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Stockman, C.A., McGilchrist, P., Collins, T., Barnes, A.L., Miller, D., Wickham, S.L., Greenwood, P.L., Cafe, L.M., Blache, D., Wemelsfelder, F. and Fleming, P.A. (2012) Qualitative Behavioural Assessment of Angus steers during pre-slaughter handling and relationship with temperament and physiological responses. Applied Animal Behaviour Science, 142 (3-4). pp. 125-133.

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Qualitative Behavioural Assessment of Angus steers during pre-slaughter handling and relationship with temperament and physiological responses

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Abstract

This study examined the behavioural expression of cattle immediately prior to slaughter through the process of Qualitative Behavioural Assessment (QBA), and compared these results to measurements of physiology and temperament. Twenty-eight Angus steers were

filmed while in a funnel chute as they were being moved towards an abattoir killing box. Footage of cattle was shown in random order to 15 observers. Observers assessed the cattle using a qualitative approach based on Free Choice Profiling (FCP) methodology, which gives observers complete freedom to choose their own descriptive terms. Data were analysed with Generalised Procrustes Analysis (GPA). There was significant consensus ($P < 0.001$) amongst observers in terms of their assessment of the behavioural expression of the cattle. Two main dimensions were identified, explaining 58.8% and 9.2% of the variation between animals, respectively. Low values for GPA dimension 1 were associated with terms such as 'calm' and 'relaxed' and high values associated with terms such as 'nervous' and 'anxious'. Low values for GPA dimension 2 were associated with terms such as 'annoyed' and 'frightened' and high values associated with terms such as 'curious' and 'interested'. We found no significant correlations between the QBA scores and three temperament measures (taken at weaning). However, cattle slaughtered towards the end of the line ($r_s = 0.45$, $P = 0.016$) and cattle with a greater plasma lactate concentration (measured at exsanguinations) ($R_{24} = 0.45$, $P = 0.020$) were attributed higher GPA dimension 1 scores (i.e. more 'nervous'/'anxious'). The only animal with an ultimate muscle pH > 5.7 (classified as a 'dark cutter') had a low value for GPA dimension 2 (scored as relatively more 'annoyed'/'frightened'). The findings from this study suggest that QBA could contribute to assessing pre-slaughter animal handling, highlighting potential issues to be followed up with additional measures. The significant correlations between GPA dimension 1 with slaughter order and plasma lactate warrant further investigation, comparing behavioural expression with aspects of meat quality.

Keywords: Cattle; Crush agitation score; Free choice profiling; Flight speed; Generalised Procrustes Analysis; Lairage; Tension score; Temperament testing

Introduction

Livestock are exposed to a range of challenging stimuli during the slaughter process, including herding, movement from extensive systems to confined conditions, handling and transport. In addition lairage (holding livestock in pens or stockyards prior to slaughter) involves increased human contact, exposure to novel/unfamiliar environments, food and water deprivation, changes in social structure (i.e. through separation and mixing), and changes in climatic conditions (Ferguson and Warner, 2008). These challenges can significantly alter the animals' physiology and have detrimental effects on meat quality (Ferguson and Warner, 2008).

An autonomic response is initiated in reaction to acute stressors that require a rapid response, typical of the slaughter process (Ferguson and Warner, 2008 and Tarrant, 1989). This response can lead to low muscle glycogen at the time of slaughter, resulting in elevation in the ultimate pH of muscles. How an animal responds to the acute stressors around slaughter will therefore have significant consequences for meat quality. Muscle is required to reach a $\text{pH} < 5.70$ otherwise the carcass will be graded as a 'dark cutter' (Kenny and Tarrant, 1987 and McVeigh and Tarrant, 1981), a classification attributed to around 8% of Australian carcasses graded by Meat Standards Australia in 2007. In addition to high ultimate pH, dark cutting is characterised by reduced shelf life (Gill and Newton, 1981), undesirable meat colour (Lawrie, 1958) and flavour (Dransfeld, 1981).

Previous studies have found behavioural responses to the processes immediately prior to slaughter are linked to the animal's physiological responses and therefore to meat quality

attributes. Several studies have shown that mixing unfamiliar cattle together during the slaughter process results in fighting, mounting and other physical activity that increases the incidence of dark cutting (Grandin, 1980, Kenny and Tarrant, 1987 and Tennessen and Price, 1980). Immonen et al. (2000) assessed temperament during the slaughter process (0 = calm, 1 = slightly restless, 2 = berserk) and found that more temperamental bulls (i.e. higher score) had more lactate accumulation, less glycogen and less residual glycogen at the time of slaughter, and had recorded worse appetite and less weight gain prior to slaughter. The reduced glycogen availability suggested that under adverse conditions, ultimate muscle pH was likely to be affected (Immonen et al., 2000). Another study found a negative correlation between temperament test scores (flight speed and crush score) and feedlot growth rates, feed intake, time spent feeding, carcass weight and objective measures of meat quality (Cafe et al., 2011).

The potential link between cattle behaviour and physiological responses and therefore measures of production and meat quality (e.g. Müller and von Keyserlingk, 2006, Petherick et al., 2002 and Petherick et al., 2009) has been reflected in changes in management during the slaughter process, including design of livestock facilities and animal handling practices (Grandin, 2006). However, little has been done to develop practical behavioural measures to assess animal responses during handling in commercial slaughter plants. Such a measure would prove useful in pinpointing animal welfare issues at specific points in the slaughter process, and help to identify differences between slaughter plants or between particular cohorts of cattle.

Qualitative Behavioural Assessment (QBA) has previously been used to differentiate between cattle exposed to varying types of challenges (indicated by behavioural and physiological responses to social and physical stressors, Rousing and Wemelsfelder, 2006 and Stockman et al., 2011). During the QBA process, human observers integrate perceived details of behaviour and its context into their judgement of an animal's overall style of behaviour or 'behavioural expression', using terms such as 'nervous', 'bold', and 'calm' to describe animals. QBA studies show that observers can reach significant agreement in their assessment of behavioural expression in pigs (Temple et al., 2011a, Temple et al., 2011b, Wemelsfelder et al., 2001, Wemelsfelder et al., 2009 and Wemelsfelder et al., 2012), cattle (Brscic et al., 2009, Rousing and Wemelsfelder, 2006, Stockman et al., 2011 and Stockman et al., under review), buffalo (Napolitano et al., 2012), sheep (Wickham et al., 2012), horses (Minero et al., 2009 and Napolitano et al., 2008), poultry (Wemelsfelder, 2007) and dogs (Walker et al., 2010), suggesting that these assessments were based on commonly perceived and systematically applied criteria. The benefits of QBA are that it is a quick and non-invasive assessment that is relatively easy to implement, and provides a useful measurement of an animal's wellbeing, capturing how it reacts to its environment at a specific time point. The aims of this experiment were to:

1. determine observer agreement in their assessments of the behavioural expression of cattle exposed to pre-slaughter handling; and
2. determine if there is a correlation between behavioural expression and physiological and behavioural (e.g. temperament scoring) measures.

Materials and methods

Animals

Eighty-one Angus steers were followed from birth through to slaughter at 2 years of age (when they had a live weight of 523 ± 40 kg). The animals formed part of an experimental herd used under another experiment and had been measured for temperament as part of that study (McGilchrist, 2011). The herd comprised of lines which had been selected for high or low muscling since 1990 (McKiernan, 2001) at the Industry and Investment New South Wales (I&I NSW) Angus herd based at the Glen Innes Agricultural Research and Advisory Station, Glen Innes, NSW, Australia ($29^{\circ}44'S$, $151^{\circ}42'E$, altitude 1057 m). The steers were born during a 92-day period starting on 1st August 2007 and had an average birth weight of 39 ± 4 kg. On the 20th December 2008 they were moved to a second property located at Ebor, NSW, Australia ($30^{\circ}24'S/152^{\circ}21'E$, altitude 1350 m) where they grazed on pasture until slaughter.

All 81 cattle were transported for 3 h (approximately 270 km from Ebor), to a commercial abattoir (Casino, NSW, Australia) by a commercial transport company the day prior to slaughter and left in lairage overnight (approximately 12 h) in four adjacent outdoor pens. While in lairage, water was available ad libitum but feed was withdrawn. We were able to obtain adequate footage (for QBA) of a random selection of 28 individuals as they passed through an undercover funnel chute (Fig. 1) immediately prior to the abattoir killing box.

The experiment was approved by the I&I NSW Animal Care and Ethics Committee, Orange, NSW (Permit number ORA 08/016), jointly with the Animal Ethics Committee at Murdoch University, Perth, Western Australia (Permit number: R2272/09).

Temperament testing

Temperament was assessed using crush agitation scores, tension scores and flight speed. Temperament measurements were recorded when the cattle were being handled through yards for other management or data collection purposes (e.g. weighing, ultrasound scanning for body composition, vaccination and blood sampling) and were made by the same experienced observer (LMC). Temperament assessments were measured over a period of 3 weeks around weaning between 7 and 8 months of age. All the animals were passed through the testing process in approximately 1 h and this process was repeated three times over the 3 weeks; these three measurements were averaged for each individual.

Crush agitation was scored when cattle were confined without head restraint for a few seconds in a single weighing crate. This scale is based on the scoring system applied by Grandin (1993) to cattle restrained in a squeeze chute and head bail. Modifications were made to this scale so that it was more suitable for loosely restrained cattle as a hydraulic squeeze crush was not available. The assessment was made visually using a 5-point scale, where:

1 = calm, standing still, head mostly still or moving slowly;

2 = slightly restless, looking around more quickly, moving feet;

3 = restless, moving backwards and forwards, shaking crate;

4 = nervous, continuous vigorous movement backwards and forwards, snorting; and

5 = very nervous, continuous violent movement, attempting to jump out.

Tension score was assessed individually while the animal was in the weighing crate using a 4-point scale (Cafe et al., 2011), where:

1 = comfortable, body not tense, eyes blinking, little movement, may show curiosity;

2 = uncomfortable, some tension in body, head up, eyes not soft (i.e. staring, not blinking), may be moving or still;

3 = nervous, body tense, head high, eyes either staring or rapid blinking, may be moving or still; and

4 = afraid, body tense, may be moving or still, eyes staring, shaking.

When the cattle were released from the weighing crate, flight speed was recorded (Burrow et al., 1988). The yard design required the cattle to make a 90° right turn into a side yard upon release from the crate. Flight time was measured after the animals had made the turn and were travelling in a straight line for a distance of 1.7–2.2 m, and converted to flight speed (m/s) for analysis.

Physiology

Muscle samples were taken from the semimembranosus (SM) and semitendinosus (ST) of all cattle via biopsy (7 days prior to slaughter) and again immediately post-slaughter. The steers remained on the same type of pasture for the 7 days following muscle sample biopsy, prior to slaughter. Muscle glycogen concentration was determined for each muscle sample. Glycogen concentrations in each muscle sample were measured using the enzymatic method of Chan and Exton (1976), modified by removing the filter paper step. Net glycogen loss from biopsy to slaughter was calculated (McGilchrist, 2011). Following slaughter, carcasses were chilled for 20 h, at which time they were quartered at the 12th/13th rib, and ultimate muscle pH was measured as described by Perry et al. (2001).

A blood sample was taken from cattle at exsanguination. The blood tubes were stored on ice and within 20 min of collection from the last animal they were centrifuged for 15 min at $604 \times g$ and the plasma removed. The harvested plasma was frozen ($-80\text{ }^{\circ}\text{C}$) for later laboratory determination of plasma cortisol and vasopressin concentration as described by McGilchrist (2011). Of the 28 cattle analysed for QBA, 26 of these animals had sufficient quantities of blood taken for laboratory determination of plasma β -hydroxy butyrate, glucose, lactate, and nonesterified fatty acid (NEFA) concentrations. Laboratory analyses of plasma were carried out as a batch sample by enzymatic methods using the Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Melville, NY) and reagent kits for β -hydroxy butyrate (Randox Laboratories kit, Ranbut, Cat. No. RB1007, County Antrim, United Kingdom), glucose (Olympus Diagnostics, Tokyo, Japan, Cat. No. OSR6121), lactate (Olympus

Diagnostics, Tokyo, Japan, Cat. No. OSR6193) and NEFA (C Kit Wako Pure Chemical Ind., Osaka, Japan; modified for the Olympus AU400 Automated Chemistry Analyser)

Qualitative Behavioural Assessment

Video footage (15 frames/s) was recorded of the cattle in a forcing pen after they had been washed down and were being moved through a funnel chute towards the abattoir for slaughter (Fig. 1). The 81 cattle moved, as a group, through a series of pens of decreasing dimensions towards the abattoir. In the corridor leading up to the funnel chute, cattle are washed with mist sprayers and hand held high-pressure hoses which were also used to turn cattle around and encourage them to move forward. In the funnel chute (holding around 8–12 individuals at a time), cattle rearranged themselves and passed out of the chute in single file into a final race towards the killing box. Footage of animals was collected for the QBA sessions as they passed through this funnel chute. Suitable footage required that the focal individual could be identified from its ear tag numbers at a distance. Also, each animal was filmed for 1 min and the cattle moved through at a fast pace: the 81 cattle moved through the abattoir in less than 60 min and individuals where less than 1 min of footage was collected had to be discarded from analyses. Within these constraints, suitable footage was obtained for an effectively random-selection of 28 of the 81 cattle that moved through the chute. The slaughter order of all 81 animals was recorded.

Fifteen observers were recruited from University staff and students and members of the public by advertising via email and accepting all those that responded (n = 11 university students, n = 2 livestock industry professionals and n = 2 general public; two male and 13

female). Importantly, a recent study has demonstrated that despite vastly different backgrounds and experience, people can reach consensus in their assessments using QBA (Wemelsfelder et al., 2012). Observers were given detailed instructions on completing the sessions. It was necessary to explain to the observers that the cattle were moving through a holding facility since the footage could not be edited to remove evidence of such. Additionally, footage was collected immediately after the cattle had passed through a wash down; this was explained to the observers to ensure they would focus on the animal's behaviour rather than the reasons why the cattle were wet. The fact that this holding facility preceded slaughter was not mentioned. Each observer was required to complete a term generation session and a subsequent quantification session by correspondence. The two sessions are detailed below and follow a procedure derived from a Free Choice Profiling (FCP) methodology developed by Wemelsfelder et al. (2001).

Session 1 – term generation

Observers were each shown eight, 1-min video clips (also included in session 2) of the experimental cattle. These clips were selected by the researchers, as the animals exhibited contrasting behaviours and therefore observers had the opportunity to document a range of behavioural expressions that they could later use in scoring animals in session 2. After watching each clip, observers were given 2 min to write down any words that they thought described the animal's behavioural expression. There was no limit imposed to the number of descriptive terms an observer could generate, but terms needed to describe not what the animal was doing, but how the animal was doing it. Subsequent editing of the observer terms was carried out by the researchers to remove terms which described physical actions (e.g. walking, chewing, tail flicking), while terms that were in the negative form were transformed

to the positive for ease of scoring (e.g. 'unafraid' became 'afraid'). Terms were arranged so that terms with similar meaning (e.g. calm and relaxed) were not deliberately listed together. The terms were printed in a list, with each term attached to a 100-mm Visual Analogue Scale (0 = minimum to 100 = maximum) for quantification during session 2.

Session 2 – quantification

Observers used their own terms to quantitatively score (by marking on the Visual Analogue Scale) the behavioural expression of individual cattle shown in the 28 video clips (shown in random order). Each of the cattle was scored on every term generated by that observer. Once the animals had been scored, the distance (mm) from the start of the Visual Analogue Scale to where the observer had made a mark was measured and these measurements were entered into individual observer Excel (Microsoft Excel 2003, North Ryde, NSW, Australia) files.

Statistical analyses

The measurements (mm) on the Visual Analogue Scale were submitted to statistical analysis with Generalised Procrustes Analysis (GPA) as part of a specialised software package written for Françoise Wemelsfelder (Genstat 2008, VSN International, Hemel Hempstead, Hertfordshire, UK, Wemelsfelder et al., 2000). For a detailed description of its procedures, see Wemelsfelder et al. (2001). Briefly summarised, GPA calculates a consensus or 'best fit' profile between observer assessments through complex pattern matching. Because each observer scores the same footage, the analysis captures the similarity in scoring patterns between observers. Through Principle Components Analysis (PCA), the number of dimensions of the consensus profile is reduced to several main dimensions (usually two or three) explaining the variation between animals.

Validity of the QBA consensus

The Procrustes Statistic represents the level of consensus (i.e. the percentage of variation between observers explained) that was achieved. Whether this consensus is a significant feature of the data set, or, alternatively, an artefact of the Procrustean calculation procedures, is determined through a randomisation test (Dijksterhuis and Heiser, 1995). This procedure rearranges, at random, each observer's scores and produces new permuted data matrices. By applying GPA to these permuted matrices, a 'randomised' profile is calculated. This procedure is repeated 100 times, providing a distribution of the Procrustes Statistic indicating how likely it is to find an observer consensus based on chance alone. Subsequently a one-way *t*-test is used to determine whether the actual observer consensus profile falls significantly outside the distribution of randomised profiles.

Interpreting the GPA dimensions

GPA dimensions were interpreted by correlating the consensus dimensions with the individual observers' scoring patterns, producing lists of terms for each observer that were strongly correlated with either axis of each GPA dimension. These lists of terms can be compared for linguistic consistency between observers, and a list of terms was produced, describing each consensus dimension.

Handling QBA scores for individual animals

Each animal receives a quantitative score on each GPA dimension. For each individual, the physiological measurements and temperament tests were correlated with the individual's GPA scores for each dimension using Pearson's correlation (R ; Microsoft Excel), or Spearman rank order correlation (r_s ; Statistica 8.0; StatSoft-Inc 2001) for categorical (tension score, crush score) and non-normally distributed (Levene's test) data (muscle pH, slaughter order).

Results

The 15 observers participating in this study generated a total of 75 unique terms to describe the cattle they were shown (average 13 ± 5 terms per observer, range: 8–27). The level of consensus between observer assessment profiles, indicated by the Procrustes Statistic, was 43.7%, and this differed significantly from the mean randomised profile ($30.94 \pm 0.52\%$; $t_{99} = 24.67$, $P < 0.001$). Two main GPA dimensions were described, with GPA dimension 1 explaining 58.8% and GPA dimension 2 explaining 9.2% of the variation between animals.

Fig. 2 shows an example of one observer's word chart, where the observer's terms were graphed against the two GPA consensus dimensions. Table 1 lists terms with the highest correlations with GPA dimensions 1 and 2 axes. Low values for GPA dimension 1 were associated with terms such as 'calm' and 'relaxed', and high values associated with terms such as 'nervous' and 'anxious'. Low values for GPA dimension 2 were associated with terms such as 'annoyed' and 'frightened', and high values associated with terms such as 'curious' and 'interested'. Although observers each used a different set of descriptive terms, because they reached consensus in how they scored the individual animals, they were using the groups of terms shown in Table 1 in a similar way. For example, while one observer used

the term 'calm' to describe an animal, other observers instead used terms with similar semantic meaning (e.g. 'relaxed', 'content').

Table 2 shows the correlations between GPA dimensions and physiological measurements. Plasma lactate at the time of slaughter was significantly positively correlated with GPA dimension 1 scores ($R_{24} = 0.45$, $P = 0.020$; Fig. 3a), indicating that cattle with high plasma lactate were scored as more 'nervous'/'anxious'; plasma lactate was not correlated with GPA dimension 2 (Fig. 3b). Ultimate muscle pH was not significantly correlated with the GPA dimensions. Only one individual (number 2) was identified as a dark cutter (ultimate muscle pH > 5.7), and this animal was attributed a negative GPA dimension 2 score (i.e. was scored as one of the most 'annoyed'/'frightened' animals; Fig. 3b).

Of the temperament measures taken, crush score and tension score were significantly correlated ($r_s = 0.66$, $P < 0.001$), but neither were correlated with flight speed ($P > 0.05$). In terms of comparison between QBA and the behavioural measures, temperament scores (flight speed, crush score and tension score) were not significantly correlated with either GPA dimension 1 or 2. However, slaughter order was positively correlated with GPA dimension 1 ($r_s = 0.45$, $P = 0.016$; Fig. 4a), with cattle that were slaughtered later perceived as more 'nervous'/'anxious'. A trend for tension score to correlate with GPA dimension 2 ($r_s = -0.31$, $P = 0.069$) suggests that cattle that were more tense in the crush at weaning also tended to be animals perceived to be more 'curious'/'interested' pre-slaughter (Fig. 4b).

Discussion

We set out to test two main objectives with this study. Firstly, we investigated whether observers could reach agreement in their assessments of the behavioural expression of cattle filmed during pre-slaughter handling. We recorded high observer agreement in their assessments. Although observers used different terms to describe the cattle, they used them in a similar way so that their meanings were comparable. Using QBA, our group of observers could discern behavioural patterns in cattle being moved through a funnel chute towards the slaughterhouse. Two main dimensions of behavioural expression were recorded. GPA dimension 1 was defined by terms such as ‘calm’/‘relaxed’ versus ‘nervous’/‘anxious’ while GPA dimension 2 was defined by terms including ‘annoyed’/‘frightened’ versus ‘curious’/‘interested’. It is interesting that some terms appear on both dimensions 1 and 2. For example, ‘calm’ and ‘relaxed’ were associated with low values on GPA dimension 1 and high values on GPA dimension 2. This illustrates the layered effect of QBA in that animals can be curious and interested while being calm and relaxed, as appears in this study.

The second aim of the study was to determine if there is a correlation between behavioural expression and physiological and behavioural (e.g. temperament scoring) measures. We found correlation with behaviour that was captured at the same time as the footage was collected (pre-slaughter sorting order), but no correlations with multiple measures of temperament that were collected between 16 and 17 months before slaughter (at weaning). We also found a positive correlation between behavioural expression and plasma lactate concentration immediately post-slaughter. We conclude that QBA could be a valuable method of assessing cattle welfare under the conditions tested, in that it provided an integrative characterisation of cattle behavioural expression pre-slaughter.

The 15 observers were shown footage of animals that had been exposed to a number of stressful procedures (commonly experienced by cattle prior to slaughter) over the preceding 24 h. These included the stressors associated with transport to the abattoir (Knowles and Warriss, 2000) and exposure to a new environment during lairage (Le Neindre, 1989). In the present study, cattle were filmed as they passed through the final funnel chute prior to moving to the killing box. This environment included stressors such as noise, smells, people and novel surroundings. One of the most striking findings of this study was that observers distinguished differences in the behavioural expression of cattle that were significantly correlated to the order in which they were slaughtered. Footage of cattle was shown to observers in random order; nevertheless, cattle that were slaughtered at the beginning of the line were attributed lower scores on GPA dimension 1 (i.e. scored as more 'calm'/'relaxed') compared with cattle that were slaughtered later (more 'nervous'/'anxious'). Grandin (1980) made similar observations of cattle moving through a handling race, where the wildest and most difficult to handle individuals tended to move through the race at the end of the group. Another factor contributing to this finding may be that cattle slaughtered near the end of the line may have simply been exposed to the abattoir race and the handling and noises associated with it for a longer period than those at the beginning of the line. A study by Orihuela and Solano (1994) found that the time taken for cattle moving down a race towards an abattoir was negatively correlated with their order in the race (cattle at the beginning traversed the race more quickly). Both noise and handling or forcing animals up in a race is a major stressor for cattle (Ferguson and Warner, 2008 and Pearson et al., 1977) and increased stress responses (e.g. heart rate) have been found to have a positive correlation with the time taken for cattle to move into an abattoir (Bourguet et al., 2010). Therefore in the

present study it is likely that longer exposure would result in amplified stress responses and therefore resistance to move down the race.

Another interesting result of this study was that cattle with high plasma lactate concentrations were also attributed significantly higher GPA dimension 1 scores (i.e. scored as more 'nervous'/'anxious'). High plasma lactate is indicative of a corticosteroid-mediated stress response (Hemsworth and Barnett, 2001) and plasma lactate concentration may be strongly correlated with flight speed as well as other temperament measures (Petherick et al., 2009). The increased stress response of animals to the slaughter process, in the form of pronounced ante mortem glycolysis, can result in an elevation of ultimate pH of muscles leading to dark cutting (Kenny and Tarrant, 1987 and McVeigh and Tarrant, 1981). This can be amplified through lairage procedures such as washing cattle down prior to slaughter (Ferguson and Warner, 2008). In the present study, only one individual had high ultimate muscle pH > 5.7 resulting in it being classed as a dark cutter. This animal was attributed the lowest GPA dimension 2 score (i.e. was scored as one of the most 'annoyed'/'frightened' individuals).

One of the challenges of this study was in comparing different measures which were also collected at different times. In respect to the lack of correlation with temperament scores, the meaning of many temperament measures may potentially be ambiguous (Petherick et al., 2002). For example, it is generally accepted that animals with more excitable, reactive temperaments will have higher flight speed and crush score, but a particularly fearful animal may also become immobile and balk or freeze leading to a lower flight speed, as found by Burrow and Corbet (2000). Another issue was in terms of when these various measures are collected. Although temperament scores should be consistent over time (since they

purport to capture consistent responses that can transpose to new situations), this is not always the case. Some studies have found that temperament tests around weaning are most accurate in terms of heritability as the animals are not yet habituated to handling and thus the results are less likely to be confounded by level of habituation to the facilities (Burrow and Corbet, 2000 and Petherick et al., 1998); therefore this study used temperament scores collected at weaning.

We found little evidence of correlation between the temperament measures collected at weaning and the animals' behavioural expressions at slaughter some 16–17 months later. The tendency towards significance ($P = 0.069$) between tension score and GPA dimension 2 (Fig. 4b) suggests that animals with a higher tension score at weaning may be scored as more 'curious'/'interested' when observed during the slaughter process. The term 'curious' has been used to describe cattle that exhibit behaviours such as sniffing, baulking or backing up when exposed to novel environments or objects, and has been observed in cattle during lairage prior to slaughter (Grandin, 1996, Grandin, 1998 and Le Neindre, 1989). These behaviours can translate to difficulty in driving the animals, a problem that has been linked to temperament (Grandin, 1993).

We recognise the difficulties in carrying out meaningful assessments of animal welfare under commercial conditions, such as slaughter, where there are significant time constraints in the assessment process. Using fixed lists of terms, QBA may prove easy to implement and provide a useful measurement of an animal's wellbeing in an integrative sense (e.g. Brscic et al., 2009). It may be valuable as a guide to interpretation of more detailed welfare assessment methods or to highlight situations that require more intensive welfare assessment, particularly

in animal production scenarios where more invasive welfare assessments are difficult to carry out.

In conclusion, QBA has previously been applied to cattle during transport (Stockman et al., 2011 and Stockman et al., under review) and under various social conditions (Rousing and Wemelsfelder, 2006); the present study extends the application of QBA to behaviour before slaughter. We recorded high agreement among observers in how they assessed the animals, and significant correlation of plasma lactate and slaughter order to QBA. Further studies are required to extend the results of this study, investigating the link between QBA and aspects of meat quality. Further experimental assessment of the behavioural expression of dark cutters pre-slaughter would be beneficial, but requires large numbers of animals initially to ensure inclusion of sufficient numbers of these animals (which make up a minor proportion of all animals slaughtered). In the commercial setting, our preliminary results suggest that screening could allow identification of high-fear individuals before slaughter, therefore allowing targeted management of these animals to minimise stress responses of these animals.

Acknowledgements

Thanks to M. Farbos for her technical assistance during the experiment. Thanks also to Murdoch University Veterinary Clinical Pathology and M. Blackberry at The University of Western Australia, School of Animal Biology for blood analyses. Thanks to the observers who participated in this study. This study was funded by Meat and Livestock Australia and Meat & Wool New Zealand, in a collaborative study funded by the Beef CRC

for Genetic Technologies. The project was approved by the Murdoch University Animal Ethics Committee.

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Fig. 1. Diagram of the funnel chute used to move cattle into the abattoir. Cattle were moved as a group through a series of pens of decreasing dimensions towards the abattoir and were filmed when they were in the final funnel chute (holding 8–12 individuals at a time).

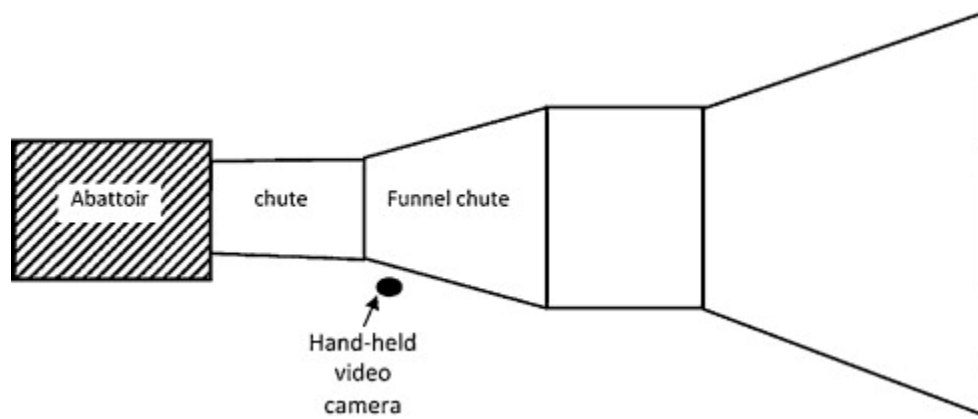


Fig. 2. Word chart of consensus profile for one observer showing how terms used by this observer correlated with the two GPA dimensions.

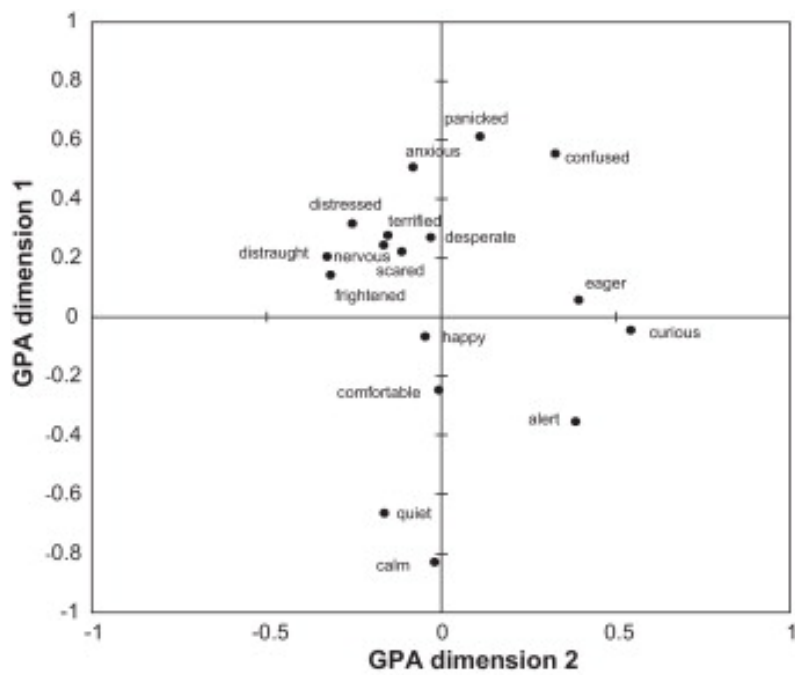


Fig. 3. Correlations of plasma lactate at slaughter with means of all observers' GPA scores on (a) GPA dimension 1 and (b) GPA dimension 2. Positions of particular cattle are indicated by their numbers. The plot represents each of the cattle once, where its position indicates its scores on each GPA dimension.

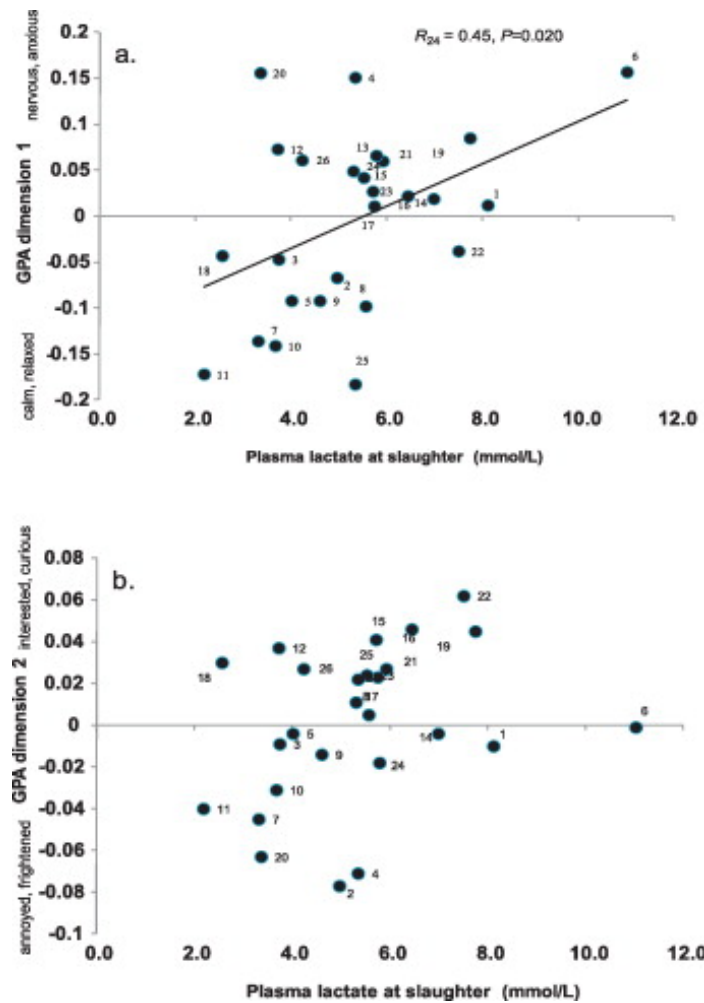


Fig. 4. Correlations of (a) slaughter order of cattle with means of observers' scores on GPA dimension 1 and (b) weaning tension scores with means of observers' scores on GPA dimension 2. Positions of particular cattle are indicated by numbers.

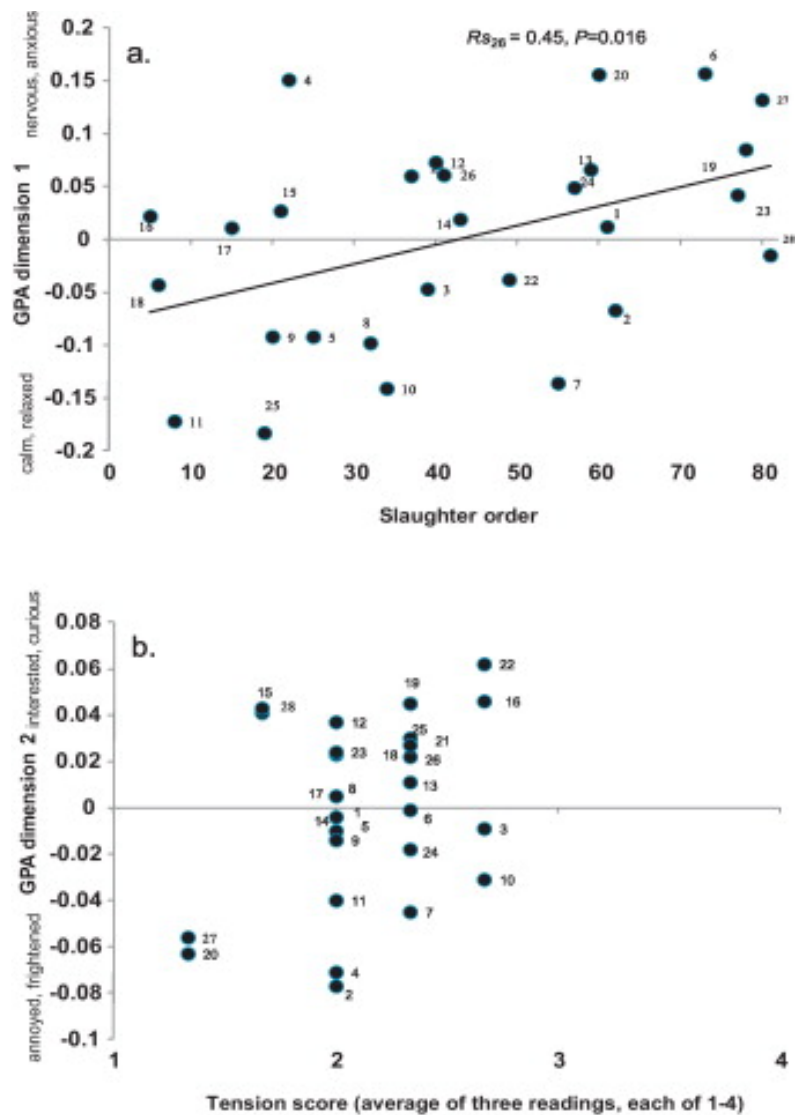


Table 1. Terms showing the highest negative and positive correlation with GPA dimensions 1 and 2 of the consensus profile. Order of terms is determined firstly by number of observers (of the 15 in total) to use that term (in brackets if greater than one) and secondly by correlation coefficient of each term with the consensus dimension. The difference in cut-off for the correlation values reflects differences in the strength of each GPA dimension (dimension 1 describes the greatest proportion of variation in the data).

GPA dimension	Low values	High values
1 (58.8%)	$R < -0.5$ Calm (9), relaxed (5), content (3), comfortable (3), quiet (2), settled, unphased, aware, passive, willing, placid, bored, submissive, happy	$R > 0.5$ Nervous (7), anxious (6), scared (4), agitated (4), distressed (4), alert (4), confused (4), unsure (3), stressed (3), frightened (3), worried (3), panicked (2), on edge (2), flighty (2), tense (2), unsettle, toey, uncertain, wants to leave, restless, energetic, dissatisfied, apprehensive, trapped, avoiding, powerless, stimulated, frustrated, edgy, alarmed, terrified, reactive, responsive, observant
2 (9.2%)	$R < -0.4$ Annoyed, frightened, submissive, stressed, agitated, disturbed, scared	$R > 0.4$ Interested (2), curious (2), alert (2), wary, anxious, comfortable, agitated, relaxed, contemplating, calm, dominant, wondering, impatient

We have not edited out terms that may appear on multiple lists because this would introduce subjectivity (as to which list to remove the term from), but the reader should be mindful that each individual observer had a unique list of terms and therefore how they scored the same term may to some degree be influenced by the remaining terms in their repertoire.

Table 2. Correlations between GPA dimensions and physiological or behavioural measures ($n = 26$ for plasma metabolites at slaughter and $n = 28$ for all other parameters). The coefficient of variation (CV) for each variable is indicated. Parametric data were analysed by Pearson's R correlation (R_{26}) except for plasma metabolites (R_{24}).

	CV (%)	GPA dimension 1		GPA dimension 2	
		_____		_____	
		<i>R</i> or <i>r_s</i>	<i>P</i>	<i>R</i> or <i>r_s</i>	<i>P</i>
Hormones at slaughter (plasma)					
Cortisol (nmol/L)	20	0.28	0.147	0.07	0.713
Vasopressin (pg/mL)	21	-0.02	0.926	0.18	0.349
Metabolites at slaughter (plasma)					
β -Hydroxy butyrate (mmol/L)	30	-0.21	0.299	-0.13	0.512
Glucose (mmol/L)	19	0.07	0.731	0.19	0.361
Lactate (mmol/L)	36	0.45	0.020*	0.32	0.117
NEFA (mmol/L)	30	0.10	0.642	-0.20	0.320
Metabolites (muscle)					
Net glycogen loss semimembranosus	215	-0.10	0.596	0.06	0.764
Net glycogen loss semitendinosus	574	-0.14	0.472	-0.26	0.185
<i>Ultimate muscle pH</i> ^a	2	<i>0.19</i>	<i>0.322</i>	<i>-0.19</i>	<i>0.337</i>
<i>Slaughter order</i> ^a	NA	<i>0.45</i>	<i>0.016*</i>	<i>-0.12</i>	<i>0.552</i>
Temperament (weaning)					
Flight speed	25	0.02	0.909	-0.29	0.135
<i>Crush agitation score</i>	27	<i>-0.24</i>	<i>0.220</i>	<i>0.26</i>	<i>0.187</i>
<i>Tension score</i> ^a	17	<i>-0.31</i>	<i>0.105</i>	<i>0.35</i>	<i>0.069</i>
Birth weight (kg)		-0.30	0.118	0.11	0.568

^a

Non-parametric data were analysed by Spearman rank order (r_s) correlation (shown in italics).

*

Significant correlations are indicated in bold, $P < 0.05$.