withdrawal stress. Even the highest maximum levels recorded (kill groups 9Cmar and 7CHals), indicate a normal and physiological response to feed withdrawal.

**Table 4:** The mean, standard deviation, minimum and maximum measurements of plasma D-3-hydroxybutyrate (mmol/L) for each kill group

| Kill Group | Mean | Std Dev | Minimum | Maximum |
|---|---|---|---|---|
| 1YGTam | 0.29 | 0.128 | 0.1 | 0.67 |
| 2YGTam | 0.38 | 0.154 | 0.11 | 0.81 |
| 3YGTam | 0.23 | 0.116 | 0.09 | 0.56 |
| 4YGTam | 0.24 | 0.130 | 0.08 | 0.56 |
| 5YGVas | 0.31 | 0.128 | 0.09 | 0.78 |
| 6CMar | 0.22 | 0.129 | 0.04 | 0.55 |
| 7CHals | 0.45 | 0.220 | 0.07 | 0.93 |
| 8CAlb | 0.23 | 0.164 | 0.09 | 0.85 |
| 9CMar | 0.46 | 0.244 | 0.11 | 1.06 |
| 10SSDer | 0.33 | 0.131 | 0.17 | 0.58 |

*Plasma Lactate*

Kill group explained the largest proportion of the variance in plasma lactate across all animal types (Table 5). Variation within a kill group was also large. Plasma lactate did not correlate with distance travelled or time from dispatch to slaughter. There was a significant relationship between plasma lactate and ultimate $pH_u$ showing that as plasma lactate increased from 5 to 25mmol/l, $pH_u$ decreased from 5.62 to 5.57. This is consistent with the level of glycogen in muscle being a significant determinant of subsequent lactate release associated with the acute stressor around stunning. However this connection is not supported at least in the yearling cattle (Figure 2). There was also a significant curve-linear negative correlation between BHB and plasma lactate, which began to plateau when BHB increased above 0.7mmol/l. As BHB increased from 0.1 to 0.7mmol/l, plasma lactate decreased by 73% from 15.86 to 4.31mmol/l. This is possibly due to a feedback inhibition of glycolysis not allowing for lactate production at high levels of BHB.

The levels of plasma lactate found in this study are relatively high especially when compared to the level in a resting undisturbed animal where you would expect less than 1mM. Similar levels have been found in a related study (Gruber at al. 2010) and so it appears that there is considerable acute stress pre stunning. Gruber et al. 2010 concluded this would impact on mechanical tenderness (shear force) however further work is needed to confirm this finding.

**Table 5:** The mean, standard deviation, minimum and maximum measurements of plasma lactate (mmol/L) for each kill group

| Kill Group | Mean | Std Dev | Minimum | Maximum |
|---|---|---|---|---|
| 1YGTam | 10.20 | 4.92 | 2.91 | 24.67 |
| 2YGTam | 8.96 | 4.33 | 2.97 | 24.49 |
| 3YGTam | 13.79 | 6.01 | 3.82 | 25.84 |
| 4YGTam | 10.08 | 4.53 | 3.82 | 23.56 |
| 5YGVas | 9.51 | 5.03 | 3.13 | 37.96 |
| 6CMar | 15.44 | 6.85 | 5.27 | 30.17 |
| 7CHals | 8.76 | 4.96 | 2.19 | 19.6 |
| 8CAlb | 10.76 | 5.71 | 2.53 | 28.55 |
| 9CMar | 8.49 | 3.54 | 3.01 | 16.24 |
| 10SSDer | 8.72 | 3.92 | 2.78 | 17.26 |

In the yearling cattle, as flight speed increased from 1 to 5 m/s, plasma lactate also increased by 44.8% from 9.67 to 14 mmol/l (see appendix). There was no effect of sex, HGP status, dentition, carcass weight or P8 fat depth on plasma lactate. Overall the average lactate concentration of all groups was high, but also had massive ranges in concentrations.

Meat colour and $pH_u$ measured on all cattle was possibly the best indicator of long term stress of the cattle and/or the difference in age of the yearling and export cows. In all the yearling cattle, variation in plasma lactate was as larger or larger than the export cows but the highest rate of dark cutting in the yearling was 4.55% as opposed to 66.67% in the export cows (Table 6). Thus it appears that $pH_u$ and meat colour are the best indicators of length of time from dispatch to slaughter or distance travelled, however a more balanced design is needed to test this assertion with more rigour.

**Table 6:** The mean, standard deviation, minimum and maximum measurements of ultimate pH for each kill group, the percentage of cattle with an ultimate pH≥5.7 and the percentage with a Meat colour higher than 3 as graded by an MSA grader

| Kill Group | Mean | Std Dev | Minimum | Maximum | % pHu≥5.7 | % MC>3 |
|---|---|---|---|---|---|---|
| 1YGTam | 5.57 | 0.063 | 5.5 | 5.79 | 4.08% | 4.08% |
| 2YGTam | 5.56 | 0.054 | 5.5 | 5.8 | 1.52% | 1.52% |
| 3YGTam | 5.59 | 0.046 | 5.51 | 5.69 | 0.00% | 0.00% |
| 4YGTam | 5.60 | 0.058 | 5.52 | 5.79 | 4.55% | 4.55% |
| 5YGVas | 5.55 | 0.034 | 5.5 | 5.66 | 0.00% | 0.00% |
| 6CMar | 5.68 | 0.141 | 5.46 | 5.94 | 30.00% | 33.33% |
| 7CHals | 5.72 | 0.128 | 5.48 | 5.92 | 40.00% | 66.67% |
| 8CAlb | 5.71 | 0.140 | 5.49 | 5.97 | 36.67% | 46.67% |
| 9CMar | 5.70 | 0.121 | 5.51 | 5.98 | 36.67% | 43.33% |
| 10SSDer | 5.62 | 0.115 | 5.48 | 5.91 | 10.00% | 10.00% |

*Muscle glycogen*

Muscle glycogen concentration was measured only in the yearling cattle and there was no difference in average muscle glycogen in the striploin between kill groups (Table 7).

**Table 7:** The mean, standard deviation, minimum and maximum measurements for muscle glycogen concentration in the *longissimus thoracis et lumborum* (g/100g) for each kill group

| Kill Group | Mean | Std Dev | Minimum | Maximum |
|---|---|---|---|---|
| 1YGTam | 1.34 | 0.288 | 0.97 | 2.16 |
| 2YGTam | 1.27 | 0.285 | 0.51 | 2.10 |

| | | | | |
|---|---|---|---|---|
| 3YGTam | 1.35 | 0.210 | 0.98 | 1.81 |
| 4YGTam | 1.36 | 0.226 | 0.96 | 1.88 |
| 5YGVas | 1.37 | 0.232 | 0.61 | 1.90 |

In the yearling cattle, as flight speed increased from 1 to 5 m/s, muscle glycogen and lactate concentration significantly increased (see appendix 1). There was no effect of sex, HGP status, dentition, carcass weight or P8 fat depth on either parameter. The responses of the different metabolites to flight speed require further interpretation. However it is clear that more flighty cattle have a stronger acute stress response (more plasma and muscle lactate formed) but this does not translate to a lowered muscle glycogen content of elevated pHu. Clearly most of the lactate mobilised from muscle glycogen in the acute period pre-slaughter in fact stayed within the muscle cell (the pool of plasma lactate is <1% of total whole body lactate) and so contributed to decreasing the pHu to acceptable levels. In other words more flighty cattle did have a more acute stress response but this was not translated into a chronic response sufficient to deplete muscle glycogen concentration.

There was also no correlation between muscle glycogen and BHB or plasma lactate (Figure 2).

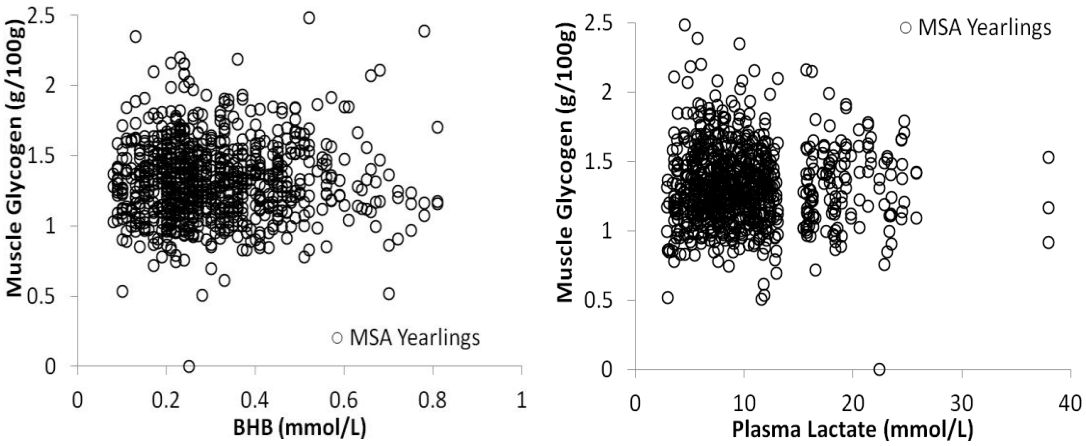


**Figure 2:** The effect of muscle glycogen concentration on plasma concentration of D-3-hydroxybuturate and lactate in yearling MSA eligible cattle

# 6 References

Gruber SL, Tatum JD, Engle TE, Chapman PL, Belk KE and Smith GC (2010) Relationships of behavioural and physiological symptoms to preslaughter stress to beef longissimus muscle tenderness. Journal of Animal Science 88,1148-1159.

McLennan L (2005) Pre-slaughter hydration of beef cattle. Honours thesis, Murdoch University, WA (available on request from David Pethick, d.pethick@murdoch.edu.au).

# 7 Recommendations

The results of this study showed that plasma lactate and BHB concentration, and Usg had large variations across a kill group, type of animal and were mostly affected by day of slaughter.

It appears these measures are not good indicators of time from dispatch to slaughter or distance travelled. Meat colour and $pH_u$ were the best indicators of time from dispatch to slaughter but this result is highly confounded by animal age and feed types. Regardless, ultimate pH and meat colour are already measured as part of standard MSA grading procedures so they already capture those cattle which don't comply to protocols. Thus carcass side measurements of BHB, Usg or plasma lactate will not provide any further accurate information about the origin or time spent in lairage by the cattle.

The levels of plasma lactate are very high in cattle at stunning and previous work (Gruber at al. 2010) suggests a relationship to mechanical tenderness (shear force). It is recommended that plasma lactate is measured in future eating quality studies to see if there is a relationship to the consumer eating quality outcome.

Further work on the relationships between Usg, feed type and production systems and carcase yield should also be considered.

# 8 Summary

Proposed indicators of pre-slaughter stress due to transport distance were measured in 294 yearling cattle and 130 export cows harvested in 5 and 4 separate lots respectively. Plasma lactate, urine specific gravity (Usg), D-3-hydroxybutyrate (BHB), carcass weight, meat colour and pHu measurements were taken on all animals with muscle glycogen and muscle lactate also measured in all yearlings. Yearlings were also MSA graded. There was a strong effect of kill group on plasma lactate, BHB and Usg but none of these measures had a strong linear relationship (positive or negative) with distance travelled or time from despatch to slaughter. This indicates that blood lactate or BHB and Usg can't be accurately used as a predictor of long term stress or time in transit. Increased "flightiness" in the yearling cattle was associated with increased muscle and plasma lactate concentration, but more glycogen in the *longissimus thoracis et lumborum*. It is concluded that cattle with increased flight speed have a stronger acute stress response immediately preslaughter but that this response is so short term that it does not affect the final ultimate pH at grading.

# 9 Appendices

Stephanie Coombes (2011) Cattle with flighty temperaments have increased muscle glycogen in the *longissimus thoracis et lumborum* at slaughter compared to cattle with calm temperaments. Honours thesis, Murdoch University, WA.