

final report

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Long distance transport trial

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Executive Summary

The purpose of this project was to investigate whether specific blood and/or urine measures could be applied to assess the eligibility of cattle for MSA grading when the time from despatch (ie farm to slaughter) exceeded the current threshold of 36 h.

A total of 460 cattle were sampled at one abattoir over 6 days of slaughter. The cattle were selected based on their marketing background (directly consigned n=236; saleyard n=224), distance they were transported and/or the time between farm and slaughter. The sample therefore represented a wide range of different pre-slaughter pathways. Supplemental samples of a further 20 head travelling a more extreme distance by road and 30 head subject to extended distance and time by rail were also collected at two further abattoirs. Blood and urine samples were collected at the time of slaughter. The blood was analysed for beta hydroxybutyrate and lactate concentrations and the urine analysed for specific gravity and osmolality. The relationship between the various measures and the time from farm to slaughter after adjustment for the fixed effects of day of slaughter, age class and sex was determined for the 460 head.

The general conclusion was that measures of plasma BHB, an indicator of fasting, and urine specific gravity or osmolality, indicators of water deprivation, lacked the sensitivity required for this application when the time from farm to slaughter extends up to 50 h. It was recommended that rather than try and pursue measures that reflect the duration of exposure to pre-slaughter stress, further investigation be undertaken to quantify the effects on beef eating quality when the current time from despatch threshold is extended from 36 to 48 h. Extending beyond an upper limit of 48 h is not recommended.

Finally, significant variation in plasma lactate concentration at slaughter was observed. This variability in the acute pre-slaughter stress response may in fact be a cause for significant variation in beef eating quality. If the results are representative in other abattoirs, further investigation of strategies to optimise pre-slaughter handling and management of cattle is warranted. It is also recommended that further consideration be given to the assessment of the quality and variation in pre-slaughter handling and management in abattoirs slaughtering MSA cattle.

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1 Background

As the MSA program gains popularity, producers located greater distances from processing plants are looking to supply cattle for MSA grading. Currently, strict pre-slaughter protocols are imposed on cattle presented for MSA grading, as current knowledge does not allow reliable prediction of the eating quality of meat produced from animals for which certain criteria are not met. For example, time from despatch (maximum 36 h), direct consignment from the farm of origin, and mixing of mobs are strictly controlled. Consequently, saleyard cattle and those travelling for long periods are currently excluded from MSA grading. However, in view of the increasing producer interest in supplying MSA, the application of these protocols requires further investigation and consideration. Some of the issues that could be further explored include: (i) quantifying the effects of longer durations from farm to slaughter (ie > 36 h) or longer transport distances on beef eating quality; (ii) exploring whether pre-transport strategies could be applied on farm to minimise pre-slaughter losses in yield and eating quality; (iii) establishing abattoir management procedures that enhance the recovery of cattle following long distance transport and thus allow MSA grading and (iv) investigating whether there are blood or urine measures that could be used to determine acceptance/rejection within MSA. Clearly, identifying practices to identify animals suitable for MSA grading and to improve the eating quality of beef derived from cattle travelling longer distances will benefit the Australian industry by improving the MSA grading outcomes and increasing the number of cattle eligible to grade MSA. It is important to note that any new protocols must be based on sound scientific principles and maintain the integrity of the MSA system.

The purpose of this project was to identify blood and urine measures that could be applied to assess the eligibility of cattle sourced from greater distances under the MSA beef grading system. Blood and urine samples were collected from slaughter cattle sourced over a wide range of transport distances and/or different pre-slaughter pathways. They were analysed for parameters that *a priori* are indicative of the animals' response to pre-slaughter stressors.

2 Project Objectives

To investigate the utility of blood and urine measures in cattle as indicators of the stress associated with increased time and/or transport distance to slaughter.

3 Methodology

The study was carried out at the Northern Co-operative Meat Company, Casino NSW (Processor A) over the period April to June 2011 with supplementary collections at Caboolture (Processor B) and Dinmore (Processor C) abattoirs. All readings taken on animals was completed post slaughter.

3.1 Sample collection

Target mobs of cattle presented for slaughter were selected based on their reported background specifically; how they were marketed and the transport distance to the abattoir. The aim was to sample cattle from the following pre-slaughter groups:

- Saleyard cattle, travelling long distances (>12 transport)
- Saleyard cattle sourced locally (< 6 h transport)
- Direct consigned, travelling long distance (>12 transport)
- Direct consigned, sourced locally (< 6 h transport)

From each target mob, 10 cattle were randomly sampled. A free-flow blood sample was collected from the sticking wound into a heparinised sample tube (BD vacutainer, Plymouth, UK, ref 367885). The kill number and animal ID number were recorded, and the carcass tagged and followed through to evisceration, where a urine sample was taken by sterile puncture of the bladder wall, into a plain vacutainer tube (BD, Plymouth, UK, ref 367895), using a 20G 1" vacutainer needle (BD, Plymouth, UK, ref 360214).

Blood samples were immediately removed to the on-site laboratory area, where they were centrifuged at 3300 RPM for 10 minutes at 4°C using a Technospin R benchtop centrifuge (Sorvall Instruments). The plasma supernatant was then aspirated and transferred into a 5mL screw cap container, and immediately deep frozen at -20°C. Urine samples were similarly deep frozen. The frozen samples were subsequently transferred to CSIRO, Chiswick, Armidale, for analysis.

The following day, as many carcasses as possible were graded by an accredited MSA grader. This included the measurement of ultimate pH (pHu). pH and temperature decline data was also collected on selected subgroups. Grading data was not able to be collected from some carcasses due to abattoir logistics.

3.2 Sample analyses

Plasma samples were analysed for L-lactate and β-hydroxybutyrate using an biochemical autoanalyzer (AU400, Olympus, Japan). Urine samples were tested for Osmolality (Vapor Pressure Osmometer, model 5500; Wescor, Utah) and specific gravity (Portable Refractometer, DLC Australia 1702231).

3.3 Livestock information collection and analysis

Groups were selected for sampling on the basis of slaughter group planning information at the abattoir. Subsequent information relating to vendors, source and the transportation of the animals was gathered through contact with abattoir stock personnel, buyers, National Vendor Declarations (NVD) and telephone interviews with agents and the vendors. Attempts were made to trace all cattle back through the chain in order to estimate the time off feed, the approximate distance travelled and time of transport. Standardised estimations of distance and transport time were made using the 'directions' facility of Google Maps [™].

3.4 Statistical analysis

The data were analysed using ANOVA in SAS's GLM Procedure. While the desired sampling protocol was achieved in regard to distance an unexpected divergence was present between time and distance due to widespread holding of saleyard cattle for a range of reasons. Separate analyses were conducted for the directly consigned and saleyard groups due to the lack of time from farm to slaughter overlap and because of the variation in the management of the saleyard consignments (see Section 4.2). The models included the fixed effects of day of slaughter, age class (beef or vealer) and sex plus the covariate time from farm to slaughter.

4 Results and Discussion

4.1 Livestock and sample information

In total, 460 animals were sampled at the primary abattoir (Processor A) over 6 days of slaughter (Table 1) as groups meeting the desired distance criteria became available. The abattoir operated two slaughter chains, a veal chain handling animals up to approximately 200kg liveweight, and a beef chain handling larger animals. Attempts were made to categorise animals by sex for analysis, but sex was not recorded on the veal chain. It was possible to identify the sex in some of the consignments based on the information from the National Vendor Declaration (NVD). However, there was still a reasonable proportion of animals were sex could not be determined (denoted as N). The breakdown of sex class by marketing type for each chain is shown in Table 1.

	Slaughter Date								
	12-Apr	13-Apr	14-Apr	23-May	24-May	25-May	Total		
Direct	48	47	52	40	49		236		
Beef	30	10	52	40	20		152		
F	10	2	11		10		33		
М	20	8	41	40	10		119		
Veal	18	37			29		84		
F	11	13					24		
М	6						6		
Ν	1	24			29		54		
Saleyard	60	51	7		46	60	224		
Beef	20	31	7			30	88		
F	9	11				27	47		
М	11	20	7			3	41		

Table 1 Number of animals sampled within marketing method, class and sex

Veal	40	20			46	30	136
F	34	14			33	2	83
М	6				2		8
Ν		6			11	28	45
Total	108	98	59	40	95	60	460

Of the total 460 animals sampled, 460 blood samples and 439 urine samples were collected for analysis. Twenty-one animals had no urine present in the bladder and a further 23 yielded insufficient urine to determine osmolality. At carcase grading, 353 pHu measurements were made and unfortunately, no grading data could be obtained for the 60 animals slaughtered on the 25th May.

In addition, 20 animals were sampled at Processor B, and 30 at Processor C, on 2nd June 2011. The animals sampled at Processor B were grass-fed beef animals, 9 male and 11 female, sent directly from the farm of origin. Those sampled at Processor C were all male, direct from the farm of origin.

4.2 Livestock movements

The animal movements particularly for the saleyard cattle were far more complex than initially envisaged. The assumption at the outset of the project was that animals would arrive at the abattoir, and be held for a set period of time (typically overnight) prior to slaughter. This occurred for the majority of the directly consigned cattle. However, when arrival and slaughter times were investigated for the saleyard cattle, animals spent variable amounts of time at the abattoir or en-route prior to slaughter, from no holding period (ie slaughtered on the same day of arrival) to 10 days (Table 2). Furthermore, for the saleyard cattle, it was expected that animals would journey from the farm of origin to a single saleyard and from there to the abattoir. In reality, it appears that a number of animals may have gone through multiple saleyards, and there was evidence that the cattle within saleyard mobs presented for slaughter were not uniform in terms of the day of sale. Random sub sets of carcasses selected for sampling from single slaughter lots included cattle from up to 3 saleyards and 12 vendors. It appears that animals from a particular saleyard were held for a few days, and added to a subsequent mob passing through the same saleyard on a different day. Full analysis of the livestock movements lies outside the scope of this report but every animal was traced from the abattoir through the saleyard to original vendor property.

	Days held at the abattoir or en-route										-
	0	2	3	4	5	6	7	8	10	Total	
Direct	227	9								236	-

 Table 2 Days held at the abattoir prior to slaughter

Saleyard	24	11	11	10	16	84	61	5	2	224
Total	251	20	11	10	16	84	61	5	2	460

During the extended time at the abattoir, cattle were supplemented with hay in pastured paddocks but it was not possible to obtain the exact details of the supplementation regime (eg. feed type and amount offered, frequency of feeding etc.) The pasture availability in the holding paddocks was generally low.

As a result of the varied holding periods, particularly for the saleyard cattle, it was decided to use 'time from farm to slaughter' as the key variable in statistical analysis, rather than the planned 'distance travelled' groupings. Time from farm to slaughter was itself used as a continuous variable in the analysis, and a classification of 'time from farm to slaughter' (TFS) was applied, which grouped animals into TFS classes '<24h'; '24-36h'; '36-48h'; '48-96h'; and '>96h' (Table 3). There were 11 saleyard animals for which the time of departure from the property of origin was not able to be identified.

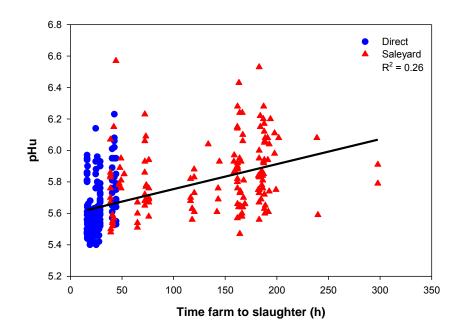
The animals sampled at Processor B travelled approximately 1100 km by road train and were held for 2 days prior to slaughter (70 hours farm to slaughter). Those sampled at Processor C travelled approximately 1600 km by road and rail, with an overnight stop at the rail head (73 hours farm to slaughter).

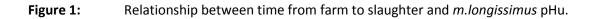
Time from farm to slaughter (h)							
	Unknown	<24	24-36	36-48	48-96	>96	Total
Direct		109	97	30			236
Saleyard	11			20	26	167	224
Total	11	109	97	50	26	167	460

 Table 3
 Numbers of direct consigned and saleyard cattle across the time groups for farm to slaughter

4.3 *M.longissimus* ultimate pH (pHu)

The positive association (P<0.001) between time from farm to slaughter and pHu is shown in Figure 1. Significant variation was observed in pHu within the two marketing methods and many carcasses failed the MSA criteria pHu>5.7 (21% Direct; 65% Saleyard).





When the data were analysed within marketing method, the association remained significant (P<0.05) for the direct consignment group only (Table 4). The slope of the relationship for the saleyard group was not significantly different from zero. The lack of association for the saleyard group was probably due to the fact that the cattle had access to feed (hay plus some pasture) whilst being held at the abattoir. This would have arrested some of the pre-slaughter glycogen loss and possibly enabled some muscle glycogen synthesis to occur.

pHu was significantly higher in vealers compared to the beef age class, in both marketing groups. Females had significantly higher pHu (P<0.001) in the directly consigned group.

Table 4 Least square means for age class and sex and slopes for time from farm to slaughter

<i>m.longissimus</i>	BHB	Lactate	Urine
pHu	(mMol/L)	(mMol/L)	Osmolality

			1	
Direct Consignment				
	0.0020	0.0016	0.0004	12.0
Slope (TFS)	0.0039	-0.0016	0.0034	13.6
	P<0.05	NS	NS	P<0.05
Age class	P<0.001	NS	NS	P=0.08
Vealer	5.89	0.35	8.93	536.2
Beef	5.53	0.28	8.14	720.3
Sex	P<0.001	NS	NS	NS
Μ	5.66	0.32	8.93	638.0
F	5.73	0.31	8.14	618.5
Saleyard				
Slope (TFS)	0.0001	0.0001	0.005	-2.14
	NS	NS	NS	P<0.001
Age class	P<0.01	NS	NS	NS
Vealer	5.89	0.43	8.46	489.0
Beef	5.69	0.44	8.34	563.1
Sex	NS	NS	NS	NS
Μ	5.81	0.45	7.81	513.1
F	5.77	0.42	8.99	539.0

TFS – Time from farm to slaughter (h)

4.4 Plasma beta hydroxybutyrate (BHB)

The measurement of plasma ketones such as BHB provides an indication of mobilisation of body fat reserves. During periods of reduced feed intake (ie. pre-slaughter) or energy imbalance, glucose levels decline resulting in lipolysis or mobilisation of body fats yielding an increase in circulating NEFA levels. The increase in NEFAs to the liver stimulates ketogenesis resulting in the production of ketone bodies. Ketones act as alternative substrates to glucose for the production of energy in tissues.

As expected, a positive association was found between time from farm to slaughter and plasma BHB concentrations (Figure 2). However, within each marketing group, the slope of the relationship was not significantly different from zero (Table 4). The effects of age class and sex were also not significant.

The majority of the cattle sampled had BHB concentrations within the normal expected range for nonlactating cattle (0.2 - 0.6 mMol/L) and there were only four cases that had levels which would be indicative of subclinical ketosis (1.0 - 1.2 mMol/L).

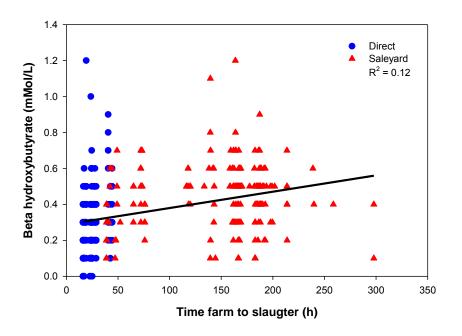


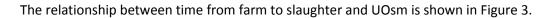
Figure 2: Relationship between time from farm to slaughter and serum BHB concentration.

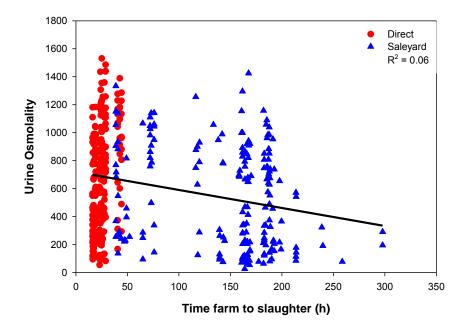
The results obtained tend to align with those from published feed deprivation and/or transport duration studies in beef cattle. It would appear that BHB is a useful indicator of fasting but it may lack the sensitivity required to differentiate between groups of animals exposed to different lengths of feed deprivation up to 48 h similar to that encountered during the normal pre-slaughter management of cattle. For example, in bulls subjected to periods of feed deprivation from 12 to 36 h following a 4 h transport event, BHB increased from 0.32 to 0.41 mMol/L however, the differences were not found to be significant (Schaefer et al 1990). Ferguson et al (2011) transported cattle that were not deprived of feed or water prior to transport for 6, 12, 30 and 48 h and found inconsistent differences in plasma BHB concentrations after transport.

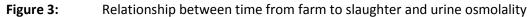
4.5 Urine osmolality (UOsm)

During the pre-slaughter phase, cattle are exposed to periods of water deprivation. Animals have the opportunity to rehydrate after arrival at the abattoir as they typically have access to water whilst in lairage. However, it is recognised that even with access to water, not all animals will drink. Limited access to and unfamiliarity with watering facilities will contribute to the variability in drinking behaviour in novel environments. Therefore assessments of hydration status at the point of slaughter may provide an indication of the duration of pre-slaughter water deprivation.

An indication of the hydration status of an animal can be determined via the measurement of specific gravity or osmolality of blood (ie. serum) or urine. Although the measurement principles differ, both measures essentially quantify the solute concentration of blood or urine which increases commensurate with duration of water deprivation. A high correlation exists between the measures as exemplified in the current study where the $R^2 = 0.9$ and the slope (b) = 0.999. Therefore, for the purposes of the discussion, only the results for UOsm are presented.







An inverse trend was observed which suggests that the saleyard cattle rehydrated during the holding period at the abattoir. However, significant variation was still observed within both marketing groups and in particular within the direct consigned group. There were some cattle in both groups with UOsm>1200 mOsmol/kg which would be indicative of mild to moderate dehydration (8% Direct; 2% Saleyard). The results for the directly consigned group support the evidence by Jacob et al (2006a) in lambs that not all animals rehydrate during their time in lairage. Furthermore, in another study Jacob et al (2006b) demonstrated that water deprivation up to 48 h had a significant effect on water holding characteristics in lamb muscle and therefore, we can speculate that this may influence eating quality.

In contrast to the overall trend in Figure 3 which is misleading, a positive significant association (P<0.05) was observed between time from farm to slaughter and UOsm for the directly consigned group (Table 4). Age class or sex did not significantly influence UOsm in both marketing groups (Table 4).

4.6 Plasma lactate

Plasma lactate concentration at slaughter provides an indication of exposure to stress in the immediate preslaughter period. Not surprisingly therefore, there was no correlation between lactate concentration at slaughter and time from farm to slaughter overall (Figure 4) or within marketing group (Table 2). Lactate concentration also did not differ due to age class or sex (Table 2).

The reason for examining plasma lactate variation in a sample of slaughter cattle was predicated on recent results from Gruber et al (2010) who showed a positive association between plasma lactate post-transport (arrival at the abattoir) and *m.longissimus* shear force in cattle. This association was independent of pHu and the effect was attributed to stress-induced retardation of post-mortem tenderisation.

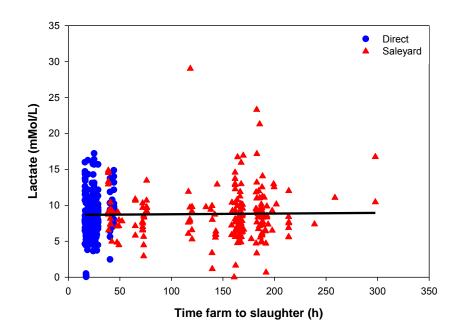


Figure 4: Relationship between time from farm to slaughter and plasma lactate concentration

The mean lactate concentration in their study was 11.97 mMol/L (range 5.71 – 20.3 mMol/L) which was higher than that observed in the directly consigned group in the current study (8.73 mMol/L; Range 0.50 – 17.22 mMol/L). By way of a contrast, in a controlled cattle study at a commercial abattoir by Warner et al (2007) where cattle were either subjected to 6-8 prods with an electric prodder 15 min prior to slaughter or not (ie. control), the plasma lactate concentrations at slaughter were 4.12 and 7.29 mMol/L for the control and electric prodder groups, respectively. Interestingly, the electric prodder treatment did not elicit as high a plasma lactate response as that observed in the current study or that conducted by Gruber et al (2010). Warner et al (2007) also demonstrated that shear force was not significantly influenced by pre-slaughter

treatment however, there were significant effects on water holding properties (eg. drip loss, cooking loss) and MSA consumer panel scores.

Clearly, these results suggest that exposure to stressors (eg. handling/human contact, activity, exposure to novel stimuli such as noise and odours etc.) during the immediate pre-slaughter period can have an influence on beef quality. Moreover, if the lactate results from the current study are representative of other abattoirs processing MSA cattle, then some consideration should be given to exploring avenues for reducing the variation in pre-slaughter stress given the putative benefits to beef eating quality. Further investigation of how this might be captured within the MSA grading protocols is also warranted.

5 Success in Achieving Objectives

The project objectives were successfully achieved.

6 Impact on Meat and Livestock Industry – now & in five years time

The outcomes of this project will require consideration by the MSA Pathways Committee and their recommendations will ultimately determine what changes are made to the MSA Beef Grading System in the short and medium term.

7 Conclusions and Recommendations

The purpose of this project was to investigate whether specific blood and/or urine measures could be applied to assess the eligibility of cattle for MSA grading when the time from despatch (ie farm to slaughter) exceeded the current threshold of 36 h. The general conclusion was that measures of plasma BHB, an indicator of fasting, and urine specific gravity or osmolality, indicators of water deprivation, lacked the sensitivity required for this application when the time from farm to slaughter extends up to 50 h. Beyond this threshold, measures like BHB may be more useful. However, the value of this is questionable as it could be argued that if the time between farm and slaughter exceeds 50 h, beef quality and potentially animal welfare has already been compromised.

Whilst the total duration of the pre-slaughter phase is important, the exposure and duration of exposure to the individual pre-slaughter stressors (eg. transport, handling events, duration of feed and/or water deprivation etc.) during this phase is probably more critical. Furthermore, because, pre-slaughter stress is multifactorial, it is very difficult to quantify the effects based on single physiological response measures.

As a general rule, cattle destined for slaughter are not transported onerous distances (typically less than 12 h). It is also known, that after an initial period (2-3 h), cattle generally habituate to the transport conditions (Warriss et al 1995; Pettiford et al 2008). Transport over longer durations will result in animal fatigue but this in itself is very difficult to quantify. In addition to assessments of lying behaviour, creatine kinase (CK) concentration been used as an indicator of fatigue during transport studies (Knowles and Warriss 2000) but the relationship between CK concentration and transport duration is not always consistent (Knowles et al 2000; Ferguson et al 2011). Ferguson et al (2011) concluded that CK was perhaps more indicative of individual animal events (e.g. muscle trauma or bruising), rather than increasing muscle fatigue associated with transport duration. The duration of food and/or water deprivation which is natural consequence of transport is probably one of the more critical pre-slaughter stressors. In a MLA commissioned review (Pethick et al 2009) on the effects of food and water deprivation it was recommended that the maximum time off feed prior should be 48 h to comply with animal welfare, food safety and meat quality standards or recommendations.

Returning to the central issue of how to accommodate cattle within MSA that exceed the current limit of 36 h from despatch, we would conclude that it is unlikely that the duration of pre-slaughter stress exposure (within a limit) can be reliably quantified using blood or urine measures. Rather we would recommend that further investigation be undertaken to quantify the effects on beef eating quality when the current time from

despatch threshold is extended from 36 to 48 h. Extending beyond an upper limit of 48 h is not recommended based on the conclusions of Pethick et al (2009).

Another salient outcome of the project was the significant variation in plasma lactate concentration at slaughter. Although the evidence is limited, the variation in the sympatho-adrenal response to stressors in the immediate pre-slaughter period may be a cause for significant variation in beef eating quality (Warner et al 2007; Gruber et al 2010). If the results are representative in other abattoirs, further investigation of strategies to optimise pre-slaughter handling and management of cattle is warranted. It is also recommended that further consideration be given to the assessment of the quality and variation in pre-slaughter handling and management in abattoirs slaughtering MSA cattle.

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V.EQT.1110 - Long distance transport trial

9 Appendix – data summary

Mean values ± standard deviation (range in parenthesis)

	<i>m.longissimus</i> pHu	BHB (mMol/L)	Lactate (mMol/L)	Urine Osmolality (mOsm/kg)	Urine pH
Processor A					
Direct Consignment					
Vealer	5.76 ± 0.18	0.36 ± 0.18	9.41 ± 2.62	756.41 ± 317.91	7.18 ± 0.91
	(5.44 – 6.23)	(0.1 – 1.2)	(2.47 – 14.87)	(82 – 1389)	(5.68 – 8.63)
Beef	5.55 ± 0.08	0.26 ± 0.15	8.27 ± 3.18	643.74 ± 402.59	7.94 ± 0.71
	(5.4 – 5.96)	(0-1)	(0.05 – 17.22)	(54 – 1531)	(6.24 – 8.83)
Processor A					
Saleyard					
Vealer	5.88 ± 0.21	0.43 ± 0.18	9.09 ± 3.34	546.10 ± 361.60	6.86 ± 0.89
	(5.56 – 6.53)	(0.1 – 0.9)	(0.67 – 23.3)	(55 – 1425)	(5.3 – 8.67)
Beef	5.74 ± 0.23	0.46 ± 0.19	8.14 ± 3.56	505.19 ± 360.90	7.00 ± 0.77
	(5.47 – 6.59)	(0.1 – 1.2)	(0.01 – 29.01)	(26 – 1256)	(5.7 – 8.67)
Processor B					
Direct Consignment					
Beef	Not measured	0.135 ± 0.12	Not measured	420.68 ± 292.79	7.41 ±0.74
		(0 – 0.5)		(46 – 1015)	(6.18 – 8.38)
Processor C					
Direct Consignment					
Beef	Not measured	0.22 ± 0.11	Not measured	561.68 ± 261.23	7.71 ± 0.58

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(0-1.1) $(98-1282)$ $(6.6-8.56)$		(0-1.1)	(98 – 1282)	(0.0 - 8.50)
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V.EQT.1110 - Long distance transport trial