final report

Project code: B.AWW.0131 & B.AWW.0130

Prepared by: Trish Fleming, Catherine Stockman, Sarah Wickham, Teresa Collins, Anne Barnes, David Miller

Murdoch University

Date published: November 2011

ISBN: 9781741916737

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Animal Welfare Objectives Measures Research Program

Qualitative Behavioural Assessment (QBA) as an Integrated Measure of Welfare

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Qualitative Behavioural Assessment (QBA) is an integrated measure that characterises behaviour as a dynamic, expressive body language. QBA can be used to quantify positive aspects of animal welfare as well as some measure of the affective state of the animals.

This project investigated the validation of QBA of animals against physiological and quantitative behavioural measurements. The validation process encompassed studies on both cattle and sheep exposed to common industry stressors that included road transport, nutritional variation, pre-slaughter handling, isolation, and exposure to novelty.

There was significant consensus between observers in their QBA scores of the animals in each of the studies. Observers were also able to distinguish between some treatment groups based on the animals' behavioural expression. Furthermore, QBA scores were correlated with key physiological and quantitative behavioural measurements. QBA is therefore a reliable, objective and valid measure of animal welfare.

QBA is a quick and non-invasive assessment method that is also versatile enough to be used under a wide range of industry situations. QBA will be useful as a tool to compare animals under a range of production scenarios where more invasive welfare assessments are difficult to implement, as a guide to interpretation of more detailed welfare measures, and to highlight situations that require more intensive welfare assessment.

Executive summary

This project validated the use of Qualitative Behavioural Assessment (QBA) as a measure for assessing the welfare of cattle and sheep under industry-relevant conditions. We tested whether QBA is a reliable, objective and valid measure of the physiological and psychological state of cattle and sheep.

The first, experimental, phase of this research programme examined cattle and sheep during a variety of transport events, using transport as a stressor that could be experimentally manipulated and controlled.

a) Transport studies were conducted on cattle to validate QBA as a measure of welfare state under three potential challenges: animals were compared according to their level of habituation to transport (naïve vs. habituated), trailer flooring type (non-grip vs. grip) and driving style (stop-start vs. continuous driving). Observers reached consensus in their use of QBA terms for each transport scenario. Observers distinguished between each of the paired transport treatments in terms of their scores of the behavioural expression of the cattle. Physiological differences were evident between transport treatments (e.g. heart rate, core temperature, hypothalamic-pituitary-adrenal (HPA) axis response and immune function) and QBA scores were correlated with physiological responses in a meaningful way.

b) Sheep were exposed to four common transport challenges: animals were compared according to their level of habituation to transport (naïve vs. habituated), trailer flooring type (non-grip vs. grip), driving style (stop-start vs. continuous driving) and degree of ventilation (trailer sides that were closed-in vs. open). Observers reached consensus in their use of QBA terms for each transport scenario. Observers distinguished between each of the paired transport treatments except in the case of the ventilation study (which may reflect that there was only minimal effect of this treatment upon the animals' physiology). Physiological differences were evident between transport treatments (e.g. heart rate, core temperature, HPA axis response and immune function response) and QBA scores were correlated with physiological responses in a meaningful way.

c) An additional experimental study was carried out to validate QBA as a measure of chronic stress. Sheep of different levels of nutritional stress (chronic stressor) were tested for their responses to road transport (acute stressor). There was consensus in the ability of observers to interpret the behavioural expressions of sheep with differing body condition scores (BCS) being road transported at different times of the year and without fasting vs. post-fasting. QBA scores were significantly correlated with physiological variables in a manner that was consistent with the interpretation that the behavioural expression of sheep reflected their physiological state.

From these three experimental studies, it was clear that various road transport scenarios resulted in altered physiological responses. Observers were able to distinguish between treatment groups using QBA and the QBA scores were correlated with physiological responses in a biologically-meaningful way.

The second part of this research programme involved working with other researchers to analyse footage of animals collected under various experimental and industry situations to validate QBA as a versatile tool for welfare assessment.

a) In collaboration with other researchers at Murdoch University, we examined QBA of cattle in lairage immediately before slaughter. This study validated QBA against measures of physiology and behaviour collected before (e.g. temperament measured since weaning) and immediately after slaughter. There was consensus between observers in their QBA scores, and correlation between GPA dimensions and physiological and temperament measurements (including slaughter order, tension

score at weaning, plasma lactate at the time of slaughter, ultimate pH and plasma glucose).

b) In collaboration with AgResearch NZ, footage of sheep tested in a behavioural demand facility was assessed using QBA. Two experiments were assessed, where lactating sheep were either of 1) differing BCS, or 2) differing rate of BCS decline. In both studies, there was consensus in the observers' assessment of the sheep and observers distinguished between sheep of different BCS treatments. QBA scores were correlated with quantitative measures of behaviour, illustrating how QBA can add an important interpretative element to quantitative analysis.

c) In collaboration with CSIRO Armidale, footage of lactating cattle of differing BCS 1) exposed to an isolation stressor and 2) in a behavioural demand facility were assessed for QBA. In both studies there was consensus between observers in their assessment of the cattle. However, observers were not able to tell the difference between BCS treatments in either experiment by QBA (matching results of the behavioural demand analysis). Quantitative behavioural assessments of the animals in the isolation stressor study indicated that temperament differences between cattle may have overridden any influence of BCS upon behaviour. QBA scores were correlated with quantitative assessments in the behavioural demand study, again adding an interpretive element to the analysis.

d) In collaboration with researchers from the Beef CRC, footage of cattle chronically exposed to varying levels of fearful (novel) cues were analysed by QBA. The study revealed differences between treatment groups with animals exposed to both chronic and acute exposure to novel stimuli showing different body language from control animals.

QBA is a reliable, objective and valid measure of animal welfare. This research programme has found consensus between observers in their qualitative assessments in all studies and (under most experimental conditions) observers were able to distinguish between treatment groups based on the animals' behavioural expressions. QBA scores are also correlated with key welfare-relevant physiological and behavioural measures.

QBA allows whole-animal assessment in an integrative sense. It is a quick and non-invasive assessment that correlates with commonly-used physiological measures of welfare (validated during road transport and in lairage). QBA may therefore be useful as an aid to interpretation of more detailed welfare measures, or to highlight situations that require more intensive welfare assessment, particularly in animal production scenarios where more invasive welfare assessments are difficult to implement.

Contents

Standard terms used in this report

AWOM	the Animal Welfare Objective Measures research programme funded by MLA
BCS	Body condition score
GPA	Generalised Procrustes Analysis – a form of multivariate analysis that analyses a diversity of inputs to create a consensus (see Appendix 1 for details of the calculations)
HPA	The hypothalamic-pituitary-adrenal axis
HR	heart rate
HRV	heart rate variability
IGF-1	Insulin-like growth factor 1 is a hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults.
PCA	Principal Components Analysis – a mathematical process of reduction of multiple dimensions to a smaller numbers of ‘consensus’ dimensions
QBA	Qualitative Behavioural Assessment - a method of capturing and quantifying the behavioural expression of animals
T_{core}	core body temperature

Values are shown as means ±1 standard deviation (SD) throughout.

Levels of statistical significance:

ns	not statistically significant
*	$p<0.05$
**	$p<0.001$
***	$p<0.001$

1 Background

Project overview

Welfare measures need to be practical, cost-effective, reliable and replicable if they are to be useful and accurately reflect the true welfare state of the animal. This research project validated Qualitative Behavioural Assessment (QBA) as a measure of the physiological and affective state of cattle and sheep. QBA is a versatile tool that enables comparative, hypothesis-driven evaluation of various industry-relevant practices. QBA provides a useful objective welfare measure that has broad applicability to the agriculture industry.

Requirement for an inclusive, integrated welfare model

Animal welfare measures require assessment that captures the complexity of animal responses. The integration of different processes (behaviour, physiology, health, molecular activity and productivity) is recognised as vital for the development of new animal welfare measures [1]. It is important, therefore, to utilise approaches that integrate and clearly represent a range of pathways, rather than measuring a single aspect of the animal's physiology or behaviour.

Most previous welfare studies have largely focused on recording behaviour or physiology responses to acute stressors. However, most behaviour or physiological measurements offer limited opportunity to assess welfare in a field situation (e.g. on-farm, transport, lairage) since there may be limitations in how these measurements are recorded or interpreted. There has also been little comparison between simultaneously-recorded measurements. Importantly, the emotional impact of exposure of the animal to an acute stressor has received negligible attention.

Objective welfare measures need to capture the animal's response to its environment

Behaviour is the outward expression of physiological changes, and since it incorporates aspects of animal perception, cognition and emotions, inclusion of behavioural assessment has been widely recognised as important in the development of future welfare measures [1]. The disadvantage of individual behaviours as an animal welfare measure, however, is that behaviour can be difficult to objectively quantify, compounded with a lack of certainty regarding what the behavioural responses may indicate about the underlying biological state of the animal. For example, tail flicking may be quantified, but without a view of the whole animal, it is difficult to interpret the meaning behind this simple measure.

Qualitative Behavioural Assessment (QBA) is a method of capturing and quantifying the behavioural expression of animals. The QBA methodology is described in detail in Appendix 1. Behavioural expression is basically the animal's body language – not *what* it is doing but *how* it goes about doing what it is doing. QBA interprets the expressive qualities, or the 'style' of behaviour demonstrated by individual animals. Because QBA assesses the whole animal, it can incorporate and interpret a wide range of body parts and behaviours. To use the previous example, it can be possible to determine if an animal is swishing its tail in a *relaxed* manner (i.e. to dislodge flies) or in an *aggressive* manner. Understanding the difference between the two is important to interpret how an animal is responding to its environment, and is a natural part of good stockmanship skills. QBA can therefore be a powerful tool to interpret individual measures of husbandry, health, physiology or behaviour.

QBA is an especially effective tool for capturing the emotional state of animals under a range of environmental situations. For example, as with many emotions, fear and anxiety are extremely difficult motivational states to measure [2-6]. Established techniques for measuring fear responses largely require that the animals are exposed to a specific controlled environment to enable measurements to be collected (e.g. the novel arena test, novel object test, time to approach a handler, restraint test). However, there are many situations that do not lend themselves to establishing specific environmental conditions or experimental test stimuli. This is particularly important in a commercial environment, where it is valuable to understand the responses of livestock to specific husbandry practices. Methods for welfare assessment therefore need to be feasible and adaptable to different environments if they are to make a valuable contribution towards production industry processes.

2 Project objectives

Experiments were conducted on cattle and sheep exposed to industry relevant psychological and physiological stressors to validate the use of Qualitative Behavioural Assessment as a welfare measure.

2.1 Primary objectives

By 1st January 2011:

1) Develop and validate a practical, convenient welfare assessment tool based on the identification and measurement of key animal behaviour that is indicative of animal welfare status in industry situations including:
    a. Identify and validate welfare-relevant behaviour in sheep that reflects psychological and physiological stress under transport conditions and validate the use of QBA for sheep welfare assessment.
    b. Identify and validate welfare-relevant behaviour in cattle that reflects psychological and physiological stress under transport conditions and validate the use of QBA for cattle welfare assessment.

2) Analyse suitable video recordings provided from other projects in the Animal Welfare Objective Measures (AWOM) program to determine the usefulness of QBA in assessing animal welfare under a range of conditions and challenge models

3) Training of 2 PhD students

2.2 Validating Qualitative Behavioural Assessment

We have tested three aspects of QBA as a measure of animal welfare. We have examined its reliability, objectivity and validity as a measure of animal welfare.

To date, QBA has been **reliably** applied to studies of pigs [7-9], horses [10,11], cattle [12,13], dogs [14], and poultry [15]. A recent review of the technique [15] indicates that observers show consensus in their terminologies and assessment of expressive behavioural style and the approach appears to reliably indicate differences between individual animals.

We have tested the **objectivity** of QBA by assessing whether we can use QBA scores to discriminate between treatment groups. Only two published studies to date have examined whether QBA can be used to distinguish between treatment groups. Minero et al. [10] revealed significant differences in QBA assessments for the same horses recorded before and after one month handling habituation: yearlings were characterised as *suspicious / nervous* and *impatient / reactive* before any handling, and as more *explorative / sociable* and *calm / apathetic* after handling. Dorman et al. [16-18] found that horses at different stages of a 160km endurance ride differed in their behavioural expression, showing behaviour that was indicative of ‘engagement’ and ‘tiredness’.

To interpret the **validity** of QBA as a measure of animal welfare, it is useful to know how animal behavioural expression correlates with physiological changes that are informative regarding the animal’s welfare state. We have selected physiological measures for comparison, since most of the understanding of quantification of animal welfare has largely been derived from physiology [19-22]. Only one previous study (unpublished) has compared QBA with the physiology of the animal [23], showing that in three test situations, individual pig’s physiological responses correlated with their behavioural expression.

This study investigated QBA as an objective measure of individual welfare for cattle and sheep exposed to different acute (behavioural, physiological and physical) and chronic (nutritional) conditions.

3 Validation of QBA against psychological and physical road transport stressors

3.1 Background

Validation of QBA as an objective, reliable and repeatable method of animal welfare assessment required testing QBA scores against known physiological and behavioural parameters. To carry out our validation process, we subjected cattle and sheep to a known stressor (transport) which allowed a reasonable degree of modification and manipulation. Please note, we did not seek to measure stress under transport conditions, since this has been extensively studied [e.g. 24,25,26] and transport reflecting commercial transport conditions (e.g. stocking rate, vehicle size) did not conform to the strictures of our experimental requirements.

The aims of these experiments are to:

1. determine whether observers could reach consensus in their assessment of the behavioural expression of cattle and sheep
2. determine whether observers could distinguish between experimental treatment groups based on their behavioural expression
3. determine how QBA scores correlate with physiological measures that are indicative of the animals physiological and affective states

3.2 General Methodology

3.2.1 Cattle treatments

Fourteen Angus steers (12 months of age; 347±11 kg) were randomly selected from a transport-naïve herd and housed in a group pen throughout the experiment. The transport treatments were 90 minutes in length (detailed in Table 1).

Table 1. The treatments cattle were exposed to in each of the transport events (which took place over a period of 27 days).

Transport event	Treatment
1	Naïve transport
2 - 7	Habituation #
8	Habituated transport
9	Non-grip flooring
10	Habituation #
11	Continuous transport
12	Stop-start transport

Physiological and behavioural responses not examined for these events

The cattle were transported in a single bay trailer with a stocking rate that was within industry recommendations (1.07 m^2/head) [27]. Environmental temperature (ºC) and relative humidity (%) were recorded (every 2 seconds) during transport to ensure that transport events were not substantially different from each other in environmental conditions.

Due to logistical constraints, cattle were transported in 2 groups of 7 (transport groups 1 and 2) with the same individuals making up each group on successive days.

Except for the continuous and stop-start events, the transport route included a mixture of main roads (speed limit: 50-70 km/h) and highways (speed limit: 70-100 km/h). The continuous driving route followed freeways and country roads with minimal stops (average speed: 85 km/hr). The stop-start driving route followed suburban streets with frequents stops at intersections and frequent turns (average speed: 40 km/hr).

All transport events except the non-grip transport event had a metal floor grate in place to provide flooring grip.

3.2.2 Sheep treatments

14 Merino wethers (14 months of age; 46.4±0.4 kg) were randomly selected from a transport-naïve flock and housed in a group pen throughout the experiment.

Table 2. Treatments sheep were exposed to in each of the transport events (which took place over a period of 26 days).

Transport event	Treatment
1	Naïve transport
2 - 7	Habituation #
8	Habituated transport 1
9	Stop-start transport
10	Habituation #
11	Closed ventilation
12	Habituation #
13	Non-grip flooring
14-15	Habituation #
16	Habituated transport 2

Physiological and behavioural responses not examined for these events

Sheep were transported as a group in a single bay trailer. Environmental temperature (°C) and relative humidity (%) were recorded (every 2 seconds) during transport to ensure that transport events were not substantially different from each other in environmental conditions. The transport treatments were 90-minutes in length (detailed in Table 2). The transport route included a mixture of main roads (speed limit: 50-70 km/h) and highways (speed limits: 70-100 km/h).

Habituated event 1 was used for comparison to the naïve transport event and habituated event 2 was used for comparison with the closed ventilation, non-grip flooring and stop-start driving events. Between each treatment event, sheep were transported as per the habitation events; to reinforce the predictability of transport treatments.

During the closed ventilation event, all sides up to the roof of the trailer were covered with clear polycarbonate sheets to minimise the amount of airflow into the trailer while travelling (the roof of the trailer was covered during all transport events).

During the stop-start driving event, the sheep were subjected to stop-start driving at the same time point they would have experienced continuous driving during a habituated event. Starting from a stationary position, the vehicle accelerated to 60 km/h over approximately 24 seconds before continuing at that speed for 15 seconds. The vehicle then decelerated to full stop over an approximate time of 13 seconds. This was repeated 10 times. This type of driving reflects part of the journey undertaken by sheep through built up areas with traffic lights. The treatment was designed to reflect the route taken to the live export shipping docks at Fremantle, Western Australia.

3.2.3 Physiological measurement of cattle and sheep

Temperature loggers were surgically implanted into the peritoneal cavity of the cattle and sheep before the study commencement, allowing time for recovery from surgery [28,29]. The loggers were set to record core body temperature (T_{core}) every 2 minutes for the duration of the experiment.

Blood was collected using jugular venapuncture while the animal was held in a crush before and after the treatment events (animals were not blood sampled for habituation events). EDTA blood was used for haematological analysis and plasma analysis of glucose, β-hydroxyl-butyrate, cortisol, prolactin and IGF-1.

Heart rate (HR, beats per minute) was recorded (every 5 seconds) using external heart rate monitors for all treatment events except habituation events. Heart rate monitors were fitted immediately after pre-transport blood sampling and were removed after post-transport blood sampling. During habituation events heart rate belts were fitted before transport and removed after transport.

For comparison of physiology with QBA scores, we calculated the change in physiological parameters in response to transport. For example, parameters derived from blood sampling were expressed as post-transport values divided by pre-transport values. Heart rate was expressed as during transport divided by before transport. Body temperature was expressed as during transport divided by the same time of day on a non-transport day (to take into account circadian rhythms). These physiological parameters were compared with QBA scores by correlation analysis.

3.2.4 QBA of cattle and sheep

Video footage was recorded during transport using four digital cameras fixed to the front and rear of the trailer, above cattle and sheep head height. Individuals were identified using numbers printed on the outside of the heart rate belts. Observers were recruited from University staff and students and members of the public to undertake QBA (see Appendix 1 for full description of the process).

Cattle QBA:

1. Term generation - Observers generated their own descriptive terms from 8 video clips of the experimental cattle demonstrating a wide range of behavioural expressions.

2. Quantification - observers were then required to attend 3 quantification sessions shown on separate days:

1. Naïve vs. habituated
2. Stop-start vs. continuous driving
3. Non-grip vs. grip flooring

One clip (15 to 30 seconds long) was chosen for each individual in each of the respective treatment events and therefore observers were shown, and scored, 28 clips in each of the sessions. Clips were chosen from footage recorded within the first 30 minutes after departure for naïve transport and for the non-grip flooring treatment. Clips were chosen from the final 30 minutes of transport for the stop-start transport event. During these time periods behavioural response to the new environment was expected to be most marked. Equivalent time points were selected from the habituated event for comparison.

Sheep QBA:

1. Term generation - observers were shown 20 video clips of the experimental sheep demonstrating a wide range of behavioural expressions.

2. Quantification - observers were then required to attend 4 quantification sessions shown on separate days:

1. Naïve vs. habituated 1
2. Closed vs. open (Habituated 2) ventilation
3. Stop-start vs. continuous (habituated 2) driving
4. Non-grip flooring vs. grip (habituated 2) flooring

Ten of the 14 sheep were clearly visible in the footage for each transport event. One clip (20-60 seconds long) of each individual was chosen from each experimental journey within the first 15 minutes after departure for the naïve and flooring transport events, within the first 15 minutes of commencing the stop-start driving treatment, and during the last 15 minutes of the closed ventilation treatment event. Equivalent time points were selected from the habituated transport events for comparison.

3.3 Results

3.3.1 Cattle: Naïve and habituated to road transport (psychological stressor)

The 40 observers participating in this study generated a total of 178 unique terms (average: 17±7 terms per observer, min: 9, max: 48).

The Procrustes Statistic was 47% and this differed significantly from a mean randomised profile (t_{99}=69.4, p<0.001).

Terms with the strongest correlation with each of the GPA dimensions are shown in Table 3; significant treatment differences are indicated in the right hand column.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 4. Table 4 also shows the correlations between GPA dimensions and physiological measurements. Cattle also assessed as more *agitated, restless* and *stressed* (GPA dimension 1) had a higher change due to transport in maximum core temperature, heart rate, plasma glucose, white blood cell count, neutrophils and neutrophil: lymphocyte ratio and a lower change due to transport in haemoglobin, haematocrit and lymphocyte count. Cattle assessed as being more *sedate, upset* and *annoyed* (GPA dimension 2) had lower change in RBC and platelet count. Cattle assessed as being more *weary, soothed* and *exhausted* (GPA dimension 3) exhibited higher change in plasma glucose levels.

3.3.2 Cattle: Altered flooring (physical stressor)

The 39 observers participating in this study generated a total of 180 unique terms (average: 17±7 terms per observer, min: 9, max: 47). The Procrustes Statistic was 44.7% and this differed significantly from a mean randomised profile (t_{99}=61.3, p<0.001).

Terms associated with each GPA dimension are shown in Table 3; significant treatment differences are indicated in the right hand column.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 5. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 5). Cattle assessed as being more *calm, comfortable* and *relaxed* (GPA dimension 1) had higher change due to transport in white blood cell count and neutrophils but lower change in neutrophil lymphocyte ratio. Mean plasma glucose was negatively correlated with GPA dimension 3 and mean heart rate was positively correlated with dimension 3.

3.3.3 Cattle: Stop-start driving (physical stressor)

The 39 observers participating in this study generated a total of 180 unique terms (average: 17±7 terms per observer, min: 9, max: 47). The Procrustes Statistic was 39.42 % and this differed significantly from a mean randomised profile (t_{99}=43.95, p<0.001).

Terms associated with each GPA dimension are shown in Table 3; significant treatment differences are indicated in the right hand column.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 6. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 6). Lymphocytes were positively correlated with GPA dimension 1 (i.e. were higher for cattle also described as more *restless, agitated* and *scared*). Cattle assessed as being *curious, interested* and *inquisitive* (GPA dimension 2) exhibited a higher change due to transport in haemoglobin, haematocrit, white blood cell count and neutrophils but lower change in neutrophil: lymphocyte ratio. Cattle assessed as being *stressed, agitated* and *afraid* (GPA dimension 3) had higher change in mean plasma cortisol.

Table 3. Terms used by observers to describe CATTLE behavioural expression during THREE TRANSPORT TREATMENTS. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column.

GPA dimension	Low values	High values	Treatment effects
Naïve vs. habituated to transport			
1 (54%)	Calm (13), comfortable (7), relaxed (7), content (4), at ease (3), bored (2), settled (2), quiet, indifferent, predictable, happy, subdued, accepting, composed, fearful, controlled	Agitated (11), restless (7), stressed (7), anxious (6), flighty (5), nervous (5), alert (3), frightened (3), scared (3), worried (3), alarmed (2), concerned (2), fearful (2), frustrated (2), panicked (2), unsure (2), wants to escape (2), claustrophobic, confused, content, distressed, evasive, excitable, fidgety, hemmed in, impatient, inquisitive, lively, on edge	$F_{1,13}$=12.07, p<0.01 - Naïve cattle scored higher cf. habituated
2 (8.5%)	Sedate, upset, annoyed, frightened, weary, nervous, fatigued, sad, bored, happy	Alert (5), curious (4), aware (4), inquisitive (3), interested (2), focused, quiet, relaxed, wary, shy, watchful	ns
3 (5.2%)	Weary, soothed, exhausted, depressed, irritated, alert, threatened, sad	Alert (3), questioning	ns
Non-grip vs. grip flooring			
1 (40.6%)	Calm (7), comfortable (5), relaxed (4), at ease (2), content (2), predictable, settled , confident, quiet, unphased, sleepy	Agitated (6), restless (4), anxious (3), unsure (2), stressed (2), toey , apprehensive, wants to leave, scared, alarmed, tense, nervous, uncertain, twitchy, confined, panicked, flighty, worried, restricted	$F_{1,13}$=15.09, p<0.01 - Cattle travelling on non-grip flooring scored higher than cattle that had a metal grid flooring
2 (11.1%)	Curious (8), interested (5), alert (5), inquisitive (3), happy (2), calm (2), aware (2), content, bored, relaxed, quiet, concentrated, bright, focussed, questioning, assessing environment, responsive, scared, worried	Angry (3), nervous (2), upset (2), shy (2), agitated (2), anxious, tired, trapped, bewildered, worried, oppressed, scared, frightened, frustrated, sad, isolated, evasive, forlorn, jumpy, alarmed, hesitant, fidgety, on edge, twitchy, depressed, annoyed	ns
3 (6.5%)	Nervous (2), bored, worried, sad, thoughtful, weary, afraid, happy, calm, comfortable, alone	Annoyed, stressed, distressed, trying to get away, inquisitive, alert, revved up, playful, evasive, caring, angry, seeking escape, intrigued, anxious	ns
Stop-start vs. continuous driving			
1 (33.3%)	Calm (17), relaxed (13), comfortable (9), content (5), at ease (3), happy (2), quiet (2), predictable , composed, afraid, bored	Restless (8), agitated (8), scared (7), anxious (7), worried (6), nervous (6), stressed (5), alarmed (5), tense (5), alert (5), distressed (4), frightened (4), fearful (4), angry (2), unsure (2), twitchy (2), bothered (2), confused (2), flighty (2), trapped (2), confined (2), upset (2), seeking companions, boxed in, excitable, at ease, toey, wants to escape, content, unnerved, cramped	$F_{1,13}$=12.63, p<0.01 - Cattle exposed to stop-start driving scored higher than cattle exposed to continuous driving
2 (16.0%)	Curious (8), interested (8), inquisitive (7), alert (4), calm (3), anxious (3), observant (2), relaxed (2), confident (2), comfortable (2), at ease (2), assessing environment, happy, bright, settled, questioning, bold, casual, agitated, focused, seeking, investigative, aware	Stressed (2), tense (2), alert, nervous, avoiding, irritated, worried, agitated, seeking comfort, looking for company, on edge, anxious, panicked, angry, apprehensive, frightened, annoyed, struggling	$F_{1,13}$=5.01, p<0.05 - Cattle exposed to stop-start driving scored lower than cattle exposed to continuous driving
3 (8.9 %)	Calm (5), bored (2), intrigued (2), relaxed (2), stressed, comfortable, annoyed, happy, worried, confined, violated, concerned, stuck, unsure, indifferent, jammed, agitated, tense, nervous	Stressed (2), agitated, afraid, frightened, comfortable, nervous, aware, anxious, curious, alert, relaxed	ns

Table 4. CATTLE: NAÏVE VS. HABITUATED TO TRANSPORT. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*, different letters indicate significant differences (p<0.05). Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Raw values				R-M ANOVA results (*p* values)			Correlation with QBA scores (Pearson's *r*) (r_{26})		
	Naïve		Habituated		Treatment (Naïve vs. habituated)	Time (Before vs. after)	Treatment x time interaction			
	Before	After	Before	After				GPA 1	GPA 2	GPA 3
Hormones										
Cortisol (ng/ml)	68.5 ± 56.3	119 ± 48.6	38.5 ± 27.5	43.5 ± 28.6	<0.001	0.021	0.074	0.182	-0.007	-0.054
IGF-1 (ng/ml)	39.6 ± 12.6	39.6 ± 13.8	48.1 ± 15.5	51.87 ± 14.19	<0.001	0.258	0.345	-0.305	-0.23	0.17
Prolactin (ng/ml)	33.4 ± 29.5	7.42 ± 5.34	25.9 ± 18.7	17.7 ± 15.6	0.462	<0.001	0.078	-0.034	-0.105	-0.304
Metabolites										
β-hydroxy butyrate (mmol/L)	0.219±0.105	0.199±0.084	0.219±0.064	0.181±0.048	0.655	0.014	0.38	0.24	-0.127	-0.153
Glucose (mmol/L)	5.37 ± 0.521[ac]	6.04 ± 0.356[b]	5.66 ± 0.389[a]	5.60 ± 0.457[c]	0.016	<0.001	0.022	0.428*	0.282	-0.408*
Haematological parameters										
Red blood cell (x 10^9/L)	8.89 ± 0.57[a]	8.52 ± 0.53[b]	8.21 ± 0.37[c]	8.15 ± 0.42[c]	<0.001	0.027	0.016	-0.031	-0.342*	0.035
Haematocrit (%)	0.383±0.023[a]	0.365±0.023[b]	0.351±0.017[c]	0.348±0.019[c]	<0.001	0.011	0.023	-0.333*	0.184	-0.205
Haemoglobin (g/L)	130 ± 7.49[a]	122 ± 6.98[b]	122 ± 6.09[b]	120 ± 6.50[b]	0.006	0.003	0.011	-0.400*	0.202	-0.126
White blood cell (x 10^9/L)	9.20 ± 1.32	12.6 ± 1.83	9.69 ± 0.797	11.4 ± 1.90	0.026	<0.001	0.055	0.649**	-0.132	0.161
Monocytes (x 10^9/L)	0.48 ± 0.48	0.33 ± 0.28	0.24 ± 0.10	0.20 ± 0.11	0.118	0.017	0.096	-0.109	-0.171	-0.305
Neutrophils (x 10^9/L)	2,47 ± 0.38[a]	6.38 ± 1.71[b]	3.01± 0.55[a]	4.62 ± 1.34[d]	0.074	<0.001	<0.001	0.535**	0.157	-0.009
Lymphocytes (x 10^9/L)	6.33 ± 1.14[a]	5.05 ± 0.79[b]	5.46 ± 1.00[c]	5.39 ± 0.83[c]	0.245	0.006	<0.001	-0.392*	-0.147	0.013
Neutrophil: Lymphocyte	0.40 ± 0.07[a]	1.30 ± 0.43[b]	0.59 ± 0.22[c]	0.88 ±0.31[d]	0.177	<0.001	<0.001	0.525**	0.167	-0.038
Platelet (x 10^9/L)	729 ±136	784 ±166	565 ±176	581 ±174	<0.001	0.030	0.176	0.001	-0.331*	0.183
Body temperature	Before [a]	During [b]	Before [a]	During [b]					(r_{24})	
Avg T_{core} (°C)	38.68 ± 0.206[a]	39.63 ± 0.488[b]	38.60 ± 0.150[c]	39.12 ± 0.238[d]	<0.001	<0.001	<0.001	0.316	0.002	-0.108
Max T_{core} (°C)	38.74 ± 0.190[a]	39.70 ± 0.513[b]	38.64 ± 0.138[c]	39.17 ± 0.229[d]	<0.001	<0.001	<0.001	0.333*	-0.007	-0.091
Heart rate avg (0-30 minutes)		127.3 ± 45.4		107.8 ± 42.3				(r_7)0.783**	0.569	-0.546

Table 5. CATTLE: NON-GRIP VS. GRIP FLOORING. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Raw values				R-M ANOVA results (*p* values)			Correlation with QBA scores (Pearson's *r*) (r_{26})		
	Non-grip		Grip/ Habituated		Treatment (Naïve vs. habituated)	Time (Before vs. after)	Treatment x time interaction			
	Before	After	Before	After				GPA 1	GPA 2	GPA 3
Hormones										
Prolactin (ng/ml)	39.29 ± 24.73	13.47 ± 9.09	25.85 ± 18.73	17.69 ± 15.56	0.064	<0.001	0.0499	-0.315	0.056	0.208
Metabolites										
β-hydroxy butyrate (mmol/L)	0.23 ± 0.08	0.22 ± 0.05	0.22 ± 0.06	0.18 ± 0.05	0.133	<0.001	0.0502	0.154	-0.224	0.078
Glucose (mmol/L)	5.60 ± 0.38	5.59 ± 0.41	5.66 ± 0.39	5.60 ± 0.46	0.644	0.582	0.769	0.207	-0.038	-0.349*
Haematological parameters										
White blood cell (x 10^9/L)	9.76 ± 1.62	10.19 ± 1.50	9.69 ± 0.80	11.37 ± 0.90	0.110	<0.001	0.018	-0.403*	-0.021	-0.003
Eosinophils (x 10^9/L)	0.88 ± 0.22	0.8 ± 0.27	0.91 ± 0.22	0.83 ± 0.21	0.018	0.968	0.070	-0.092	0.068	0.061
Monocytes (x 10^9/L)	2.84 ± 1.69	2.21 ± 1.43	2.62 ± 1.05	1.94 ± 1.10	0.318	<0.001	0.867	0.098	0.029	-0.182
Neutrophils (x 10^9/L)	32.89 ± 7.61	37.93 ± 8.51	33.31 ± 7.49	42.94 ± 8.08	0.139	<0.001	0.025	0.520**	0.014	0.182
Neutrophil: Lymphocyte	2.59 ± 0.67	2.75 ± 0.75	2.79 ± 0.73	2.31 ± 0.41	0.552	0.333	0.065	0.517**	-0.087	-0.200
Body temperature	Before [a]	During [b]	Before [a]	During [b]					(r_{24})	
Avg T_{core} (°C)	38.55 ± 0.03	39.08 ± 0.06	38.60 ± 0.04	39.12 ± 0.06	<0.001	<0.001	0.66	0.085	0.039	0.125
Max T_{core} (°C)	38.65 ± 0.13	39.12 ± 0.24	38.64 ± 0.14	39.17 ± 0.23	0.146	<0.001	0.06	0.031	0.024	0.133
Heart rate avg (0-30 minutes)		107.7 ± 70.0		107.8 ± 42.3				(r_7)0.008	0.211	0.592*

Table 6. CATTLE: STOP-START VS. CONTINUOUS DRIVING. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Raw values				R-M ANOVA results (*p* values)			Correlation with QBA scores (Pearson's *r*) (r_{26})		
	Stop-Start		Continuous		Treatment (Naïve vs. habituated)	Time (Before vs. after)	Treatment x time interaction			
	Before	After	Before	After				GPA 1	GPA 2	GPA 3
Hormones										
Cortisol (ng/ml)	26.37 ± 18.67	31.21 ± 32.27	23.82 ± 11.55	27.59 ± 23.27	0.745	0.724	0.875	0.084	-0.154	0.409*
IGF-1 (ng/ml)	58.81 ± 16.72	60.17 ± 23.49	50.91 ± 16.35	51.63 ± 14.13	0.003	0.341	0.843	0.088	-0.218	-0.019
Prolactin (ng/ml)	25.5 ± 18.21	22.89 ± 17.80	21.01 ± 20.18	14.53 ± 7.58	0.012	0.163	0.622	-0.193	-0.029	-0.023
Metabolites										
β-hydroxy butyrate (mmol/L)	0.21 ± 0.07	0.19 ± 0.05	0.21 ± 0.06	0.19 ± 0.05	0.958	0.002	0.937	-0.207	0.263	-0.207
Haematological parameters										
Haematocrit (%)	0.34 ± 0.01	0.34 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.006	0.966	0.681	-0.046	-0.345*	-0.024
White blood cell (x 10^9/L)	9.71 ± 1.03	10.93 ± 1.24	9.79 ± 1.50	10.87 ± 1.46	0.418	<0.001	0.626	-0.032	-0.342*	0.035
Eosinophils (x 10^9/L)	0.82 ± 0.15	0.78 ± 0.18	0.91 ± 0.21	0.86 ± 0.24	<0.001	0.493	0.877	-0.046	-0.180	-0.069
Monocytes (x 10^9/L)	3.89 ± 2.78	2.51 ± 2.33	4.62 ± 4.54	3.51 ± 3.72	0.183	<0.001	0.490	-0.280	0.015	0.119
Neutrophils (x 10^9/L)	31.06 ± 5.05	40.20 ± 7.54	29.54 ± 6.57	37.98 ± 7.57	0.728	<0.001	0.444	-0.027	-0.429*	0.078
Lymphocytes (x 10^9/L)	60.25 ± 5.38	52.59 ± 6.59	60.84 ± 6.66	53.91 ± 6.11	0.123	0.106	0.886	0.347*	0.045	-0.032
Neutrophil: Lymphocyte	3.03 ± 0.99	2.88 ± 0.37	3.14 ± 1.18	2.86 ± 0.96	0.821	0.311	0.742	0.126	0.345*	-0.151
Body temperature	Before [a]	During [b]	Before [a]	During [b]					(r_{24})	
Avg T_{core} (°C)	38.62 ± 0.10	39.04 ± 0.32	38.63 ± 0.10	38.85 ± 0.38	<0.001	<0.001	<0.001	-0.038	0.215	-0.121
Max T_{core} (°C)	38.67 ± 0.11	39.07 ± 0.34	38.67 ± 0.10	38.91 ± 0.36	0.008	0.008	0.002	-0.033	0.219	-0.041

3.3.4 Sheep: Naïve and habituated to road transport (psychological stressor)

The GPA consensus profile explained 53.03 % of the variation among the 63 observers, and this differed significantly from the mean randomised profile (t_{99}=87.5, p<0.001).

Terms associated with each GPA dimension are shown in Table 7; significant treatment differences are indicated in the right hand column.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 8. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 8). Correlations were observed between the GPA scores and heart rate (HR and HRV), T_{core}, IGF-1 concentration, neutrophil: lymphocyte ratio, monocyte and basophil numbers.

3.3.5 Sheep: Open or closed ventilation (physiological stressor)

The GPA consensus profile explained 48.7 % of the variation among the 57 observers and this differed significantly from the mean randomised profile (t_{99}=67.3, p<0.001).

Terms associated with each GPA dimension are shown in Table 7. There were no significant differences between sheep during the closed and open ventilation treatments on any of the dimensions.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 9. Correlations were observed between the GPA scores and heart rate, leptin, RBC, neutrophils and lymphocytes (and the neutrophil: lymphocyte ratio).

3.3.6 Sheep: Non-grip vs. grip flooring (physical stressor)

The GPA consensus profile explained 48.2 % of the variation among the 56 observers. This differed significantly from the mean randomised profile (t_{99}=61.57, p<0.001).

Terms associated with each GPA dimension are shown in Table 7; significant treatment differences are indicated in the right hand column.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 10. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 10). Correlations were observed between the QBA scores and heart rate (HR and HRV), T_{core}, IGF-1 concentrations, neutrophil: lymphocyte ratio, monocyte and basophil numbers.

3.3.7 Sheep: Stop-start vs. continuous driving (physical stressor)

The GPA consensus profile explained 51.0 % of the variation among the 52 observers and this was significantly different from the mean randomised profile (t_{99}=86.64, p<0.001).

Terms associated with each GPA dimension are shown in Table 7; significant treatment differences are indicated in the right hand column.

Significant *time*, *treatment* and *time x treatment* interaction effects for physiological data are shown in Table 8. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 11).

Table 7. Terms used by observers to describe SHEEP behavioural expression during FOUR TRANSPORT TREATMENTS. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column.

GPA dimension	Low values	High values	Treatment effects
Naïve vs. habituated to transport			
1 (42.6 %)	Calm (17), relaxed (12), happy (9), bored (9), comfortable (6), content (4), sleepy (3), quiet (2), at ease (2), steady (2), chilled out (2), sure (2), coping,, peaceful, balanced, accepting, chilled, mellow, assured, settled, tired, at peace, confident, in control, reassured	Anxious (17), nervous (12), worried (11), agitated (9), frightened (6), distressed (6), alert (6), scared (5), stressed (5), fearful (4), tense (4), concerned (3), apprehensive (3), alarmed (3), panicked (2), jittery (2), aware (2), perturbed (2), jumpy (2), distracted, flighty, attentive, disgruntled, intense, awake, startled, fretting, upset, terrified, wary, afraid, on guard, confused, defensive, looking for a way out	ns
2 (9.8 %)	Comfortable (3), tired (2), confident (2), happy (2), scared (2), anxious (2), annoyed (2), content (2), angry, concerned, avoiding, restful, determined, placid, calm, pissed off, defensive, mischievous, fearful, resigned, irritable, grumpy, upset, assured, busy, panic, aloof, withdrawn, absorbed, quiet, weary, occupied.	Alert (15), anxious (5), aware (5), curious (5), interested (4), watchful (3), confused (3), attentive (3), nervous (2), concerned (2), lost (2), observant (2), worried (2), frightened (2), awaiting, defensive, settled, thinking, expectant, inquisitive, defeated, wanting to escape, agitated, tense (2), penned, apprehensive, distressed, scared, bright, questioning, intrigued, afraid, cornered, dominant, definite, confident, fearful, wary	$F_{1,9}$=9.01, p=0.015* - naïve sheep scored higher cf. habituated sheep
3 (8.2 %)	Curious (7), alert (6), comfortable (5), aware (3), interested (3), relaxed (3), wary (3), inquisitive (3), sure (2), content (2), stressed (2), observant (2), happy (2), calm (2), tense (2), watchful, expectant, stable, conscious, in control, enduring, quizzical, certain, pleasant, dominating, satiated, pleased, looking for escape, confined, mad, active, sleepy, surprised, nervous, concerned, resigned, hesitant, satisfied, worried, confident, purposeful.	Frightened (4), agitated (3), afraid (2), tired (2), scared (2), disturbed (2), nervous (2), worried (2), stoic, sad, distracted, puzzled, depressed, anxious, comfortable, sleepy, terrified, certain, enclosed, steady, calm, lethargic, on edge, annoyed, secure, despair, alert, stressed, resigned, concerned, tense.	ns
Closed vs. open ventilation			
1 (36.3 %)	Relaxed (6), calm (4), sleepy (4), bored (3), comfortable (3), content (3), tired (2), quiet, doughy, resigned, settled, complacent, at ease, gentle, in control, steady, happy, pissed off, accepting	Alert (7), anxious (6), responsive (4), restless (3), aware (2), interested (2), agitated (2), curious (2), nervous (2), attentive, annoyed, bothered, flustered, confused, on guard, bright, on edge, wary, tense, concerned, worried, apprehensive, lost, startled, stressed, scared, trying to find a way out, jittery, watchful	ns
2 (15.6 %)	Happy (13), alert (12), curious (7), confident (6), calm (5), aware (4), content (4), attentive (3), interested (3), inquisitive (3), comfortable (3), at ease (3), relaxed (3), dominant (2), bright (2), sure (2), watchful (2), steady (2), watching, certain, wondering, awaiting, cool, balanced, authoritative, engaged, hungry, social, seeking distraction, restful, tolerating, responsive, collected	Worried (7), frightened (7), nervous (5), tired (4), scared (3), lonely (2), submissive (2), distressed (2), stressed (2), startled (2), sleepy (2), anxious (2), fatigued, apprehensive, doomed, lost, lethargic, overcome, wary, seeking comfort, cautious, defensive, trapped, confused, bored, flighty, terrified, panicked, despair, hopeless, seeking to escape, bewildered, tense, inhibited, irritated, oblivious, disorientated, exhausted, drowsy, concerned	ns
3 (6.8 %)	Frightened (2), nervous (2), stressed (2), tranquil, fed up, not fussed, exposed, at ease, calm, anxious, vulnerable, patient, sad, timid, happy, perturbed, relieved,	Curious (3), confused (2), flustered, frightened, excited, nervous, fed up , scared, timid, confident, distressed, sedate, depressed, happy, exhausted, dejected, terrified, annoyed,	ns

	wary, bossy, disturbed, content, trapped, antisocial, taking it easy, comfortable	bossy, calm, anxious, stable, composed, docile, concerned, adventurous, panicking, stressed, wondering, sleepy, assured, bored	
Non-grip vs. grip flooring			
1 (40.6 %)	Calm (13), relaxed (10), comfortable (7), content (6), happy (5), bored (5), sleepy (3), steady (2), settled (2), sure (2), composed, non phased, patient, doughy, compliant, balanced, satisfied, placid, relieved, tired, coping, pleased	Anxious (9), agitated (7), worried (5), alert (5), nervous (4), stressed (4), confused (3), scared (3), frightened (3), concerned (2), distressed (2), defensive (2), afraid, distracted, wary, jittery, alarmed, petrified, fearful, panicked, questioning, active, braced, apprehensive, disorientated, bewildered, seeking to escape, seeking, restless, twitchy, fidgety, startled, excited, lost, jumpy	ns
2 (11.1 %)	Tired (3), passive (2), terrified (2), frightened (2), timid, apathetic, resigned, overcome, sedate, irritable, stressed, anxious, flighty, sleepy, depressed, fatigued, trapped, doomed, weary, defeated, scared, hopeless	Alert (17), curious (13), aware (6), interested (4), inquisitive (3), attentive (2), observant (2), anxious (2), calm (2), lost, bright, dominant, sure, protective, responsive, confused, stable, looking for a way out, exploring, stressed, concerned, nervous, searching, distracted, comfortable, content, wondering, sleepy	$F_{1,9}$=9.25, p=0.014* - grip flooring scored higher cf. non-grip flooring event
3 (6.5 %)	Interested (3), happy (2), aware (2), concerned (2), comfortable (2), scared (2), calm (2), depressed, inquisitive, despair, hungry, curious, playful, oblivious, nervy, frightened, exposed, thirstily, restful, alert, confused, responsive, dejected, aloof, observant, sleepy, bored, constricted, relaxed, wary, stiff, tired, worried	Worried (2), confused (2), tense (2), patient, claustrophobic, mad, alert, enclosed, comfortable, depressed, awaiting, taking it easy, sure, relaxed, distressed, concerned, defensive, investigative, fed up, at ease, watching, peaceful, jumpy, anxious, afraid, stoic, interested	ns
Stop-start vs. continuous driving			
1 (54.8 %)	Calm (12), relaxed (9), sleepy (6), comfortable (5), bored (5), content (5), happy (4), accepting (3), tired (3), settled (3), at ease (3), certain (2), sure (2), compliant, quiet, oblivious, reassured, submissive, lethargic, chilled out, composed, tranquil, doughy, taking it easy, drowsy, sluggish, confident, coping, resigned, placid, steady, no phased, serene, chilled, subdued, mellow	Alert (20), anxious (17), nervous (11), agitated (11), stressed (9), worried (9), concerned (8), curious (8), confused (7), aware (6), tense (6), wary (6), scared (5), frightened (5), fearful (4), flighty (4), startled (4), alarmed (3), responsive (3), awake (2), upset (2), jittery (2), afraid (2), panicked (2), attentive (2), apprehensive (2), bewildered (2), observant (2), disturbed (2), on guard (2), disorientated, distressed, bright, petrified, flustered, inquisitive, enquiring, twitchy, erratic, distracted, cautious, restless, fretting, conscious, wondering, jumpy, vulnerable, watchful, tentative	$F_{1,9}$=13.88,p=0.005** - stop-start event scored higher cf. the continuous driving event
2 (9.3 %)	Alert (5), calm (4), relaxed (4), comfortable (4), happy (3), content (3), at ease (2), aware (2), responsive (2), dominant (2), enduring, assured, resigned, tolerating, curious, stable, certain, confident, chilled out, peaceful, inquisitive, restful, sure, balanced, authoritative, bright, afraid, in control, trusting, passive, patient	Scared (6), terrified (5), worried (5), depressed (5), frightened (4), stressed (3), tense (2), looking for escape (2), distressed (2), nervous (2), sleepy (2), apprehensive, overcome, withdrawn, doomed, avoiding, determined, certain, braced, disorientated, pissed off, seeking comfort, exhausted, aloof, lethargic, fed up, purposeful, on edge, content, subdued, hopeless, antisocial, fearful, sad, lonely, self preservation, anxious, defensive, isolated	ns
3 (4.6 %)	Bored, cornered, annoyed, relaxed, frustrated, comfortable, dopey, aggressive, cramped, calm, settled, expectant	Excited (2), happy (2), agitated (2), worried (2), distressed (2), at ease, irritated, restless, watching, stressed, anxious, busy, tense, wired, dominant, occupied, safe, defensive, terrified, scared, sure	ns

Table 8. SHEEP: TRANSPORT-NAÏVE VS. TRANSPORT-HABITUATED. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Naïve		Habituated		Treatment (Naïve vs. habituated)	Time (Before vs. after)	Treatment x time interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
	Before	After	Before	After						
Hormones					Results of RM ANOVA; p values			Correlation coefficients		
[ACTH] (pg/ml)	387.2 ± 245.2	126.7 ± 50.3	200.6 ± 236.3	63.9 ± 51.2	<0.001	<0.001	0.773	0.027	-0.093	-0.143
[Cortisol] (ng/ml)	51.2 ± 27.1	50.0 ± 13.9	40.1 ± 24.3	21.4 ± 11.3	0.003	0.097	0.043	-0.152	0.022	-0.114
[IGF-1] (ng/ml)	31.4 ± 10.5	27.2 ± 10.7	40.7 ±13.8	36.8 ± 12.4	<0.001	0.008	0.882	-0.385 *	-0.001	-0.189
[Insulin] (µU/ml)	5.87 ± 1.75	6.36 ± 1.09	5.82 ± 1.19	5.75 ± 1.60	0.218	0.543	0.339	0.278	-0.157	-0.082
[Leptin] (ng/ml)	1.19 ± 0.25	1.18 ± 0.24	1.62 ± 0.28	1.39 ± 0.22	<0.001	<0.001	<0.001	0.034	0.261	-0.163
Metabolites										
[β-OH] (mmol/L)	0.28 ± 0.06	0.28 ± 0.09	0.26 ± 0.06	0.24 ± 0.06	0.067	0.619	0.744	0.129	0.282	-0.099
[Glucose] (mmol/L)	4.76 ± 1.15	5.32 ± 1.35	3.91 ± 0.36	4.37 ± 0.66	<0.001	0.133	0.866	0.044	-0.012	0.217
Haematological parameters										
Haematocrit	0.39 ± 0.02	0.36 ± 0.02	0.35 ± 0.02	0.32 ± 0.02	<0.001	<0.001	0.583	-0.174	0.336	-0.017
Red blood cell (x 1012/L)	10.12 ± 0.66	9.40 ± 0.65	9.58 ± 0.51	8.77 ± 0.46	<0.001	<0.001	0.571	-0.293	0.348	0.020
White blood cell (x 109/L)	8.10 ± 1.73	8.84 ± 1.79	8.04 ± 1.58	7.59 ± 1.60	0.009	0.438	0.007	-0.067	0.115	-0.108
Eosinophils (x 109/L)	0.33 ± 0.13	0.25 ± 0.11	0.24 ± 0.14	0.16 ± 0.10	<0.001	0.002	0.845	-0.124	0.104	-0.325
Monocytes (x 109/L)	0.11 ± 0.04	0.09 ± 0.03	0.24 ± 0.17	0.16 ± 0.10	0.002	0.065	0.371	0.262	-0.409 *	-0.089
Basophils (x 109/L)	0.026 ± 0.013	0.035 ± 0.012	0.042 ± 0.016	0.039 ± 0.014	0.007	0.323	0.017	-0.223	-0.296	-0.445 *
Neutrophils (x 109/L)	2.85 ± 1.67	4.80 ± 1.68	2.22 ± 0.82	2.90 ± 1.34	<0.001	<0.001	0.002	-0.193	0.513 *	-0.384 *
Lymphocyte (x 109/L)	4.91 ± 0.98	4.13 ± 1.06	5.70 ± 1.17	4.99 ± 1.22	<0.001	0.002	0.635	0.338	-0.542 **	0.138
Neutrophil: Lymphocyte	0.60 ± 0.37	1.24 ± 0.57	0.40 ± 0.15	0.62 ± 0.34	<0.001	<0.001	<0.001	-0.320	0.673 ***	-0.399 *
	Before	During	Before	During						
HR (bpm)	65.8 ± 7.29	112.9 ± 32.7	72.6 ± 18.5	77.2 ± 11.1	0.059	0.002	0.008	0.755 ***	0.163	0.056
HRV (SDNN) (ms)	61.58 ± 31.23	84.56 ± 40.64	62.25 ± 26.79	47.52 ± 24.69	0.133	0.728	0.120	0.766 ***	-0.219	-0.038
Tcore average (ºC)	39.29 ± 0.17	40.00 ± 0.24	39.36 ± 0.16	39.50 ± 0.17	<0.001	<0.001	<0.001	0.417 *	0.390 *	-0.125
Tcore maximum (ºC)	39.36 ± 0.18	40.16 ± 0.24	39.48 ± 0.19	39.62 ± 0.15	0.001	<0.001	<0.001	0.430 *	0.425 *	0.209

HR & HRV (before): 10-5 min. before departure — Tcore (before): 40 min. at same time of day on non-transport day

HR & HRV (during): 5-10 min. after departure — Tcore (during): 0-40 min. after departure

Table 9. SHEEP: CLOSED VENTILATION VS. OPEN VENTILATION. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Open ventilation		Closed ventilation		Treatment (Closed vs. open ventilation)	Time (Before vs. after)	Treatment x time interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
	Before	After	Before	After						
Hormones & metabolites					Results of RM ANOVA p values			Correlation coefficients		
[Leptin] (ng/ml)	1.62 ± 0.28	1.39 ± 0.22	1.37 ± 0.23	1.31 ± 0.18	<0.001	<0.001	<0.001	-0.542 *	0.279	0.225
[Cortisol] (ng/ml)	40.1 ± 24.3	21.4 ± 11.3	47.6 ± 29.2	27.3 ± 12.8	0.371	<0.001	0.875	0.004	-0.161	0.068
[IGF-1] (ng/ml)	40.7 ±13.8	36.8 ± 12.4	32.1 ± 9.5	29.9 ± 10.7	<0.001	0.021	0.429	0.122	0.192	-0.012
[Insulin] (µU/ml)	5.82 ± 1.19	5.75 ± 1.60	5.50 ± 1.30	6.32 ± 1.89	0.776	0.346	0.038	-0.049	-0.038	-0.068
[β-OH] (mmol/L)	0.26 ± 0.06	0.24 ± 0.06	0.30 ± 0.07	0.34 ± 0.07	<0.001	0.427	0.031	-0.266	-0.230	0.021
[Glucose] (mmol/L)	3.91 ± 0.36	4.37 ± 0.66	3.89 ± 0.22	3.97 ± 0.27	0.054	0.009	0.020	-0.183	0.061	-0.182
Haematological parameters										
Haematocrit	0.35 ± 0.02	0.32 ± 0.02	0.35 ± 0.02	0.33 ± 0.02	0.105	<0.001	0.019	-0.261	-0.212	0.074
Red blood cell (x 1012/L)	9.58 ± 0.51	8.77 ± 0.46	9.30 ± 0.44	8.77 ± 0.62	0.209	<0.001	0.052	-0.134	-0.399 *	-0.089
White blood cell (x 109/L)	8.04 ± 1.58	7.59 ± 1.60	6.70 ± 0.87	6.99 ± 0.89	0.022	0.557	0.040	0.133	0.072	0.193
Eosinophils (x 109/L)	0.24 ± 0.14	0.16 ± 0.10	0.27 ± 0.13	0.16 ± 0.06	0.559	0.001	0.606	0.332	-0.042	0.106
Monocytes (x 109/L)	0.24 ± 0.17	0.16 ± 0.10	0.17 ± 0.07	0.15 ± 0.07	0.177	0.140	0.364	-0.015	-0.043	0.035
Basophils (x 109/L)	0.042 ± 0.016	0.039 ± 0.014	0.030 ± 0.008	0.029 ± 0.014	0.002	0.246	0.893	-0.046	0.135	0.277
Neutrophils (x 109/L)	2.22 ± 0.82	2.90 ± 1.34	2.27 ± 0.57	2.49 ± 1.09	0.380	0.033	0.044	0.228	-0.056	-0.494 *
Lymphocytes (x 109/L)	5.70 ± 1.17	4.99 ± 1.22	4.71 ± 0.73	4.91 ± 0.88	0.057	0.195	0.019	0.041	0.067	0.396 *
Neutrophil: Lymphocyte	0.40 ± 0.15	0.62 ± 0.34	0.49 ± 0.14	0.55 ± 0.32	0.772	0.051	0.018	0.074	-0.083	-0.463 *
	Before	During	Before	During						
HR (bpm)	72.6 ± 18.5	70.2 ± 12.9	77.0 ± 16.5	80.7 ± 14.4	0.160	0.893	0.565	-0.569 *	-0.237	0.223
HRV (SDNN)(ms)	62.25 ± 26.79	60.29 ± 27.53	61.65 ± 32.11	89.56 ± 51.25	0.232	0.279	0.214	-0.280	0.221	-0.091
Tcore average (ºC)	39.45 ± 0.22	39.26 ± 0.20	38.91 ± 0.18	39.36 ± 0.21	<0.001	<0.001	<0.001	0.108	0.096	0.073
Tcore maximum (ºC)	39.54 ± 0.21	39.35 ± 0.21	38.99 ± 0.17	39.49 ± 0.18	<0.001	0.031	<0.001	0.142	0.088	0.046

HR & HRV (before): 10-5 min. before departure
HR & HRV (during): 70-75 min. after departure
Tcore (before): 40 min. at same time of day on non-transport day
T_{core} (during): 40-80 min. after departure

Table 10. SHEEP: NON-GRIP FLOORING VS. GRIP FLOORING. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Non-grip flooring		Grip flooring		Treatment (Non-grip vs. grip flooring)	Time (Before vs. after)	Treatment x time interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
	Before	After	Before	After						
Hormones & metabolites					Results of RM ANOVA p values			Correlation coefficients		
[Leptin] (ng/ml)	1.62 ± 0.28	1.39 ± 0.22	1.51 ± 0.28	1.44 ± 0.20	0.309	<0.001	<0.001	0.564 *	0.241	-0.169
[Cortisol] (ng/ml)	40.1 ± 24.3	21.4 ± 11.3	59.14 ± 12.7	36.82 ± 13.6	0.005	<0.001	0.659	0.045	-0.295	0.008
[IGF-1] (ng/ml)	40.7 ±13.8	36.8 ± 12.4	36.5 ± 10.9	33.0 ± 9.4	0.042	<0.001	0.915	0.384 *	-0.277	0.038
[Insulin] (µU/ml)	5.82 ± 1.19	5.75 ± 1.60	5.93 ± 1.58	6.51 ± 1.56	0.233	0.459	0.283	0.117	0.184	0.010
[β-OH] (mmol/L)	0.26 ± 0.06	0.24 ± 0.06	0.32 ± 0.07	0.38 ± 0.08	<0.001	0.029	0.030	0.247	0.354	-0.230
[Glucose] (mmol/L)	3.91 ± 0.36	4.37 ± 0.66	4.60 ± 0.62	3.96 ± 0.32	0.135	0.258	0.002	-0.454 *	-0.332	0.007
Haematological parameters										
Haematocrit	0.35 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.33 ± 0.02	0.003	0.051	<0.001	0.468 *	0.563 *	0.104
Red blood cell (x 1012/L)	9.58 ± 0.51	8.77 ± 0.46	8.40 ± 0.38	8.75 ± 0.55	<0.001	0.035	<0.001	0.422 *	0.522 *	0.060
White blood cell (x 109/L)	8.04 ± 1.58	7.59 ± 1.60	6.72 ± 1.24	7.16 ± 1.21	0.036	0.999	0.188	0.133	0.252	-0.312
Eosinophils (x 109/L)	0.24 ± 0.14	0.16 ± 0.10	0.14 ± 0.11	0.19 ± 0.12	0.324	0.414	0.036	0.085	0.199	-0.302
Monocytes (x 109/L)	0.24 ± 0.17	0.16 ± 0.10	0.18 ± 0.08	0.21 ± 0.12	0.823	0.549	0.132	0.570 **	-0.122	-0.006
Basophils (x 109/L)	0.042 ± 0.016	0.039 ± 0.014	0.033 ± 0.014	0.036 ± 0.016	0.049	0.899	0.388	-0.042	0.094	-0.177
Neutrophils (x 109/L)	2.22 ± 0.82	2.90 ± 1.34	2.38 ± 0.99	1.97 ± 0.67	0.189	0.561	0.005	-0.045	-0.084	-0.509 *
Lymphocytes (x 109/L)	5.70 ± 1.17	4.99 ± 1.22	4.57 ± 1.07	5.35 ± 1.14	0.159	0.877	0.023	0.089	0.271	-0.188
Neutrophil: Lymphocyte	0.40 ± 0.15	0.62 ± 0.34	0.56 ± 0.32	0.39 ± 0.17	0.636	0.756	0.001	-0.087	-0.189	0.191
	Before	During	Before	During						
HR (bpm)	72.6 ± 18.5	77.2 ± 11.1	83.0 ± 12.9	98.3 ± 26.6	0.009	0.091	0.362	-0.260	0.196	-0.299
HRV (SDNN) (ms)	62.25 ± 26.79	47.52 ± 24.69	96.38 ± 40.20	76.94 ± 19.16	0.001	0.065	0.794	0.384 *	0.238	0.069
Tcore average (ºC)	39.36 ± 0.16	39.50 ± 0.17	39.05 ± 0.14	39.53 ± 0.13	<0.001	<0.001	<0.001	0.422 *	0.445 *	-0.204
Tcore maximum (ºC)	39.48 ± 0.19	39.62 ± 0.15	39.18 ± 0.14	39.63 ± 0.13	<0.001	<0.001	<0.001	0.430 *	0.390 *	-0.041

HR & HRV (before): 10-5 min. before departure — T_{core} (before): 40 min. at same time of day on non-transport day

HR & HRV (during): 5-10 min. after departure — T_{core} (during): 0-40 min. after departure

Table 11. SHEEP: STOP-START VS. CONTINUOUS DRIVING. Left hand columns give the mean (± 1 SD) hormonal and metabolite variables for hormones, metabolites and haematological parameters (collected before and after transport), heart rate and body temperature (measured before and during transport). Central columns indicate the results of Repeated-Measures ANOVA testing for the effects of *treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Continuous driving		Stop-start driving		Treatment (Stop-start vs. continuous)	Time (Before vs. after)	Treatment x time interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
	Before	After	Before	After						
Hormones & metabolites					Results of RM ANOVA *p* values			Correlation coefficients r_{18}		
[Leptin] (ng/ml)	1.62 ± 0.28	1.39 ± 0.22	1.44 ± 0.18	1.37 ± 0.19	0.009	<0.001	<0.001	0.379 *	0.203	-0.359
[Cortisol] (ng/ml)	40.1 ± 24.3	21.4 ± 11.3	48.6 ± 24.3	37.5 ± 18.2	0.107	0.005	0.254	0.007	-0.121	-0.537
[IGF-1] (ng/ml)	40.7 ±13.8	36.8 ± 12.4	35.4 ± 12.2	33.5 ± 10.4	0.004	0.020	0.386	0.045	-0.015	-0.063
[Insulin] (μU/ml)	5.82 ± 1.19	5.75 ± 1.60	5.97 ± 1.59	6.38 ± 1.15	0.192	0.656	0.356	-0.065	-0.352	0.106
[β-OH] (mmol/L)	0.26 ± 0.06	0.24 ± 0.06	0.31 ± 0.07	0.29 ± 0.08	<0.001	0.180	0.737	0.021	0.257	0.203
[Glucose] (mmol/L)	3.91 ± 0.36	4.37 ± 0.66	3.82 ± 0.20	4.53 ± 0.83	0.533	0.005	0.121	-0.281	0.443 *	-0.172
Haematological parameters										
Haematocrit	0.35 ± 0.02	0.32 ± 0.02	0.34 ± 0.02	0.32 ± 0.02	<0.001	<0.001	0.078	-0.102	0.188	0.345
Red blood cell (x 10^{12}/L)	9.58 ± 0.51	8.77 ± 0.46	9.14 ± 0.52	8.68 ± 0.46	<0.001	<0.001	0.018	0.079	0.111	0.260
White blood cell (x 10^{9}/L)	8.04 ± 1.58	7.59 ± 1.60	7.04 ± 1.22	7.32 ± 1.16	0.019	0.564	0.017	-0.038	0.111	0.040
Eosinophils (x 10^{9}/L)	0.24 ± 0.14	0.16 ± 0.10	0.22 ± 0.10	0.15 ± 0.09	0.448	0.004	0.221	-0.181	-0.334	0.088
Monocytes (x 10^{9}/L)	0.24 ± 0.17	0.16 ± 0.10	0.18 ± 0.08	0.17 ± 0.05	0.525	0.120	0.333	0.199	-0.278	-0.149
Basophils (x 10^{9}/L)	0.042 ± 0.016	0.039 ± 0.014	0.039 ± 0.020	0.036 ± 0.015	0.191	0.209	0.827	-0.405 *	-0.023	0.039
Neutrophils (x 10^{9}/L)	2.22 ± 0.82	2.90 ± 1.34	2.01 ± 0.59	2.77 ± 1.03	0.163	<0.001	0.406	-0.010	-0.012	0.361
Lymphocytes (x 10^{9}/L)	5.70 ± 1.17	4.99 ± 1.22	5.33 ± 1.07	4.90 ± 1.06	0.120	0.020	0.327	-0.407 *	-0.059	-0.186
Neutrophil: Lymphocyte	0.40 ± 0.15	0.62 ± 0.34	0.39 ± 0.13	0.61 ± 0.33	0.732	0.004	0.900	0.233	0.018	0.364
	Before	During	Before	During						
HR (bpm)	72.6 ± 18.5	72.1 ± 15.3	78.1 ± 16.6	95.2 ± 24.7	0.021	0.169	0.148	0.054	-0.128	-0.012
HRV (SDNN) (ms)	62.25 ± 26.79	77.50 ± 31.35	71.23 ± 32.04	91.56 ± 25.19	0.202	0.052	0.776	-0.402 *	-0.189	0.234
T_{core} average (ºC)	39.45 ± 0.22	39.26 ± 0.20	38.91 ± 0.18	39.49 ± 0.26	<0.001	0.015	<0.001	0.483 *	0.383 *	-0.301
T_{core} maximum (ºC)	39.54 ± 0.21	39.35 ± 0.21	38.99 ± 0.17	39.64 ± 0.32	0.002	0.007	<0.001	0.490 *	0.334	-0.267

HR & HRV (before): 10-5 min. before departure T_{core} (before): 40 min. at same time of day on non-transport day
HR & HRV (during): 45-50 min. after departure T_{core} (during): 40-80 min. after departure

3.4 Discussion

3.4.1 Cattle

There was consensus between observers in all studies in their assessment of the behavioural expression of the cattle, with the GPA consensus profile explaining around half the variation in scores between the observers.

Observers were able to distinguish between cattle exposed to both psychological (naïve vs. habituated) and physical (non-grip vs. grip flooring, and stop-start vs. continuous driving) transport stressors based on their behavioural expression. For example, naïve animals scored higher on GPA dimension 1 (i.e. observed to be more *agitated, restless, stressed*) cf. habituated animals which scored lower on this dimension (i.e. observed to be more *calm, comfortable, relaxed*).

Transport resulted in significantly altered physiological variables typically associated with the stress response. Finally, QBA scores were correlated with physiological parameters, suggesting that the QBA process was detecting behavioural manifestations of stress.

Road transport was a useful model that provided significant manifestations of stress, indicated by both physiological and behavioural measures. However, this study did not investigate the capacity for use of QBA within the road transport industry since we could not adequately replicate commercial transport conditions within the logistical constraints of our experimental design. The extension of this study to commercial conditions requires further investigation.

3.4.2 Sheep

There was consensus between observers in all studies in their assessment of the behavioural expression of the sheep, with the GPA consensus profile explaining around half the variation in scores between the observers.

Observers were able to distinguish between sheep exposed to both psychological (naïve vs. habituated) and physical (non-grip vs. grip flooring, and stop-start vs. continuous driving) transport stressors based on their behavioural expression. However observers were not able to distinguish between animals exposed to the open or closed ventilation treatments; the physiological parameters also suggest that the sheep may not have found this treatment particularly challenging.

Meaningful associations were found between the QBA scores and physiological variables. Correlations were observed between the GPA scores and leptin concentration (all treatments), glucose concentration (ventilation and flooring), IGF-1 (flooring), white blood cell profile (all treatments), body temperature (ventilation and flooring) and heart rate or heart rate variability (all treatments). For example, sheep that were described as more *nervous, worried, alert, anxious* or *aware* demonstrated reduced IGF-1 concentrations, an increased stress leukogram (i.e. elevated neutrophil: lymphocyte ratio, reduced monocyte numbers), and increased body temperature and heart rate. These physiological variables, which have been associated with a stress response, varied in response to the short (90-minute) transport events.

The substantial differences between treatments suggest that the interpretation of behavioural expression can be extremely sensitive to subtle responses to environmental changes.

4 Validation of QBA in cattle pre slaughter

4.1.1 Background

All production animals will experience some level of stress when exposed to the slaughter process and this can have detrimental effects on meat quality. Animals can be exposed to a range of challenging stimuli during this time, including mustering, transport, handling, increased human contact, novel/unfamiliar environments, food and water deprivation, changes in social structure (i.e. through separation and mixing), and changes in climatic conditions [26]. These factors can influence animal welfare and resulting physiology can have a detrimental impact on meat quality.

We investigated whether QBA could be used as a welfare measure during lairage immediately before slaughter. The present experiment was carried out in collaboration with **researchers in meat science at Murdoch University**. The aims of this experiment are to:

1. determine whether QBA-suitable footage of cattle could be collected as part of pre-slaughter handling
2. determine if there is a correlation between behavioural expression and physiological and behavioural (e.g. temperament scoring) measures

4.1.2 Methodology

Animals

28 Angus steers were slaughtered at two years of age (live weight of 523±39.7 kg). Cattle were transported to a commercial abattoir by a commercial transport company the day before slaughter and held in lairage overnight in four adjacent pens.

Temperament testing

Temperament was assessed using flight speed [30], crush agitation scores [31] and tension scores; tension score was assessed by the pressure the animal exerted on the crush when they had their head constrained by the crush. The assessment was made visually on a scale of 1 to 5. Temperament assessments were made 3 times over a period of 3 weeks around weaning and these measurements were averaged to give weaning temperament scores. Temperament tests were also carried out at 12 and 16 months.

Physiology

Muscle samples were taken from the semimebranosis (SM) and semitendonosus (ST) muscles of all cattle via biopsy (7 days before slaughter) and at slaughter. The steers remained on the same type of pasture for the 7 days following muscle sample biopsy, before slaughter. Muscle glycogen and lactate concentration were determined for each muscle biopsy, and net glycogen loss from biopsy to slaughter was calculated [32].

A blood sample at slaughter was taken at exsanguination, shortly after the animals were captive bolted, from 26 of the 28 cattle. Blood was centrifuged and the harvested plasma frozen at -80°C for later laboratory determination of glucose, lactate, β hydroxy butyrate, NEFA and cortisol concentration [32].

Qualitative Behavioural Assessment

Video footage was taken of cattle in a forcing pen as they were being moved toward the abattoir for slaughter. Cattle were moved into the forcing pen as a group and cattle that had visible tags were filmed for QBA. One video clip (between 30 seconds and 1 minute long) was chosen for each animal. In total there were 28 clips suitable for QBA.

Fifteen observers were recruited from University staff and students and members of the public. Each observer was required to complete a term generation session and a subsequent quantification session by correspondence. The two sessions are detailed below:

1. Term generation - observers were shown 8 video clips of the experimental cattle demonstrating a wide range of behavioural expressions. The 15 observers generated a total of 75 unique terms (average: 13±5 terms per observer, min: 8, max: 27)

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).

4.1.3 Results

The GPA consensus profile explained 43.7% of the variation among the 15 observers scores, and this differed significantly from a mean randomised profile (t_{99}=24.67, p<0.001).

Terms with the strongest correlation with each of the GPA dimensions are shown in Table 1; significant correlations between GPA scores and physiological and temperament measurements are indicated in the right hand column.

Table 1. Terms used by observers to describe CATTLE behavioural expression during PRE-SLAUGHTER LAIRAGE. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column.

GPA dimension	Low values	High values	Correlated with
1 (58.8%)	Calm (9), relaxed (5), content (3), comfortable (3), quiet (2), settled, unphased, aware, passive, willing, placid, bored, submissive, happy	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	- Slaughter order: cattle slaughtered later were attributed higher scores - Plasma lactate at the time of slaughter positively correlated - Plasma NEFA negatively correlated
2 (9.2%)	Annoyed, frightened, submissive, stressed, agitated, disturbed, scared	interested (2), curious (2), alert (2), wary, anxious, comfortable, alert, agitated, relaxed, contemplating, calm, dominant, wondering, impatient	- Ultimate pH negatively correlated - Tension score at weaning: cattle that exerted more pressure on the crush scored higher - yearling crush score positively correlated
3 (6.5%)	Comfortable, alert, calm, relaxed, scared, wondering	Anxious, nervous, scared, frightened, agitated, uneasy, worried	- Plasma glucose: cattle with high plasma glucose scored lower - Plasma lactate at the time of slaughter was negatively correlated - flight speed positively correlated

Table 2. Significant correlations between physiological parameters and GPA dimension 1, 2 and 3. Significant correlations are indicated in bold (r^2_{24} for plasma metabolites and biopsy cortisol and r^2_{26} for all remaining variables).

		GPA dimension 1	GPA dimension 2	GPA dimension 3
Plasma Glucose	Biopsy	-0.085	-0.306	-0.454**
	Slaughter	0.071	0.187	-0.423*
Plasma Lactate	Slaughter	0.453*	0.315	-0.425*
Plasma NEFA	Biopsy	-0.365*	-0.113	0.025
Muscle pH	Post mortem	0.065	-0.395*	0.029
Slaughter order		0.442**	-0.114	0.012
Birth weight		-0.302	0.113	0.530**
Weaning tension score		-0.313	0.349*	-0.042
Yearling crush score		-0.026	0.334*	-0.067
Flight speed (16 months)		-0.132	-0.199	0.468**

4.1.4 Discussion

Cattle demonstrated a range of behavioural expression in response to being moved through handling facilities toward an abattoir and observers' assessment of the behavioural expression of these cattle was both consistent and repeatable. One of the most marked findings of this study was that observers distinguished differences in the behavioural expression of cattle which were significantly correlated with their slaughter order. Cattle that were slaughtered towards the end of the line (scored as more *nervous, anxious* and *scared*; GPA dimension 1) 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. Since both noise and handling or forcing animals up in a race are major stressors for cattle [26,33] it is likely that longer exposure would result in amplified stress responses indicated by differences in behavioural expression.

Analysis of correlations between temperament and behavioural expression indicates that cattle that had higher tension scores following weaning were also attributed significantly higher GPA 2 scores (i.e. were scored as more *interested, curious*) when observed during the slaughter process. The curious energy observed in the cattle in the present study has been observed previously in cattle exposed to lairage and other novel environments, and is commonly associated with behaviour including sniffing, baulking or backing up when exposed to the novel environment or object [34-36]. This can translate into difficulty in driving the animals due to baulking, a problem that has been linked to temperament [37].

Cattle attributed significantly higher GPA dimension 1 scores (i.e. scored as more *nervous, anxious, scared*) also had high plasma lactate concentrations. Increased plasma lactate is indicative of a corticosteroid–mediated stress response [38]. The negative correlation of plasma glucose with GPA dimension 3 does not follow this logic, most likely because dimension 3 explains very little of the variation between the animals and the relationship was largely driven by two outliers (individuals that received extremely low GPA 3 scores).

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 [39,40]. In the present study, it was found that one individual had high ultimate muscle pH resulting in it being scored as a dark cutter. This animal was also attributed the lowest GPA 2 score (i.e. was scored as more *annoyed, frightened*) and was responsible for the significant correlation between GPA 2 scores and muscle pH.

5 Beef CRC fear experiment

5.1 QBA of cattle as part of the Beef CRC fear experiment

5.1.1 Background

Fear is a powerful motivator to action to avoid the perceived threat, i.e. the fight or flight response stimulated by the autonomic nervous system [41]. Production animals, derived mostly from ancestral prey species, are frequently exposed to novel environmental- and management-related procedures which are likely to result in fear and anxiety responses. Production animals, however, often experience restricted movement (i.e. preventing flight or fight responses) and animals may therefore have limited opportunity to respond to uncomfortable or fearful experiences. As a result of threats perceived to be uncontrollable or unavoidable, animals therefore cannot rest and remain in a state of tension or anxiety which has implications for animal welfare, management and productivity [6,25,42-45].

As with many emotions, fear and anxiety are extremely difficult motivational states to measure [2-6]. Established techniques for measuring fear responses largely require that the animals are exposed to a specific controlled environment to enable measurements to be collected (e.g. the novel arena test, novel object test, time to approach a handler, restraint test). However, there are many commercial industry situations that do not lend themselves to establishing specific environmental conditions or experimental test stimuli.

The present experiment was carried out in collaboration with the **Beef CRC at the Department of Primary Industries, Victoria**. The aims of this experiment are to:

1. determine if observers can reliably perceive the behavioural expression of beef cattle
2. determine whether there are differences in cattle behavioural expression depending on the nature or frequency of a novel stressor

5.1.2 Methodology

Nine female ~400 kg *Bos taurus* beef cattle (~12 months of age) were housed in 4x5 m individual pens (with access to an outdoor area) with visual but limited tactile contact with neighbours. Three *'chronic' fear provoking stimuli* were used in this experiment.

1. Animals were fitted with electric shock collars that delivered a stimulus similar to that used with commercial prodders that are routinely used for moving stock. The control group of animals had collars fitted but received no electrical stimulation.
2. A novel object stimulus involved dropping a multi-coloured plastic ball (200 mm diameter) and/or 10 L bucket (both attached to independent ropes) from the roof of the barn into the individual's pen. Thirty seconds later the ball and/or bucket were raised from the pen.
3. A high intensity flashing light stimulus.

Cattle were assigned into three 'chronic' treatment groups. Three individuals were assigned to the control group and were not exposed to any treatment over the four-week period. Two experimental treatment groups received a maximum of four (of the same) stimulus (between 9:00 am and 9:30 am) either three times in a week (n=3 animals) or five times in a week (n=3 animals). The animals were not constrained during this time and could choose to move or remain stationary in the pen in response to the stimulus.

Video footage and 'acute treatments'

Video footage was collected of cattle either in the absence of any stimulus (i.e. not between 9:00 am and 9:30 am; Acute treatment=0) or filmed during exposure to the novel object treatment (Acute treatment=1). For the purpose of this study, animals from all chronic treatment groups were filmed as they were exposed to this stimulus (although control animals had not previously been exposed to these stimuli during the preceding 4 weeks). In total, 17 video clips were collected (one individual was filmed only once) and were randomly sorted and presented to observers. At no point were observers given any information regarding the experimental treatments.

Qualitative Behavioural Assessment of cattle behavioural expression

Fourteen volunteer observers were recruited from around Murdoch University campus.

1. Term generation - Observers watched 9 clips (30-60 s in length) to generate their own individual descriptive terms. Observers generated between 8 to 24 terms.

2. Quantification – Observers watched and scored the 17 experimental clips shown in random order.

5.1.3 Results

There was significant consensus between observers (Procrustes statistic: 54.91%; *t*-test: t_{99}=15.76, p<0.001).

Terms associated with each GPA dimension are shown in Table 1; significant treatment differences are indicated in the right hand column. Average GPA scores for the treatment groups are shown in Figure 1.

Table 1. Terms used by observers to describe CATTLE behavioural expression during A CHRONIC FEAR TEST. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension); term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). A summary of mixed-model ANOVA testing for the affects of acute and chronic treatments upon QBA scores is shown in the right hand column; the three main consensus dimensions were each tested separately. The three levels of 'chronic' treatment were the control cattle and the cattle exposed to stimuli either 3x or 5x a week for four weeks. Acute treatments were 1=cattle filmed immediately after presentation of the novel and 0=no exposure to the novel object immediately before filming. Observer ID was included as a fixed factor in the analyses. Factors which statistically significantly influenced each GPA dimension scores are shown in bold.

GPA dimension	Low values	High values	Treatment effects:	
1 (37.0%)	Agitated (4), Apprehensive (2), Nervous (2), Tense (2), Wary (2), Alert, Annoyed, Anxious, Avoiding, Aware, Bored, Concerned, Defensive, Disturbed, Flighty, Frustrated, Hesitant, Irritated, Jumpy, Resigned, Shy, Stressed, Unsure	Comfortable (5), Relaxed (4), Happy (3), Calm (2), Content (2), At_Ease, Calm, Chilled_Out, Confident,	Chronic treatment Acute treatment Random effect – observer ID	$F_{2,26}$=21.91*** $F_{1,13}$=0.44 $F_{13,8}$<0.01
2 (27.5%)	Calm (3), At_Ease, Relaxed, Comfortable, Bored, Uninterested, Lethargic, Shy, Cornered, Defeated, Weary, Anxious	Curious (9), Alert (2), Inquisitive (2), Restless (2), Aggressive, Angry, Anxious, Aware, Bold, Bored, Bothered, Claustrophobic, Comfortable, Concerned, Confident, Content, Deranged, Distracted, Disturbed, Dominant, Frightened, Inquisitive, Irritated, Mad, Nervous, Observant, Restless, Uneasy, Watchful	Chronic treatment Acute treatment Random effect – observer ID i.e. Higher values in chronic treatment where animals were exposed to shock/novel objects 5x a week	$F_{2,26}$=14.92*** $F_{1,13}$=12.87** $F_{13,17}$<0.01
3 (9.5 %)	Bored (6), Resigned, Tired, Relaxed, Inquisitive, Dominant, Bothered, Annoyed, Angry, Curious, Confined	Agitated (3), Anxious (2), Nervous (2), At_Ease (2), Aggressive, Frightened, Defensive, Confused, Scared, Cautious, Tense, Happy, Comfortable	Chronic treatment Acute treatment Random effect – observer ID i.e. Lower values in chronic treatment where animals were exposed to shock/novel objects 5x a week, higher values in acute treatment where animals were confronted with a bucket	$F_{2,26}$=21.87*** $F_{1,13}$=28.27*** $F_{13,14}$<0.01

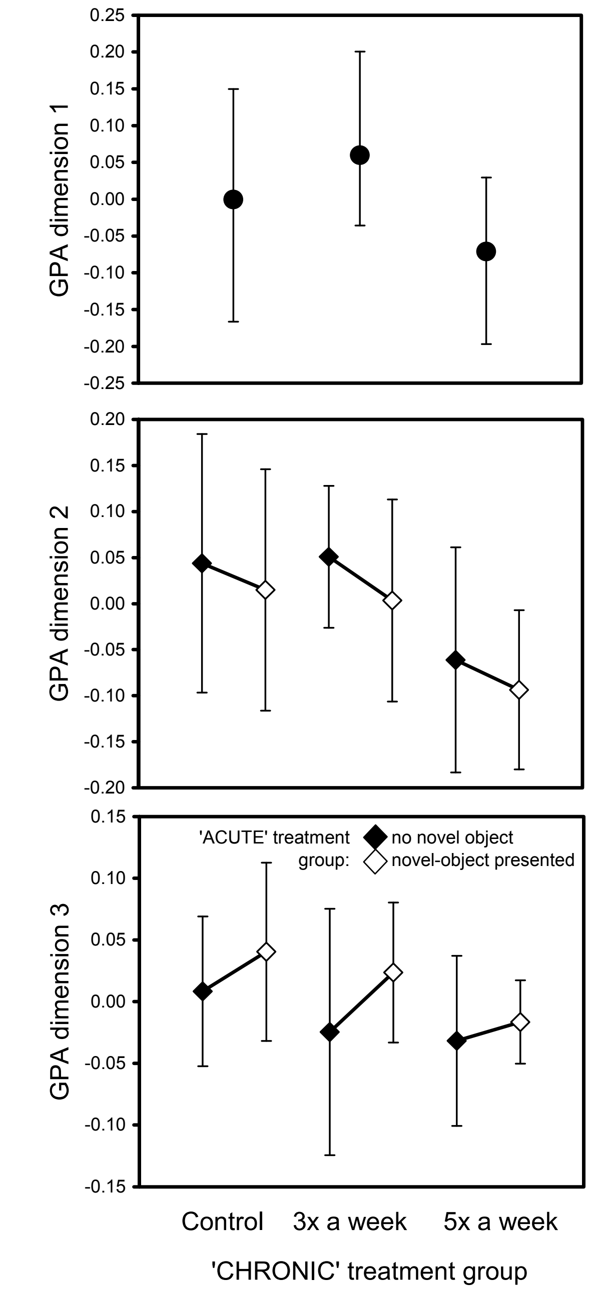


Figure 1. Average (± 1SD) QBA scores for cattle subjected to three 'chronic' treatment groups (control treatment or exposed to stimuli either three or five times a week). Cattle were either filmed immediately after they were presented with a novel object or without this additional 'acute' stimulus. The average values for each chronic treatment group are indicated for GPA dimension 1 since there was no significant effect of the acute treatment upon scores for this dimension. However, both acute and chronic treatments significantly affected scores for GPA dimensions 2 and 3 and therefore these data are shown separately.

5.1.4 Discussion

Both the QBA scores and physiology data measured one week after the end of the 4 week treatment period [46] suggest that there were significant treatment effects in this experiment, with animals in the extreme fear challenge treatment (stimuli 5x a week) demonstrating behaviour and physiology that suggests they were experiencing a greater level of fear over the experimental period. Animals in the extreme fear challenge treatment (stimuli 5x a week) demonstrated significantly higher daytime mean cortisol concentrations, an increased cortisol response to corticotrophic releasing hormone (CRH), reduced growth rate and a trend for an increased response to adrenocorticotrophic hormone (ACTH) compared to the control treatment. Cortisol and CRH response were also significantly higher for the 5x a week treatment group cf. the 3x a week treatment.

In conclusion, QBA is a potentially useful welfare assessment tool for assessment of cattle exposed to various fear-provoking stimuli. This suggests that QBA has been successful in this experimental situation and could be used as a welfare assessment tool to assess levels of fear or anxiety in cattle. The value of QBA is that this assessment may enable quantification of affective states (e.g. fear or anxiety) that are difficult to assess.

6 Validation of QBA under various nutritional stressors

It is common for livestock to be exposed to variation in nutrition throughout the year and as a consequence, body reserves can vary quite dramatically [47]. Body condition score (BCS) is a useful indicator of the quantity of body reserves available and is used widely within the animal production industry [48,49]. BCS measures are particularly important from a welfare point of view to indicate need for supplementation or as an animal health indicator, particularly in times of drought [50].

There is reasonable evidence to suggest that the behaviour of animals may be linked with their body condition.

Firstly, body condition may directly influence actions of the animals, for example feeding activity, foraging rate, walking between suitable foraging areas etc. There is evidence that high BCS ruminants eat less than low BCS ruminants and have shorter meal times [51,52].

Secondly, body condition may influence an animal's emotional state. Animals may be more tired, lethargic or hungry if they are in poor nutritional state or they may be show greater motivation for food [53,54] or demonstrate elevated HPA axis [55,56]. Nutritional status can cause chronic stress in animals, affecting the functioning of various organ systems [57] and therefore overall wellbeing.

Finally, there is also the possibility that nutritional state can influence an animal's emotional reactivity. Being in poor nutritional condition may provide a form of cognitive bias. Animals can be more emotionally reactive to an additional stressor if they have limited nutritional capacity to deal with such challenges. Activity of the HPA axis is largely regulated by caloric availability [58] with calorie-restricted animals having higher baseline corticosterone/cortisol than non-restricted animals [59,60]. Calorie-restriction may lead to a blunted stress response [55,61].

6.1 Sheep: Nutritional stress and transport 1. effect of season

6.1.1 Background

Sheep exhibit distinct photoperiod-driven cycles in feeding behaviour and adiposity aligned to changes in pasture availability and quality. For example, sheep on sparse, dry pasture in autumn are in negative energy balance, mobilising body fat reserves for energy, and are therefore in a period of live weight loss [62,63]; supplementary feeding is required at this time for sheep to maintain bodyweight [63]. By contrast, in the second half of the year in the same region, sheep are on green pasture and lay down body fat [63]. These seasonal patterns of feeding behaviour and adiposity may be linked with susceptibility to stress. For example, studies investigating causes of shipboard deaths as part of the live export trade have revealed that sheep that died of inanition have greater reserves of body fat (compared with controls and sheep that died from other causes, [62,64]) and shipboard deaths are higher in the second half of the year when sheep are coming off lusher pastures [62,65].

The aims of this experiment are to investigate the link between nutrition and response to the challenge of transport over <u>different seasons</u>:

1. Are high BCS sheep (on a static plane of nutrition) more responsive to transport at different times of the year compared with low BCS sheep (on a static plane of nutrition)?
2. Are decreasing-BCS sheep more responsive to transport at different times of the year compared with increasing-BCS sheep?

6.1.2 Methodology

Animals

32 Merino wethers (18 months of age; 35±2.6 kg) were randomly allocated to two nutrition groups and individually hand-fed one of two feeds (high and low nutrition) to achieve two treatment groups: BCS 1.5 and BCS 3.5. Sheep were weighed and assessed for BCS weekly; feeding was adjusted accordingly (Figure 1a).

The first half of the experiment was conducted between January and April 2009 (Austral summer/autumn); a period of decreasing day length. The second half of the experiment was conducted between July and October 2009 (Austral winter/spring); a period of increasing day length.

Nutrition and experimental groups

Once sheep reached their desired BCS (January), they underwent their first transport journey over a shortening day length period and then 8 animals from each group were switched diet to achieve the opposite BCS (Figure 1a). Sheep underwent their second transport event at the end of March. In June, sheep were returned (from spelling paddocks) to their individual pens and assigned to their original (BCS 1.5 and BCS 3.5) groups. The experiment was repeated as described above, with the first transport journey in July and the final transport journey in October (Figure 1b).

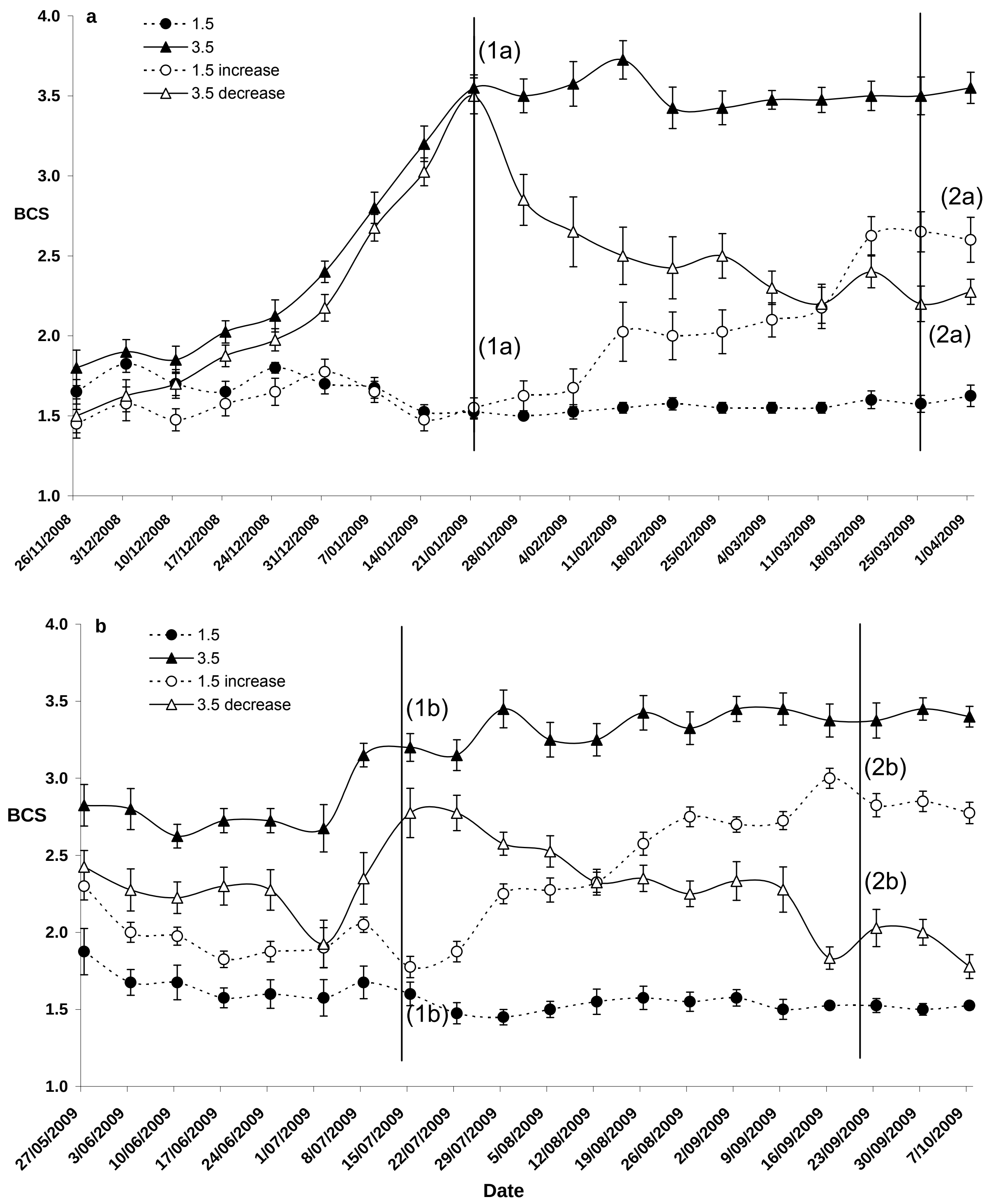


Figure 1. Change in BCS for each group of sheep a) between November and April (decreasing day length) and b) between June and October (increasing day length). Vertical lines indicate points at which transport events were carried out. For the first hypothesis, footage of the BCS 1.5 and BCS 3.5 sheep was compared for the January (1a) and July (1b) transport events. For the second hypothesis, footage of the decreasing-BCS and increasing-BCS groups were compared for the March (2a) and October (2b) transport events.

Transport and environmental measures

Footage of sheep was collected during the first 15 minutes after departure for each transport event for QBA assessment.

25 observers attended 3 sessions:

1. Term generation – Observers were shown clips captured across all experimental treatments. Observers generated an average of 17±6 descriptive terms (range 9 to 31).

2. *Two quantification sessions* – Observers watched 32 clips of individual sheep from the experimental transport events over each of the quantification sessions. The clips selected addressed each of the two hypotheses being tested:

> *1. Are high BCS sheep (on a static plane of nutrition) more responsive to transport at different times of the year compared with low BCS sheep (on a static plane of nutrition)?* Footage of the BCS 1.5 and BCS 3.5 sheep was compared for the January and July transport events.
>
> *2. Are decreasing-BCS sheep more responsive to transport at different times of the year compared with increasing-BCS sheep?* Footage of the decreasing-BCS and increasing-BCS groups were compared for the March and October transport events.

6.1.3 Results

1. Are high BCS sheep (on a static plane of nutrition) more responsive to transport at different times of the year compared with low BCS sheep (on a static plane of nutrition)?

The GPA consensus profile explained 38.56 % of the variation among the 25 observers, and this differed significantly from the mean randomised profile (t_{99}=25.8, p<0.001).

Terms associated with each GPA dimension are shown in Table 1; significant treatment differences are indicated in the right hand columns. Average GPA scores for the treatment groups are shown in Figure 2.

Significant *time*, *nutrition* and *time x nutrition* interaction effects for physiological data are shown in Table 2. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 2). Sheep described as more *agitated, nervous* and *uneasy* (GPA dimension 1) and *scared, frightened* and *confused* (GPA dimension 2) also had increased IGF-1 concentrations and decreased haematocrit and red blood cells numbers. Sheep scored as more *agitated, nervous* and *uneasy* also had decreased plasma β-OH concentration and higher neutrophil numbers and neutrophil: lymphocyte ratio. Monocytes and lymphocytes were both negatively correlated with GPA dimension 3, where sheep described as more *frustrated, anxious* and *calm* had increased monocyte and lymphocyte numbers. Finally, body mass positively correlated with GPA dimension 1, with heavier sheep described as more *calm, comfortable* and *relaxed* than lighter sheep (more *agitated, nervous* and *uneasy*). Actual BCS values for each sheep (i.e. individual values rather than nutrition treatment groups) were not correlated with any of the GPA dimensions.

Table 1. Terms used by observers to describe behavioural expression of SHEEP OF TWO NUTRITIONAL LEVELS (BCS 1.5 and 3.5) during TRANSPORT IN JANUARY (DECREASING DAY LENGTH) AND JULY (INCREASING DAY LENGTH). The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand columns.

GPA dimension	Low values	High values	Treatment effects	
1 (24.0 %)	Agitated (3), nervous, uneasy, startled, alarmed, edgy, wary, alert, afraid	Calm (7), comfortable (4), relaxed (3), doesn't care, stable, secure, acquiescent, accepting, quiet, squashed, happy, knowing, passive, patient, confident, tired, composed, obedient	*time:* $F_{1,14}$=39.9, p <0.001 *nutrition:* ns *time x nutrition:* $F_{1,14}$=4.89, p= 0.04	- Sheep transported in January (decreasing day length) scored lower cf. July (increasing day length) - BCS 3.5 sheep have a greater difference in their behavioural expressions between January and July cf. BCS 1.5 sheep
2 (21.6 %)	Scared (2), frightened (2), confused (2), stressed (2), nervous, afraid, tired, timid, submissive, jostled, lonely	Curious (7), comfortable (6), inquisitive (6), confident (5), happy (5), sure (4), alert (2), certain, intrigued, calm, wondering, assertive, secure, composed, observant, aware, bemused, in control, bright, relaxed, searching, pushy, hungry, determined	*time:* ns *nutrition:* ns *Time x nutrition:* $F_{1,14}$=9.89, p=0.007	- BCS 1.5 sheep transported in July (increasing day length) scored higher than when they were transported in January (decreasing day length) - BCS 3.5 sheep were not different between the two transport events
3 (10.2 %)	Frustrated (2), anxious (2), calm, sure, strong intent, persistent, certain, confident, lonely, stressed, tired, confused, exhausted, distressed, inquisitive	Restless, nervous, anxious, confused, sedate	*time:* ns *nutrition:* ns *time x nutrition:* $F_{1,14}$=31.6, p<0.001	- BCS 1.5 sheep transported in July (increasing day length) higher than when they had been transported in January (decreasing day length); converse true for BCS 3.5 sheep

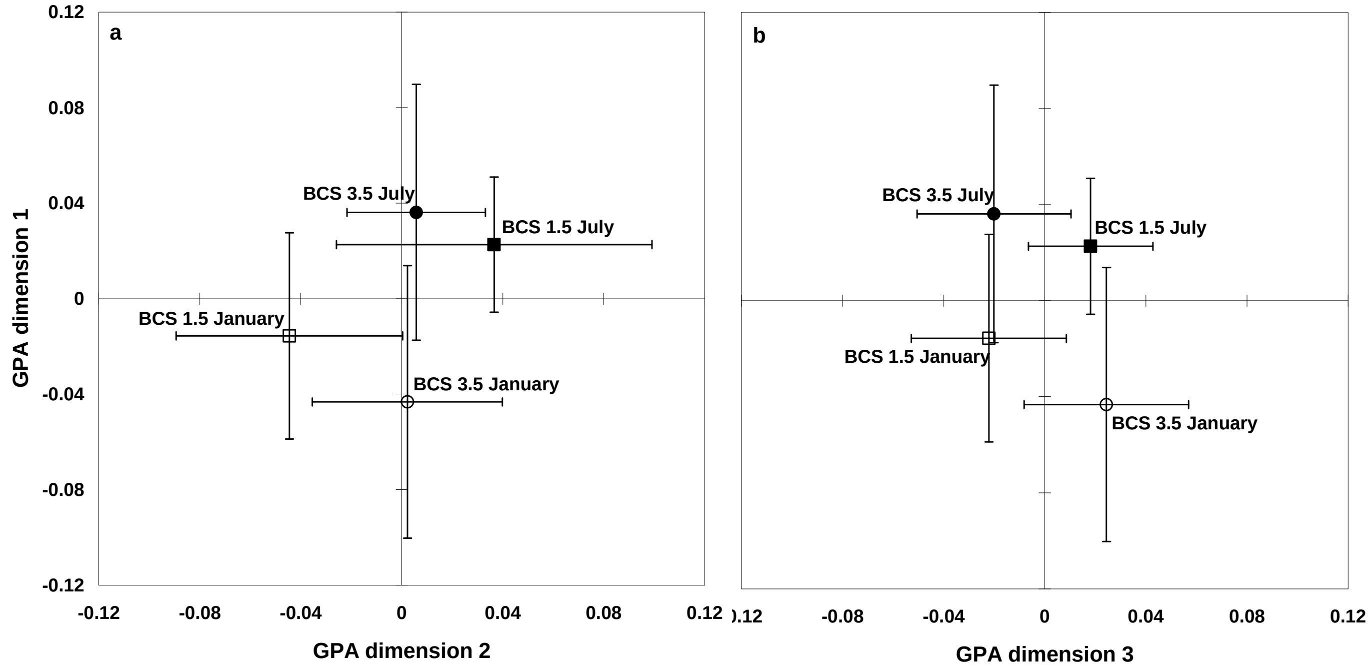


Figure 2. Positions of the four treatment groups on GPA dimensions 1 and 2 (a) and 1 and 3 (b). Values are the mean ±1SD for each treatment group.

Table 2. Left hand columns indicate the results of Repeated-Measures ANOVA testing for the effects of *nutrition treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Nutrition	Day length	Nutrition x day length interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
Hormones & metabolites	Results of RM ANOVA *p* values			Correlation coefficients r_{30}		
[Leptin] (ng/ml)	0.115	0.478	0.559	0.095	-0.150	-0.102
[Cortisol] (ng/ml)	0.435	0.165	0.435	0.197	0.151	0.016
[IGF-1] (ng/ml)	0.928	<0.001	0.906	-0.326*	-0.308*	-0.095
[Insulin] (µU/ml)	0.328	0.014	0.264	0.144	0.153	0.095
[β-OH] (mmol/L)	0.021	<0.001	0.022	0.229	0.498**	0.118
[Glucose] (mmol/L)	0.428	0.463	0.320	-0.218	-0.058	0.192
Haematological variables						
Haematocrit	0.299	0.005	0.783	0.395*	0.376*	-0.113
Red blood cell (x 10^{12}/L)	0.157	0.003	0.986	0.435**	0.374*	-0.081
White blood cell (x 10^9/L)	0.828	0.064	0.078	0.020	0.158	0.248
Monocytes (x 10^9/L)	0.106	0.427	0.269	-0.095	0.033	-0.416**
Neutrophils (x 10^9/L)	0.028	<0.001	0.011	-0.557***	-0.163	0.206
Lymphocytes (x 10^9/L)	0.656	<0.001	0.200	0.092	0.071	-0.352*
Neutrophil: Lymphocyte	0.040	<0.001	0.017	-0.586***	-0.149	0.124
T_{core} average (ºC)	0.063	0.018	0.766	-0.197	-0.228	-0.031
Weight				0.345*	0.202	-0.075
BCS				-0.044	0.065	0.144

2. Are decreasing-BCS sheep more responsive to transport at different times of the year compared with increasing-BCS sheep?

The GPA consensus profile explained 34.45 % of the variation among the 25 observers, and this differed significantly from the mean randomised profile (t_{99}=18.9, p<0.001).

Terms associated with each GPA dimension are shown in Table 3. There were no significant differences between treatment groups on any of the three GPA dimensions. Average GPA scores for the treatment groups are shown in Figure 3.

There was no significant *time x nutrition* interaction effect for physiological data in this experimental study, only two variables demonstrated a significant effect of *nutritional* status but more showed an effect of *time* (Table 4). Only two physiological variables correlated with GPA dimensions. Sheep described as more *curious, inquisitive* and *comfortable* (GPA dimension 1) had increased leptin concentrations while sheep described as more *confident, curious* and *scared* (GPA dimension 3) had increased basophil numbers.

Table 3. Terms used by observers to describe behavioural expression of SHEEP OF TWO NUTRITIONAL LEVELS (increasing-BCS and decreasing-BCS) during TRANSPORT IN MARCH (DECREASING DAY LENGTH) AND OCTOBER (INCREASING DAY LENGTH). The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). There were no significant treatment effects (ns in the right hand column).

GPA dimension	Low values	High values	Treatment effects
1 (27.9 %)	Nervous (3), confused (3), timid (2), worried (2), concerned, annoyed, stressed, agitated, crowded, lost, frustrated, sedate, scared, edgy, squashed	Curious (8), inquisitive (6), comfortable (5), confident (4), interested (3), happy (2), bored (2), alert (2), calm (2), secure (2), sure (2), certain, hungry, complacent, bemused, fine, laid back, determined, quizzical, intrigued, searching, wondering, unfazed	*ns*
2 (16.0 %)	Alert (4), confused (3), worried (2), anxious (2), wary (2), nervous (2), agitated, concerned, scared, wondering, disorientated, determined, excited, assertive, uneasy	Relaxed (5), calm (4), comfortable (2), bored (2), certain, docile, placid, happy, carefree, passive, quiet, tired	*ns*
3 (8.6 %)	Confident (2), curious (2), scared, frightened, calm, nervous, assertive	Frustrated, depressed, curious, hungry	*ns*

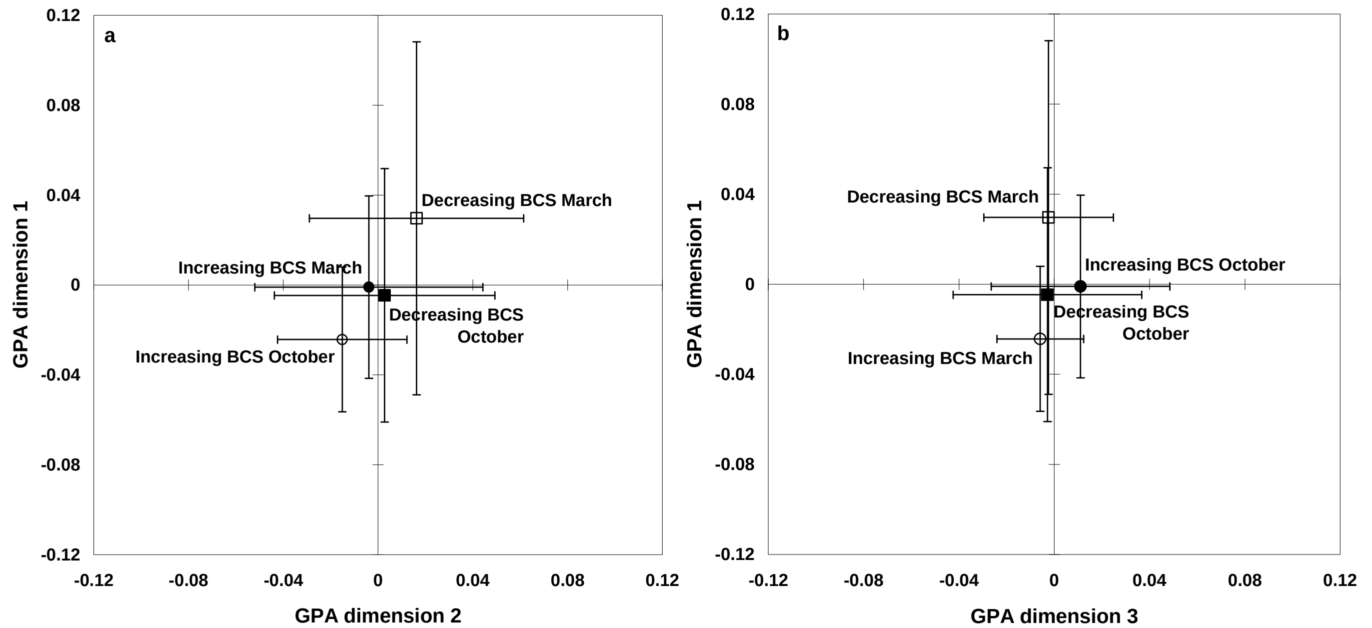


Figure 3. Positions of the four treatment groups on GPA dimensions 1 and 2 (a) and 1 and 3 (b). Values are the mean ±1SD for each treatment group.

Table 4. Left hand columns indicate the results of Repeated-Measures ANOVA testing for the effects of *nutrition treatment* and *time*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Nutrition	Day length	Nutrition x day length interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
Hormones & metabolites	Results of RM ANOVA *p* values			Correlation coefficients r_{30}		
[Leptin] (ng/ml)	0.001	0.014	0.093	0.319*	0.277	-0.045
[Cortisol] (ng/ml)	0.804	0.461	0.147	0.066	-0.094	-0.037
[IGF-1] (ng/ml)	0.610	0.378	0.467	-0.031	-0.104	0.139
[Insulin] (µU/ml)	0.610	0.015	0.918	0.084	-0.057	-0.079
[β-OH] (mmol/L)	0.251	<0.001	0.133	0.076	-0.079	-0.174
[Glucose] (mmol/L)	0.837	0.002	0.465	0.031	0.123	0.105
Haematological variables						
Haematocrit	0.450	0.445	0.799	0.132	-0.028	-0.081
Red blood cell (x 10^{12}/L)	0.549	0.502	0.686	0.110	-0.025	-0.042
White blood cell (x 10^9/L)	0.256	0.846	0.522	-0.231	-0.105	-0.209
Basophils (x 10^9/L)	0.944	0.302	0.483	-0.003	0.049	-0.303*
Eosinophils (x 10^9/L)	0.172	0.006	0.811	-0.231	-0.003	-0.066
Monocytes (x 10^9/L)	0.199	0.030	0.379	0.096	0.122	-0.202
Neutrophils (x 10^9/L)	0.565	<0.001	0.239	-0.156	0.018	-0.089
Lymphocytes (x 10^9/L)	0.099	<0.001	0.259	-0.032	-0.108	-0.032
Neutrophil: Lymphocyte	0.069	<0.001	0.335	-0.067	0.103	-0.032
T_{core} average (ºC)	0.017	<0.001	0.489	0.070	-0.127	0.018
Weight				-0.229	-0.211	0.138
BCS				-0.073	-0.102	0.128

6.1.4 Discussion

Observers described BCS 1.5 sheep as more *curious, comfortable* and *inquisitive* or *restless, nervous* and *anxious* when transported in July compared to January. BCS 3.5 sheep were described as more *calm, comfortable* and *relaxed* or more *frustrated, anxious* and *calm* when transported in July compared to January. These data suggest that sheep were differentially affected by day length when subjected to a stressor (transport). This result for BCS 3.5 sheep does not support the notion that fatter sheep respond worse to transport than thinner sheep, nor that sheep appear to be worse during transport during a period of increasing day length (July transport) compared with decreasing day length (January transport).

Although they reached consensus in their assessment of the sheep's behavioural expression, observers were not able to distinguish between sheep of an increasing- or decreasing-BCS transported either during March (decreasing day length) or October (increasing day length). Observers therefore identified differences between animals for this experimental treatment rather than differences due to treatment effects. The average BCS of the two groups were similar at the time of transport, although there were physiological differences (e.g. leptin concentrations) between the two treatment groups and the increasing-BCS sheep were receiving twice the amount of feed cf. the decreasing-BCS group. Since there were no behavioural differences observed between these two groups, it suggests that feed amount has no influence on the behavioural expression of sheep during transport.

There was a stronger effect of 'season' (i.e. increasing or decreasing day length) on the physiological variables than nutritional status in both experiments. However, although observers distinguished between sheep being transported in January or July, it was not possible to distinguish seasonal effects in this experimental design, as there was a possible confounding factor of habituation between seasons. Sheep transported during the increasing day length period had encountered a number of transport events before, whereas during the decreasing day length period, transport was still a fairly novel experience. In addition to the habituation effect, we noted differences in wool growth and health status of our animals which changed over time. The increase in T_{core} found in sheep transported in July and October (compared with January or March, respectively) is likely due to the fleece length. Sheep were shorn once, prior to the start of the study, and had ~1 cm of wool in January, ~5 cm in July and ~9cm in October. Additionally, around August, the sheep were diagnosed with an infestation of *Oestrus ovis* (nasal bots) and all individuals were treated with Ivermectin. It has been shown that nasal bots cause eosinophilia [66] so the decrease observed here from March to October could be due to the eradication of the nasal bots and not due to effects of day length.

The correlations between physiology and behaviour were marked for the first experiment, comparing sheep with BCS 1.5 or 3.5, but showed few correlations for the experiment comparing sheep of changing nutritional plane. The correlations between observer scores and the neutrophil: lymphocyte ratio, monocyte number and IGF-1 concentrations (all higher in sheep transported in January), indicate a typical stress response [67,68]. β-OH was also correlated with observer scores. β-OH has been associated with the utilisation or depletion of liver glycogen and lipid mobilisation [69] and is usually noted after a period of feed and water withdrawal. In this study BCS 1.5 sheep had increased β-OH concentrations when transported in July compared to January. There was not an excessive period of feed and water withdrawal in this study and all sheep were transported under the same conditions, with feed times the same for all sheep.

6.2 Sheep: Nutritional stress and transport 2. Effect of fasting

6.2.1 Background

During livestock transport, the aim of feed and water withdrawal (FWD) is to reduce fouling on the truck and roads and to give a more accurate measure of carcass weight [70]. However fasting may be a stressor in itself for animals, and could alter their responses to other environmental challenges, including the novel transport environment.

The aims of this experiment are to investigate the link between nutrition and response to the challenge of transport before or after fasting:

The aim of the present study was to determine whether sheep of different background nutritional states demonstrate obvious differences in behavioural expression during road transport before and after FWD, tested in two seasons (shortening day length, and lengthening day length). The hypotheses tested were:

1. Do decreasing-BCS exhibit different responses to fasting compared with low BCS (on a static plane of nutrition)?
2. Do increasing-BCS exhibit different responses to fasting compared with high BCS (on a static plane of nutrition)?
3. Do high BCS (on a static plane of nutrition) exhibit different responses to fasting compared with low BCS (on a static plane of nutrition)?

6.2.2 Methods

Animals

See section 6.3. Animals were transported without fasting prior to loading, or with a 36 hour feed and water curfew (herein termed 'post-fasting').

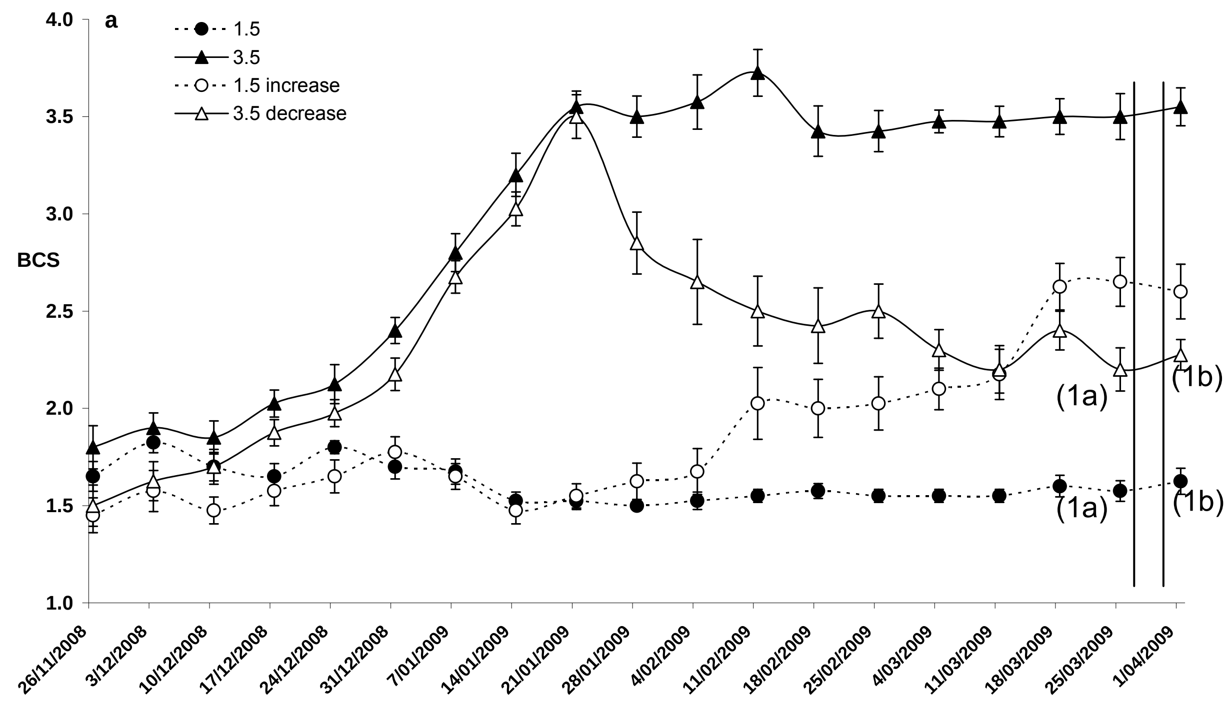
Transport and environmental measures

Footage of sheep was collected during the first 15 minutes after departure for each transport event for QBA assessment.

1. Term generation – 25 observers used the terms they had generated as part of the previous study (Section 6.3) (average 17±6 terms, range 9 to 31).

2. *Three quantification sessions* – Observers watched 32 clips of individual sheep each session from the experimental transport events (Figure 4). The clips selected addressed each of the three hypotheses being tested:

> *1. Do decreasing-BCS exhibit different responses to fasting compared with low BCS (on a static plane of nutrition)?* Footage of the decreasing-BCS and BCS 1.5 sheep was compared for the March and April transport events.
>
> *2. Do increasing-BCS exhibit different responses to fasting compared with high BCS (on a static plane of nutrition)?* Footage of the increasing-BCS and BCS 3.5 sheep was compared for the October transport events.
>
> *3. Do high BCS (on a static plane of nutrition) exhibit different responses to fasting compared with low BCS (on a static plane of nutrition)?* Footage of the BCS 1.5 and BCS 3.5 groups were compared for the October transport events.



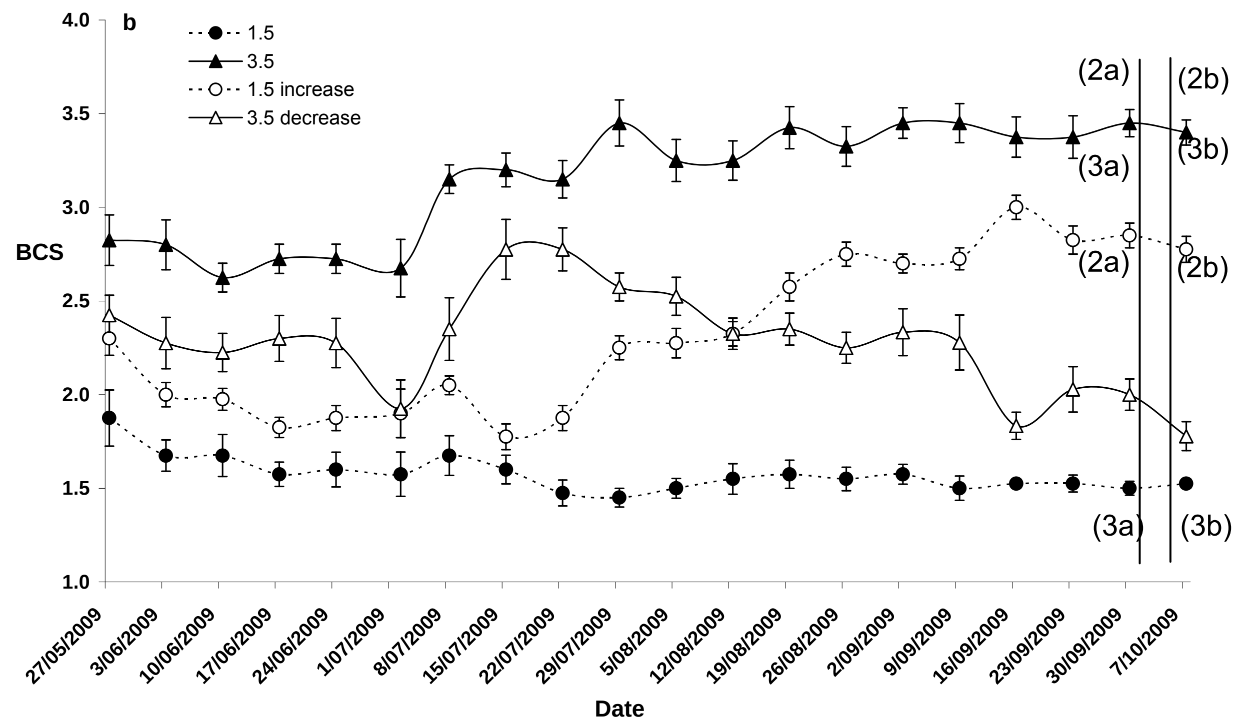


Figure 4. Change in BCS for each group of sheep a) between November and April (decreasing day length) and b) between June and October (increasing day length). Vertical lines indicate points at which transport events were carried out. For the first hypothesis, footage of decreasing-BCS and BCS 1.5 sheep was compared for the pre-fast (March) (1a) and post-fast (April) (1b) transport events. For the second hypothesis, footage of the increasing-BCS and BCS 3.5 sheep were compared for the pre-fast (2a) and post-fast (2b) October transport events. For the third hypothesis, footage of BCS 3.5 and BCS 1.5 sheep were compared for the pre-fast (3a) and post-fast (3b) October transport events.

6.2.3 Results

1. Do decreasing-BCS exhibit different responses to fasting compared with low BCS (on a static plane of nutrition)?

The GPA consensus profile explained 37.64 % of the variation among the 25 observers, and this differed significantly from the mean randomised profile (t_{99}=27.8, p<0.001).

Terms associated with each GPA dimension are shown in Table 5; significant treatment differences are indicated in the right hand column. Average GPA scores for the treatment groups are shown in Figure 5.

Significant *fasting*, *nutrition* and *fasting x nutrition* interaction effects for physiological data are shown in Table 6. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 6). Sheep described as more *confident, sure* and *comfortable* had increased monocyte numbers. Sheep described as more *relaxed, passive* and *quiet* had increased cortisol concentration and lower eosinophil numbers, and also had a lower BCS. Sheep scored as more *hungry, alone* and *isolated* had increased insulin and glucose concentrations and increased eosinophil numbers.

Table 5. Terms used by observers to describe behavioural expression of SHEEP OF TWO NUTRITIONAL LEVELS (BCS 1.5 and decreasing-BCS) during TRANSPORT WITHOUT FASTING IN MARCH AND POST-FASTING IN APRIL (DECREASING DAY LENGTH). The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column.

GPA dimension	Low values	High values	Treatment effects
1 (23.8 %)	Nervous (4), frightened (4), confused (4), stressed (3), afraid, concerned, tired, worried, scared, lost, cautious, edgy, depressed, timid, restless	Confident (5), sure (4), comfortable (4), happy (4), calm (3), relaxed (2), inquisitive (2), unfazed, laid back, assertive, alert, content, pensive, certain, safe, coping, bored, knowing, secure, composed, hungry	*time x nutrition:* $F_{1,14}$=4.92, p=0.04 - BCS 1.5 sheep scored lower when transported post-fast - decreasing-BCS scored higher when transported post-fast
2 (20.9 %)	Curious (8), anxious (4), interested (4), alert (3), inquisitive (2), awards (2), worried (2), annoyed (2), agitated (2), assertive, cheeky, relaxed, intrigued, bright, perplexed, tense, restless, quizzical, intent, nervous, wary, bewildered, startled, questioning, excited, incredulous, comfortable, cautious, distressed, confident, observant, concerned	Relaxed (2), passive (2), quiet (2), calm (2), scared, bored, disappointed, at ease, obedient, docile, sedate, confused	*nutrition:* $F_{1,14}$=8.03, p=0.01 - BCS 1.5 scored higher than decreasing-BCS
3 (10.9 %)	Crowded (2), scared, nervous, confined, jostled, comfortable, squashed, secure, frightened, alert, watchful, pressured	Hungry (2), alone, isolated, overcome, deserted, sad, searching, anxious, certain	*ns*

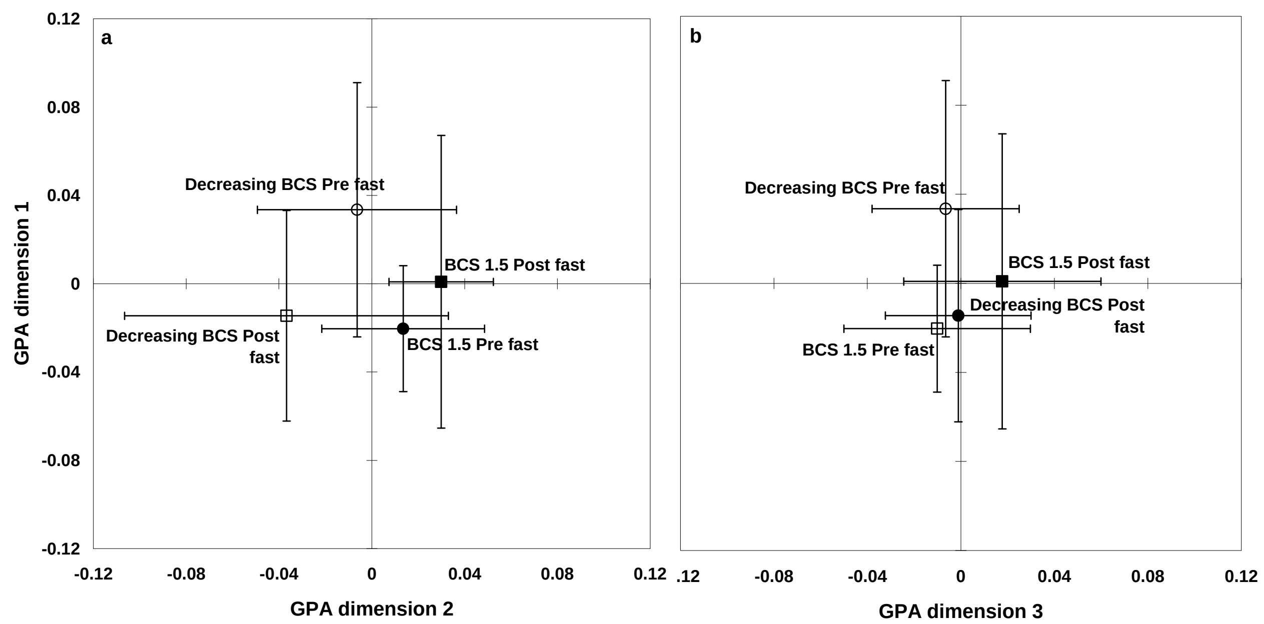


Figure 5. Positions of the four treatment groups on GPA dimensions 1 and 2 (a) and 1 and 3 (b). Values are the mean ±1SD for each treatment group.

Table 6. Left hand columns indicate the results of Repeated-Measures ANOVA testing for the effects of *nutrition treatment* and *fasting*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Nutrition	Fasting	Nutrition x fasting state interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
Hormones	Results of RM ANOVA *p* values			Correlation coefficients r_{30}		
[Leptin] (ng/ml)	0.047	0.748	0.152	-0.067	-0.286	-0.073
[Cortisol] (ng/ml)	0.575	0.324	0.607	-0.127	0.297*	0.035
[IGF-1] (ng/ml)	0.904	0.583	0.636	0.239	0.192	-0.015
[Insulin] (μU/ml)	0.795	0.043	0.789	-0.191	-0.078	0.354*
Metabolites						
[β-OH] (mmol/L)	0.164	0.091	0.320	0.075	0.194	-0.026
[Glucose] (mmol/L)	0.356	0.136	0.814	-0.202	-0.131	0.401*
Haematological variables						
Haematocrit	0.022	0.877	0.813	-0.072	-0.289	-0.044
Red blood cell (x 10^{12}/L)	0.171	0.094	0.565	-0.014	-0.058	-0.252
White blood cell (x 10^9/L)	0.365	0.627	0.986	0.083	-0.042	-0.055
Basophils (x 10^9/L)	0.592	0.032	0.758	-0.059	-0.066	0.237
Eosinophils (x 10^9/L)	0.097	0.589	0.375	-0.107	-0.325*	0.302*
Monocytes (x 10^9/L)	0.130	0.472	0.376	0.300*	0.106	-0.068
Neutrophils (x 10^9/L)	0.194	0.441	0.697	-0.079	0.001	-0.087
Lymphocytes (x 10^9/L)	0.375	0.082	0.910	0.116	-0.099	0.052
Neutrophil: Lymphocyte	0.535	0.214	0.606	-0.103	0.027	-0.124
T_{core} average (ºC)	0.399	0.240	0.578	-0.088	0.008	0.069
Weight				0.077	-0.237	0.142
BCS				0.230	-0.441**	-0.089

2. Do increasing-BCS exhibit different responses to fasting compared with high BCS (on a static plane of nutrition)?

The GPA consensus profile explained 38.18 % of the variation among the 25 observers, and this differed significantly from the mean randomised profile (t_{99}=35.9, p<0.001).

Terms associated with each GPA dimension are shown in Table 7; significant treatment differences are indicated in the right hand column. Average GPA scores for the treatment groups are shown in Figure 6.

Significant *fasting*, *nutrition* and *fasting x nutrition* interaction effects for physiological data are shown in Table 7. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 8). Sheep described as more *calm, docile* and *passive* also had a higher BCS. Sheep described as more *nervous, scared* and *distressed* had increased glucose concentration while sheep described as more *calm, happy* and *comfortable* had increased concentrations of leptin and glucose, increased haematocrit and an increased neutrophil: lymphocyte ratio, and increased numbers of red blood cells, monocytes and neutrophils. Sheep scored as more *relaxed* and *bored* had increased leptin concentration.

Table 7. Terms used by observers to describe behavioural expression of SHEEP OF TWO NUTRITIONAL LEVELS (increasing-BCS and BCS 3.5) during TRANSPORT WITHOUT FASTING AND POST-FASTING IN OCTOBER (INCREASING DAY LENGTH). The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column.

GPA dimension	Low values	High values	Treatment effects
1 (35.4 %)	Curious (12), alert (7), inquisitive (6), wondering (3), interested (3), confident (3), sure (3), comfortable (3), nervous (3), happy (2), concerned (2), aware (2), worried (2), searching (2), assertive (2), anxious (2), quizzical, disorientated, questioning, bewildered, incredulous, bright, intrigued, bored, excited, confused, safe, watchful, secure, certain, scared, troubled, tense, calm, alarmed, vulnerable, frustrated	Calm (3), docile (2), passive (2), stressed (2), placid (2), nervous, accepting, acquiescent, compliant, certain, carefree, comfortable, confident, obedient, afraid, confused, patient, relaxed, sad, timid, squashed, content, wary, concerned, suspicious	*time:* $F_{1,14}$=5.17, p=0.04 - sheep transported post-fast scored lower than sheep transported post-fast
2 (16.3 %)	Calm (4), happy (3), comfortable (2), confident (2), relaxed (2), bored (2), composed, doesn't care, docile, awaiting, content, passive, sure	Nervous (5), scared (3), distressed (3), agitated (2), confused (2), frightened (2), edgy, frustrated, vulnerable, apprehensive, wary, tense, scattered, stressed, lost, worried, clingy, restless, intimidated	*ns*
3 (6.9 %)	Frightened, jumpy, distressed, squashed, comfortable, confined, certain	Relaxed, bored	*time x nutrition:* $F_{1,14}$=8.85, p=0.01 - BCS 3.5 sheep transported post-fast scored lower - increasing-BCS transported pre-fast scored higher

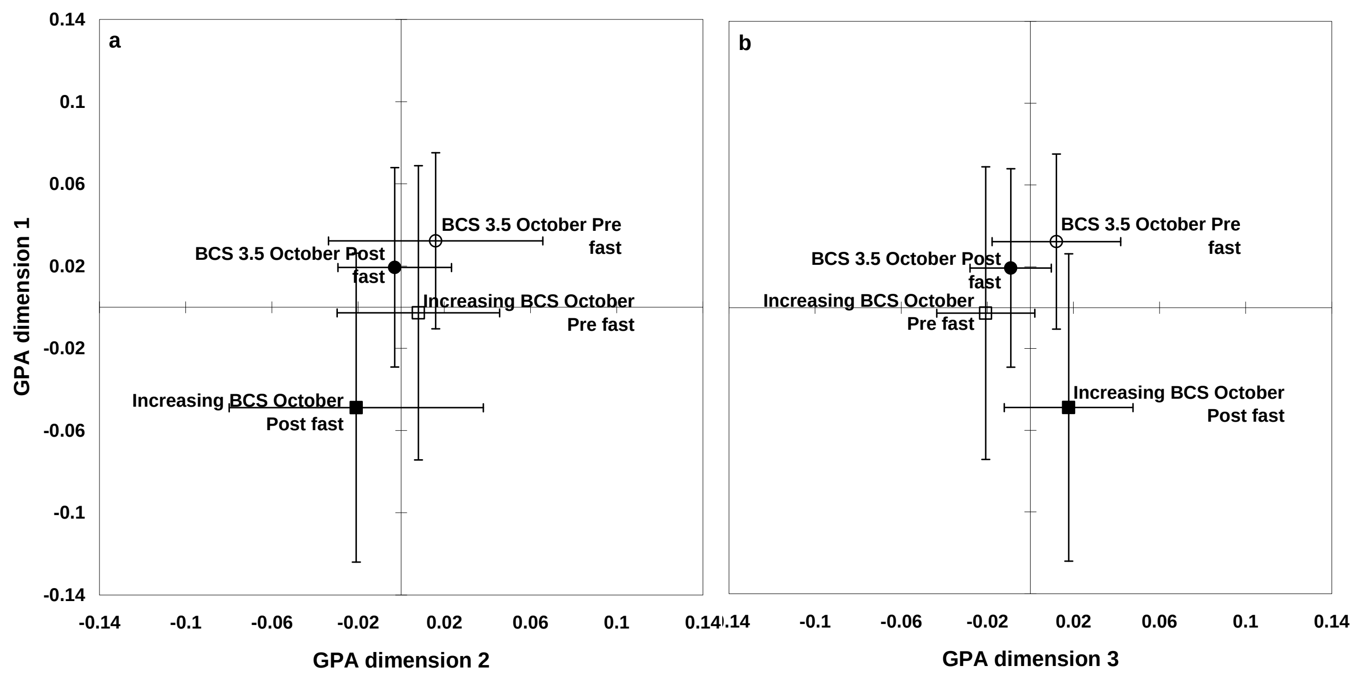


Figure 6. Positions of the four treatment groups on GPA dimensions 1 and 2 (a) and 1 and 3 (b). Values are the mean ±1SD for each treatment group.

Table 8. Left hand columns indicate the results of Repeated-Measures ANOVA testing for the effects of *nutrition treatment* and *fasting*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Nutrition	Fasting	Nutrition x fasting state interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
Hormones	Results of RM ANOVA *p* values			Correlation coefficients r_{30}		
[Leptin] (ng/ml)	0.619	0.003	0.056	-0.083	-0.395*	0.397*
[Cortisol] (ng/ml)	0.868	0.281	0.310	-0.119	0.103	-0.039
[IGF-1] (ng/ml)	0.318	0.049	0.553	0.096	0.156	0.028
[Insulin] (µU/ml)	0.969	0.626	0.737	-0.255	0.145	0.060
Metabolites						
[β-OH] (mmol/L)	0.059	0.133	0.152	-0.127	-0.116	-0.160
[Glucose] (mmol/L)	0.089	0.028	0.861	-0.098	0.323*	0.137
Haematological variables						
Haematocrit	0.321	0.019	0.313	0.208	-0.428**	0.195
Red blood cell (x 10^{12}/L)	0.328	0.011	0.197	0.206	-0.433**	0.166
White blood cell (x 10^9/L)	0.786	0.028	0.797	0.236	-0.194	-0.038
Basophils (x 10^9/L)	0.671	0.318	0.939	0.109	-0.155	<0.001
Eosinophils (x 10^9/L)	0.041	0.722	0.175	0.242	0.053	0.224
Monocytes (x 10^9/L)	0.508	0.949	0.152	-0.025	-0.373*	0.169
Neutrophils (x 10^9/L)	0.528	<0.001	0.224	-0.096	-0.353*	0.120
Lymphocytes (x 10^9/L)	0.747	0.058	0.134	0.190	0.010	-0.294
Neutrophil: Lymphocyte	0.936	<0.001	0.196	-0.246	-0.354*	0.258
T_{core} average (ºC)	0.368	0.118	0.908	0.097	-0.015	0.211
Weight				0.102	0.024	-0.209
BCS				0.564***	-0.038	0.135

3. Do high BCS (on a static plane of nutrition) exhibit different responses to fasting compared with low BCS (on a static plane of nutrition)?

The GPA consensus profile explained 34.52 % of the variation among the 25 observers, and this differed significantly from the mean randomised profile (t_{99}=17.7, p<0.001).

Terms associated with each GPA dimension are shown in Table 9; there were no significant treatment differences. Average GPA scores for the treatment groups are shown in Figure 7.

Significant *fasting*, *nutrition* and *fasting x nutrition* interaction effects for physiological data are shown in Table 10. A number of physiological variables were significantly correlated with the GPA dimension scores (Table 10). Sheep described as more *calm, passive* and *placid* had increased leptin concentration and lower T_{core}. Sheep described as more *curious, confident* and *certain* had higher eosinophil and monocyte numbers and sheep described as more *scared, nervous* and *frightened* were heavier and had a greater BCS. None of the physiological variables correlated with GPA dimension 3.

Table 9. Terms used by observers to describe behavioural expression of SHEEP OF TWO NUTRITIONAL LEVELS (BCS 1.5 and BCS 3.5) during TRANSPORT WITHOUT FASTING AND POST-FASTING IN OCTOBER (INCREASING DAY LENGTH). The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). There were no significant treatment effects (ns in the right hand column).

GPA dimension	Low values	High values	Treatment effects
1 (29 %)	Curious (9), inquisitive (4), alert (3), wary (2), nervous (2), agitated (2), aware (2), worried (2), wondering (2), concerned (2), scared, afraid, cautious, uneasy, observant, assertive, disorientated, confident, quizzical, confused, alarmed, edgy, sure, restless, startled, anxious, apprehensive, searching, interested	Calm (6), passive (3), placid (2), quiet (2), patient (2), relaxed (2), docile, content, bored, acquiescent, frightened, tired, at ease, scared, composed, carefree, comfortable, certain, accepting, exhausted, vulnerable, sedate	*ns*
2 (17.3 %)	Curious (5), confident (3), certain (3), interested (2), bored (2), happy (2), alert (2), calm (2), content, laid back, comfortable, stable, unfazed, bemused, watchful, safe, fine, sure, relaxed	Scared (3), nervous (3), frightened (2), lonely, submissive, bewildered, wary, stressed, afraid, confused, annoyed, worried, lost, anxious, tired	*ns*
3 (8.2 %)	Sure, afraid, stressed, bored, confused	Happy, cautious, secure, certain, sure, safe, confident, curious, confused, wondering, calm, agitated, scattered	*ns*

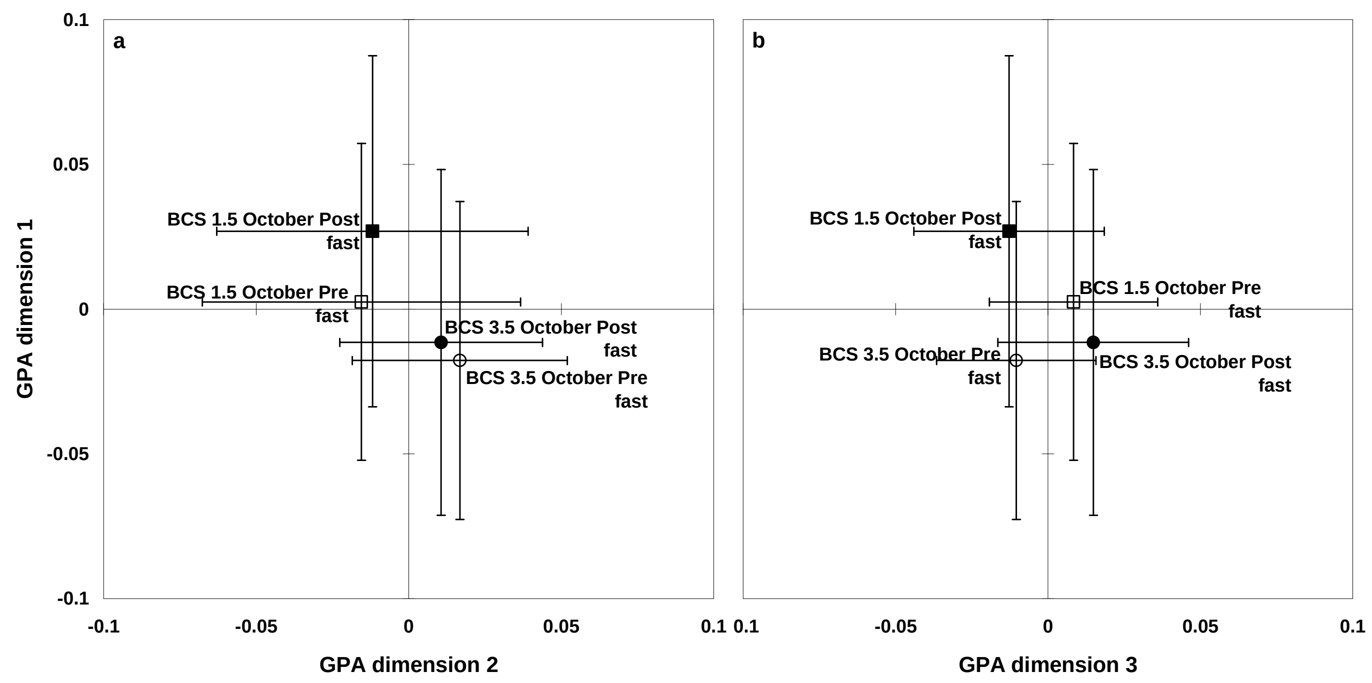


Figure 7. Positions of the four treatment groups on GPA dimensions 1 and 2 (a) and 1 and 3 (b). Values are the mean ±1SD for each treatment group.

Table 10. Left hand columns indicate the results of Repeated-Measures ANOVA testing for the effects of *nutrition treatment* and *fasting*. Right hand columns indicate the correlation between GPA dimension scores and the change due to transport in each physiology measure. Significant effects are indicated in bold.

	Nutrition	Fasting	Nutrition x fasting state interaction	GPA dimension 1	GPA dimension 2	GPA dimension 3
Hormones	Results of RM ANOVA *p* values			Correlation coefficients r_{30}		
[Leptin] (ng/ml)	0.827	0.021	0.181	0.317*	-0.173	-0.071
[Cortisol] (ng/ml)	0.333	0.073	0.339	-0.122	0.131	-0.132
[IGF-1] (ng/ml)	0.209	0.045	0.418	-0.102	0.074	-0.055
[Insulin] (μU/ml)	0.036	0.423	0.670	0.155	0.238	0.124
Metabolites						
[β-OH] (mmol/L)	0.404	0.005	0.005	-0.193	-0.028	-0.044
[Glucose] (mmol/L)	0.829	0.573	0.229	-0.162	-0.228	-0.014
Haematological variables						
Haematocrit	0.184	0.148	0.173	-0.060	-0.089	-0.147
Red blood cell (x 10^{12}/L)	0.290	0.205	0.392	-0.048	-0.090	-0.120
White blood cell (x 10^9/L)	0.479	0.148	0.733	0.026	-0.142	-0.046
Basophils (x 10^9/L)	0.274	0.209	0.515	0.210	-0.002	0.275
Eosinophils (x 10^9/L)	0.912	0.181	0.992	0.202	-0.297*	0.122
Monocytes (x 10^9/L)	0.441	0.012	0.118	0.130	-0.368*	-0.160
Neutrophils (x 10^9/L)	0.093	0.036	0.583	0.118	-0.026	-0.008
Lymphocytes (x 10^9/L)	0.937	0.616	0.470	-0.008	-0.261	-0.030
Neutrophil: Lymphocyte	0.282	0.031	0.675	0.166	0.078	0.042
T_{core} average (ºC)	0.014	0.965	0.081	-0.447*	0.046	-0.157
Weight				-0.246	0.345*	0.012
BCS				-0.254	0.322*	0.017

6.2.4 Discussion

Observers reached consensus in how they scored the behavioural responses of sheep of different nutritional states to a short transport event pre- and post fasting. Observers were also able to distinguish between BCS 1.5 and decreasing-BCS sheep; they described BCS 1.5 sheep post-fasting and decreasing-BCS sheep without fasting as more *nervous, frightened* and *confused* (cf. *confident, sure* and *comfortable*). Observers also described BCS 1.5 sheep as more *relaxed, passive* and *quiet* and decreasing-BCS sheep as more *curious, anxious* and *interested*. These data suggest that sheep were differentially affected by fasting as well as by nutritional status when subjected to a stressor (transport).

Observers also reached consensus in how they scored BCS 3.5 sheep and increasing-BCS sheep transported without fasting and post-fast. Observers described sheep transported without fasting as more *calm, docile* and *passive* compared to post-fasting. Observers also described BCS 3.5 sheep transported without fasting and increasing-BCS sheep post-fasting as more *relaxed* and *bored* (cf. *frightened, jumpy* and *distressed*).

Although observers reached consensus in their agreement of the behavioural expression of BCS 1.5 and BCS 3.5 sheep, they were not able to distinguish between sheep without fasting or post-fast. QBA scores for this experiment therefore reflect differences between-animals rather than differences due to treatment effects. Since there were no behavioural differences between sheep with stable BCS (1.5 and 3.5 BCS) it appears that fasting has a greater effect on sheep with a changing BCS and this is regardless of season.

There was a stronger effect of ‘fasting’ (i.e. transport without fasting or post-fast) on the physiological variables than nutritional status in all three experiments. QBA scores demonstrated significant correlations with physiological variables in a manner that was largely consistent with the interpretation that the behavioural expression of sheep reflected their current physiological state. QBA scores were correlated with neutrophil: lymphocyte ratio, indicating a typical stress response [67,68]. Unlike the previous study (Section 6.1), there was no correlation of β-OH with observer scores. β-OH is usually noted after a period of feed and water withdrawal and has been associated with the utilisation or depletion of liver glycogen and lipid mobilisation [69].

6.3 Sheep: Behavioural demand of pregnant sheep of (1) differing condition score, and (2) exposed to different rates of decline in BCS (Ag Research, New Zealand)

6.3.1 Background

Behavioural perception of hunger has been previously studied using motivation tests [53]. For example, Schutz et al. [53] found that cattle with a lower body weight had a higher motivation for food than heavier cattle and Jackson et al. [54] found that sheep deprived of food showed more motivation for food than those that were not deprived.

We examined whether groups of sheep of different body conditions show a different response to food restriction. We also investigated whether we could quantify differences in affective state between treatment groups which may inform us of how these sheep experience hunger. In this study, a feed motivation test was used to test whether pregnant sheep of different BCS display significant differences in behavioural expression when exposed to a food reward.

The aims of this experiment are to:

1. determine if observers can reliably perceive the behavioural expression of sheep during their activities within a behavioural demand facility
2. determine whether there are differences in sheep behavioural expression according to their body condition score treatment groups
3. correlate QBA scores with specific behavioural actions

6.3.2 Methods

Study 1: Behavioural demand of pregnant sheep of differing body condition score (BCS)

Feed motivation was assessed in 22 pregnant Coopworth ewes with different BCS (BCS 2, n= 8; BCS 3, n= 8, BCS 4, n= 6). Sheep reached these set BCS as shown in Figure 8. Sheep were kept on pasture from day -60 to day 70 of gestation and were supplemented with two different pellets, each providing half of the daily metabolisable energy to achieve the desired BCS. Ewes were housed indoor in group pens bedded with sawdust from day 70 until one week after lambing and were supplemented with the same pellets and received an additional handful of hay per sheep per day. Sheep always had free access to water.

Bodyweight and BCS were measured weekly and the feeding level adjusted according to BCS change throughout gestation. The width and depth of the eye muscle, the fat covering the eye muscle and fat on top of the rib (GR site) on the 12th rib were also measured by ultrasound scanning on day -36, 1, 35, 57, 76, 119 and 157 of gestation to get an accurate assessment of body reserves.

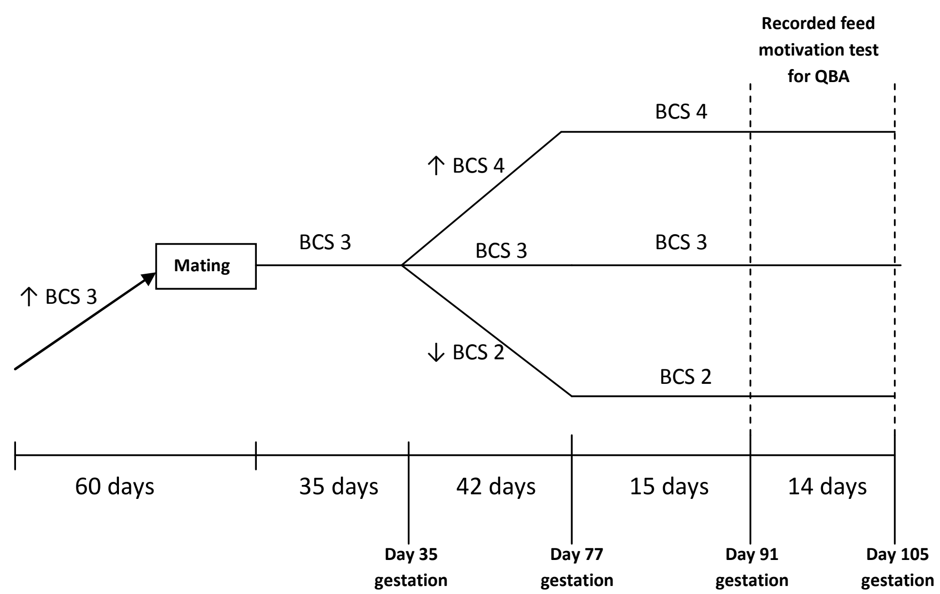


Figure 8. Recording of feed motivation test for QBA analysis in relation to the timeline of gestation and body condition score (BCS) treatments.

Study 2: Behavioural demand of pregnant sheep exposed to different rates of decline in BCS

Starting 60 days before mating 21 pregnant Coopworth ewes (2 to 5 years of age) were fed to BCS 3. Once mated, ewes were maintained at BCS 3 until day 35 of gestation and then randomly allocated to three different feeding treatments. In one treatment ewes were maintained at BCS 3 (maintained M; n=7). Ewes in the remaining 2 treatments were exposed to a decreasing plane of nutrition resulting in a decrease in 1 BCS over 4 to 6 weeks (fast F; n=7) or a decrease in 1 BCS over 10 to 12 weeks (slow S; n=7) (Figure 9). A loss of 1 BCS corresponded to an average loss of 5 kg. For the F group, this corresponded to a loss of 0.8 to 1.3 kg/ week, and for the S group to around 0.4 to 0.5 kg/ week. Feed motivation was assessed between days 48 and 70 of gestation (Figure 9).

During the study ewes were kept on pasture and supplemented with pellets. Ewes were body condition scored by palpation of the lumbar region by a trained assessor and weighed once a week. Stocking rate and pellet supply were adjusted to reach the desired BCS within the desired timeframe.

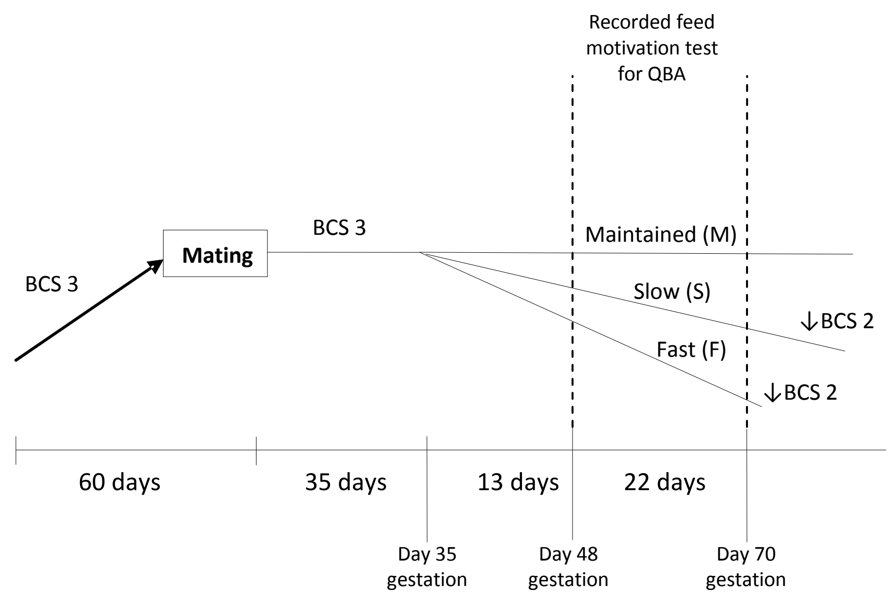


Figure 9. Recording of feed motivation test for QBA analysis in relation to the timeline of gestation and body condition score (BCS) treatments.

Feed motivation test for studies 1 and 2

The feed motivation test was designed using four identical testing races, each used to test one ewe at a time (Figure 10).

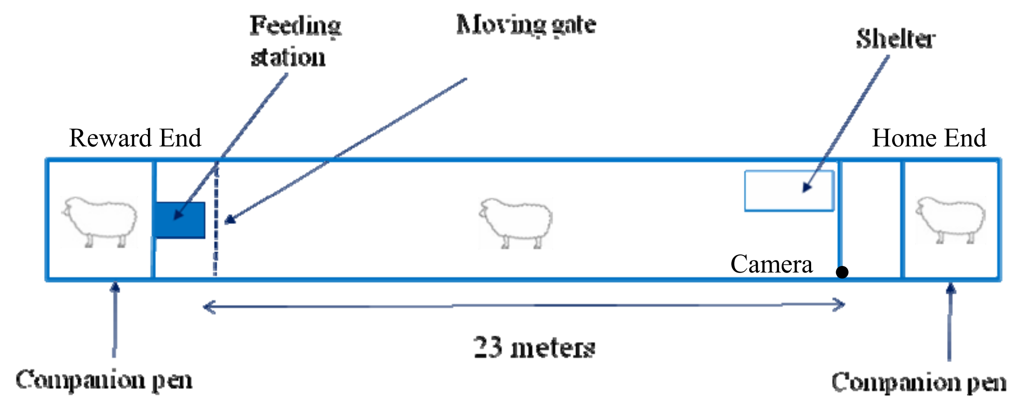


Figure 10. One of the four motivation testing races (adapted from [71]).

Sheep were trained in the feed motivation test races over 5 weeks to familiarise them with the apparatus, food reward and procedure. The apparatus consisted of a reward end and a home end. In order to minimise isolation stress during the tests, companion sheep were confined in a separate enclosure at each end of each race. The race was transversed by a metal gate that was programmed to give the sheep access to a feeding station at the reward end. A sensor placed above the feeder could detect the presence of a sheep at the feeder. Upon detecting a sheep, a 4.5 gram food reward was delivered. After 20 seconds, an auditory signal was sounded for 2 seconds and then the gate was programmed to move the animal slowly away from the reward and toward the home end of the pen. After the gate transversed a specified distance (termed the cost level), the gate returned to the reward end to once again allow access to pellets. The animal could choose to walk back to the feeding station at the reward end to find its food. The reward size was kept constant for all cost levels and BCS treatments. The sheep had the opportunity to repeat this process without restriction during the 23 hour test period. Test and companion sheep had *ad libitum* access to water throughout the test period. Test sheep were housed in individual pens on the day before the test and were fed their daily ration.

QBA for studies 1 and 2

A video camera was positioned so as to allow view of the sheep during the feed motivation test. One video clip was chosen within the first 30 minutes of the feed motivation test for each sheep. The video clips encompassed one feed motivation sequence (gate moving from home end to reward end, feeding period and gate moving from reward end back towards the home end). The clips were an average of 2 minutes in length. The selection process was based on selecting the first available clip of the animal, or if this clip did not have the sheep clearly in view during this time or the behaviour of the sheep was influence by outside factors (a person walking past the race) the next suitable clip was chosen. Observers were given detailed instructions on completing the sessions but were not told about the experimental treatments. The two sessions are detailed below:

1. Term generation – Observers were shown 22 clips of sheep including 6 clips selected from the 22 clips chosen for the feed motivation QBA (present study); the remaining clips were of sheep from other nutritional studies carried out as part of this project.

2. Quantification – Observers were shown the 22 clips of the BCS sheep in random order. Each observer used their own terms to quantitatively score (by marking on the visual analogue scale) the behavioural expression of individual sheep. Each of the sheep were scored on every term generated by each observer.

Quantitative behavioural assessment for studies 1 and 2

Following QBA sessions each clip was observed by the research group to assess links between quantitative behaviours and QBA dimension scores. The presence of particular behaviours during the clips was assessed by a yes/no process as follows:

Approaching the feeder

- moved to the feeder
- did not move initially toward the feeder
- chased the gate the whole way to the feeder
- moved halfway down the race to feeder, stop, and then moved to feeder

At the feeder

- put its head immediately into the feeder
- pawed at the feeder
- looked about while at the feeder
- sniffed and looked for more feed

Upon return from feeder

- pushed back by gate after feeding
- move away from the gate after feeding before being forced back by the gate
- Was the clip hand held recorded or recorded from a tripod

6.3.3 Results

Study 1: Differing BCS

The 21 observers generated a total of 147 unique terms to describe the sheep they were shown (average 19±6 terms per observer, min: 9, max: 31). The GPA consensus profile explained 53.5% of the variation among the 21 observers, and this differed significantly from the mean randomised profile (t_{99}=38.15, p<0.001).

Terms associated with each GPA dimension are shown in Table 11; significant treatment differences are indicated in the right hand column. Quantitative behaviours associated with each GPA dimension are indicated.

Table 11. Terms used by observers to describe SHEEP (3 BCS) behavioural expression during TESTING IN A BEHAVIOURAL DEMAND FACILITY. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column (mixed-model ANOVA including all observers' GPA scores as the dependent variable and including observer number as a fixed effect). The GPA dimension scores for individual animals were examined by *t*-test (grouped as yes or no for each behaviour); significant results are shown as 'sheep behaviour'.

GPA dimension		Low values	High values	Treatment effects
1 (49.5%)	QBA Terms	relaxed (3), interested (2), confident (4), intrigued, inquisitive, comfortable (4), curious (3), happy (2), hungry (5), sure (2), laid back, calm (3), determined, safe, certain, keen, secure, bright, assertive (2), eager	awaiting, passive, troubled, tense, worried, pensive, apprehensive, resigned, cautious, vulnerable, anxious (3), alarmed, curious (1), wondering (2), frightened (2), lonely (2), stressed (3), isolated, bewildered, wary (2), uneasy (2), alert, watchful, reticent, clingy, questioning, nervous (6), lost (2), incredulous, scared (3), confused (5), distressed (3)	*BCS:* $F_{2,439}$=31.10, p<0.01 - BCS 4 sheep had a higher scores than BCS 2 and BCS 3
	Sheep behaviour	Moved toward feeder (p<0.01) Chased down gate (p<0.05) Head into feeder immediately (p<0.01) Pushed back by gate as it moved back to home end of race (p<0.05)	Did not move toward feeder at all (p<0.01) Did not move to feeder straight away (p<0.05)	
2 (8.4%)	QBA Terms	patient, bewildered, nervous, in control, thrilled, tired, relaxed (2), wary, calm, anxious (2), comfortable, confident	frustrated, annoyed (3), vulnerable, settled, agitated (2), pushy, impatient, bossy, inquisitive, distressed, sure, focussed, intent, jostled, bright, confident, comforted, relaxed, persistent	*BCS:* $F_{2,439}$=12.46, p<0.01 - BCS 2 scored higher than BCS 3 and BCS 4
	Sheep behaviour	Voluntarily moved away from gate after feeding before gate started to move back to home end (p<0.01)	Pawing at feeder (p<0.01) Pushed back by gate as it moved back to home end of race (p<0.05)	
3 (6.3%)	QBA Terms	Concerned, inquisitive, aware, alert, searching, sedate	Confined, exhausted, frustrated, overstimulated, stressed, alert, confused, scared, claustrophobic, isolated, sad, knowing, certain, overcome, apprehensive, sure	*BCS:* $F_{2,439}$=11.27, p<0.01 - BCS 3 scored highest
	Sheep behaviour		Stopped half-way down the race upon return from feeding and then pushed by the gate the remainder of the way back to home end of race (p<0.01)	

Study 2: Different rates of BCS decline

The 11 observers participating in this study generated a total of 58 unique terms (average: 11±4 terms per observer, min: 7, max: 19). The GPA consensus profile explained 44.7% of the variation among the 11 observers, and this differed significantly from the mean randomised profile (t_{99}=4.69, p<0.001).

Terms associated with each GPA dimension are shown in Table 12; significant treatment differences are indicated in the right hand column. Quantitative behaviours associated with

GPA dimension 2 are indicated (GPA dimension 1 and 3 scores were not significantly different between the behaviour groups).

Table 12. Terms used by observers to describe SHEEP (3 RATES OF DECLINING BCS) behavioural expression during TESTING IN A BEHAVIOURAL DEMAND FACILITY. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Significant treatment effects are shown in the right hand column. The GPA dimension scores for individual animals were examined by *t*-test (grouped as yes or no for each behaviour); significant results are shown as 'sheep behaviour'.

GPA dimension		Low values	High values	Treatment effects
1 (26.5%)	QBA terms	Calm (2), bored, comfortable, quiet, positively occupied, relaxed, patient	Interested (4), anxious (3), excited (2), frustrated (2), searching (2), skittish, pushy, curious, unsure, attentive, worried, nervous, motivated	BCS: $p<0.05$ - Slow declining BCS scored higher than ewes maintained at BCS 3
2 (21.4%)	QBA terms	Hungry (2), searching (2), excited (2), interested (2), relaxed (2), inquisitive (2), confident, sure, eager, motivated, purposeful, resigned	Curious (2), intimidated, uneasy, tense, indecisive, cautious, confused, unsure, nervous, anxious, scared, aware, watchful, distressed	ns
	Sheep behaviour	Sniffing and looking for more feed ($p<0.05$)	Did not walk directly to food reward (stopping along way) ($p<0.01$)	
3 (11.1%)	QBA terms	Hungry (2), bold, interested, pushy, calm, frustrated, purposeful, anxious	Curious (2), concerned, reserved, interested, watchful, frustrated, eager, anxious, dominant, scared	ns

6.3.4 Discussion

Observers' assessment of behavioural expression of pregnant sheep of differing BCS and exposed to different rates of BCS decline were both consistent and repeatable. Furthermore, our observers detected differences in behavioural expression that were consistent with the behaviours recorded and which differed between BCS treatments. There was consensus between observers in regard to the behavioural expression of the sheep, such that the GPA consensus profile explained half the variation in scores between the observers. The observers used most terms in the same way, and it was possible to identify distinct clusters of words with similar meanings on the different dimensions.

The relative position of sheep on the QBA dimensions varied depending on their BCS, indicating that observers could differentiate between the behavioural expressions of high and low condition score sheep exposed to food. QBA scores were significantly associated with the expression of particular behaviours (measured as the exhibition of particular behaviours or the time spent in each behaviour). The significant association between quantitative and qualitative assessments found in this study illustrated how the qualitative assessments add an interpretative element to the quantitative analysis, and in that sense also validate the meaning of this analysis.

6.4 Cattle: Body condition score and handling stressor (CSIRO, Armidale)

6.4.1 Background

We examined footage of cattle to determine whether their response to a psychological stressor (being separated from their calf while being handled in isolation through a crush for examination) differed between body condition score treatment groups.

The aims of this experiment are to:

1. determine if observers can reliably perceive the behavioural expression of cattle filmed during an isolation challenge
2. determine whether there are differences in cattle behavioural expression according to their body condition score treatment groups
3. correlate QBA scores with specific behavioural actions

6.4.2 Methodology

Animals

Behavioural response of cows to isolation from their calves and fellow herd members was assessed in 24 Hereford cows of BCS 1 (n=8), BCS 2 (n=8) and BCS 3 (n=8). The BCS 1, 2 and 3 cows had a fat target depth of 2, 4 and 10 mm respectively. The cows were individually run through a race and held in a crush before being released into an isolation yard where they could see, but not access their calves and fellow herd members.

A hessian screen was erected against the fence line of the isolation yard. The animals were videoed from behind this screen to minimise the influence of the human observer on the animal's behaviour. Each of the cows was videoed being released from the crush and moving into the isolation yard. The yard was bare with little ground cover and was large enough for the animal to run freely. Each animal was filmed for a total of 3 minutes. From this footage, a video clip between 30 seconds and 1 minute long was chosen for each animal while it was in the isolation yard. This clip did not include release from the crush. The clips for the 24 individuals were randomly ordered and shown to 39 observers for QBA assessment. These observers scored the animals based on their own unique set of terms generated using free choice profiling (session 1) before the quantification session 2.

Observers were recruited from University staff and students and members of the public to attend term generation session and a subsequent quantification session on campus or by correspondence. Observers were given detailed instructions on completing the sessions but were not told about the experimental treatments. The two sessions are detailed below:

1. Term generation – Observers were shown 15 video clips of the experimental cattle demonstrating a wide range of behaviours (some of these clips were the experimental footage and also used in session 2). The 39 observers generated a total of 180 different terms (average 17±7 terms per observer, min: 9, max: 47).

2. Quantification - observers used their own terms to quantitatively score the behavioural expression of individual cattle shown in the 24 video clips.

Quantitative behavioural assessment

Following QBA sessions, each clip was observed by the research group. Behaviours assessed were:

- Time (seconds) taken for animal to move from the crush (point A) once opened to animal moving completely past the end of the race (point B) (a distance of approx 1 metre). See Figure 1.
- Cumulative time (seconds) spent running from the time of opening the crush till 2 minutes thereafter.
- Number of tail swishes from the time of opening the crush till 2 minutes thereafter.

6.4.3 Results

The GPA consensus profile explained 59.0% of the variation among the 39 observers, and this differed significantly from the mean randomised profile (t_{99}=126.38, p<0.001).

Terms associated with each GPA dimension are shown in Table 13; significant treatment differences are indicated in the right hand column. Quantitative behaviours associated with each GPA dimension are indicated.

There was no significant effect of BCS on GPA dimensions.

Table 13. Terms used by observers to describe CATTLE behavioural expression during ISOLATION. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). Correlations between quantitative behavioural assessments and GPA dimension 1, 2 and 3 are shown in the right hand columns; significant correlations (R_{24}) are indicated in bold.

GPA dimension	Low values	High values	Treatment effect (all ns) Correlated behaviour	
1 (73.2%)	Calm (31), relaxed (18), comfortable (17), happy (9), content (7), at ease (5), bored (3), confident (2), quiet (2), settled (2), unphased, casual, sure, predictable, used to it, aware, observant, composed, accepting, certain, indifferent	Agitated (17), nervous (15), anxious (14), stressed (12), alert (10), frightened (9), scared (9), worried (7), restless (7), distressed (6), flighty (5), afraid (5), fearful (5), upset (5), tense (4), alarmed (4), wary (3), on edge (3), concerned (3), annoyed (3), apprehensive (2), wants to escape (2), unsure (2) uptight (2), bothered (2), panicked (2), evasive (2), confused (2), disturbed (2), avoiding (2), frustrated (2), boxed in, aware, toey, watchful, responsive, threatened, seeking escape, excitable, twitchy, isolated, trying to get away, guarded, excited, oppressed, trapped, lively, skittish, perplexed, angry, uncertain, confined, unable to relate to environment, swamped	ns Time taken to leave the crush (from point A to point B) Number of vocalisations Number of tail swishes Time spent running in isolation pen	 -0.720*** -0.484** 0.066 0.787***
2 (4.0%)	Curious, concerned, indifferent, isolated, hemmed in, interested, lonely, bright	Curious (3), alert (2), confident, concentrated , strong, playful, thoughtful, sure, inquisitive, comfortable, confined , focused, interested, stand offish	ns ns	
3 (3.1%)	Inquisitive (2), quizzical, curious, wondering	Fidgety, flighty, content, jittery, curious, twitchy, nervous, confident, tense, cramped, jumpy, submissive, apprehensive, alert, anxious, angry, intimidated	ns ns	

6.4.4 Discussion

This experiment supported that observer's assessment of behavioural expression of cattle is consistent and repeatable. However, observers were not able to detect differences in behavioural expression between cattle of different BCS. Ferguson [46] indicated that highly variable temperaments of the tested cattle may have confounded the results. Unfortunately no temperament tests were carried out on these animals.

The research group undertook quantitative behaviour assessment of the cattle using the available footage. This allowed insight into behaviour that may have influenced QBA assessments. It also allowed an assessment of the temperament of cows by using footage not shown to observers (while the animal was in the crush and leaving the crush) to determine the time taken for the cattle to leave the crush. QBA scores were correlated with behaviour that was consistent with a measure of 'agitation'. Animals that were scored as more *agitated, nervous* and *anxious* (GPA dimension 1) were also recording a faster exit time from crush ($p<0.01$) and spent more time running in the isolation pen ($p<0.01$). The findings of this study suggest a link between QBA and flight speed.

The study also found that that the time animals spent running in the isolation pen was correlated with the QBA scores. It is likely that observers integrated this behaviour into their QBA assessment and therefore it is not surprising that there was a correlation.

6.5 Cattle: Body condition score, lactation and behavioural demand (CSIRO, Armidale)

6.5.1 Background

Although we could not distinguish between BCS treatment groups in the previous study (Section 6.4), one of the possible reasons for this result may have been that the context in which the footage was recorded was not relevant to the experimental treatments being examined. The aim of the present study was to undertake QBA in a context relevant to the experimental treatment being examined. It was predicted that carrying out QBA of cattle in a behavioural demand facility may capture the expression of behaviour more relevant to their body condition treatment (e.g. hunger, motivation to feed).

The aims of this experiment are to:

1. determine if observers can reliably perceive the behavioural expression of cattle during their activities within a behavioural demand facility
2. determine whether there are differences in cattle behavioural expression according to their body condition score treatment groups
3. correlate QBA scores with specific behavioural actions

6.5.2 Methodology

Animals

21 Angus cows (4 to 4.5 years of age) gave birth while at BCS 3 and 4-6 weeks later were randomly allocated to 3 treatments. In one treatment cows were maintained at BCS 3 (n=7) for 5 months. Cows in the remaining 2 treatments were given a decreasing plane of nutrition over a 4 month period to reach either a BCS of 2 (n=7) or a BCS of 1.5 (n=7) and then maintained at these condition scores for a further month. Behavioural demand was assessed in all cows 1 month and 4.5 months post calving.

During the study cows were held in treatment group paddocks with calves and maintained on a pellet ration. Cows were body condition scored by palpation of the lumbar region by a trained assessor and weighed once a week. Pellet supply was adjusted to reach the desired BCS within the desired timeframe.

Behavioural demand test

The behavioural demand test was designed using a 50 metre testing race (Figure 11). Cows were trained in the behavioural demand race before calving to familiarise them with the race, food reward and procedure. The race consisted of a reward end that had a 2 sided feeder. The cow was required to walk a minimum of 1.5 metres to access the other side of the feeder (Figure 11). A specified weight of the pellet reward was delivered into the opposite feeder when the cow attempted to feed (via an infrared beam located on each of the feeders). The cow was therefore required to walk to the other side of the feeder to access the feed reward. The cow had opportunity to repeat this process without restriction. In order to minimise stress of isolation from the calf during the tests, the calf was confined in a separate enclosure at the reward end of the race. The reward size was kept constant for all BCS treatments. Cows had *ad libitum* access to water throughout the test period.

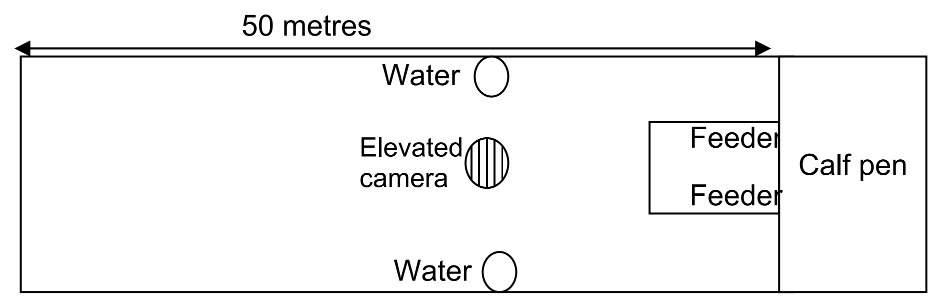


Figure 11. Behavioural demand testing races (adapted from [46])

QBA

A video camera was positioned on a pole in the middle of the race approximately 2 metres from the feeders (Figure 11) and recorded each cow for the first 1.5 hours of its behavioural demand assessment. One video clip was chosen within the first 10 minutes of the behavioural demand test for each cow. The video clips encompassed the cow feeding and then moving to the opposite side of the feeder for a food reward, feeding and then moving to the opposite feeder again. The clips were an average of 1 minute in length. The selection process was based on selecting the first available clip of the animal, or if this clip did not have the cow clearly in view during this time or the behaviour of the cow was influence by outside factors (a person walking past the race) the next suitable clip was chosen.

Observers were recruited from University staff and students and members of the public. Observers were given detailed instructions on completing the sessions but were not told about the experimental treatments. The two QBA sessions are detailed below:

Session 1 - Term generation - observers were shown 6 clips of cows from the present study (also shown in session 2). The 11 observers generated a total of 60 unique terms (average: 10±4 terms per observer, min: 5, max: 17).

Session 2 – Quantification – Observers were shown the 21 clips of the cows in random order. Each observer used their own terms to quantitatively score (by marking on the visual analogue scale) the behavioural expression of individual cows. Each cow was scored on every term generated by each observer.

Quantitative behavioural assessment

Following QBA sessions, each clip was observed by the research group. Behaviours assessed were:

Cumulative time (seconds) spent:
- Walking
- Feeding
- Looking around at surroundings
- Moving from one feeder to the other feeder
- Self grooming

Number of vocalisations

6.5.3 Results

The GPA consensus profile explained 50.4% of the variation among the 11 observers, and this differed significantly from the mean randomised profile (t_{99}=15.36, p<0.001).

Table 14 shows terms with the strongest correlations of observer's terms to each of the GPA dimensions; significant treatment differences and correlated behaviours are indicated in the right hand column.

There was no significant effect of BCS on GPA dimensions. Behavioural demand measurements (number of feeding events and feed intake) made by Ferguson et al. (unpublished) for the entirety of the behavioural demand period were also not different between treatment groups. However, the number of feeding events for the entirety of behavioural demand assessment was positively correlated with GPA dimension 1.

Table 14. Terms used by observers to describe CATTLE behavioural expression during TESTING IN A BEHAVIOURAL DEMAND FACILITY. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension). There were no significant treatment effects (ns in the right hand column). Significant correlations between QBA scores and quantitative behaviours are shown in the right hand column.

GPA dimension	Low values	High values	*Treatment effect* - Correlated behaviour
1 (43.2%)	Bored (2), curious (2), watchful (2), interested (2), explorative, distracted, disturbed, restless, alert, confident, bold, listening, attentive	Hungry (4), purposeful, interested, motivated, eager, searching, comfortable, excited, happy	*BCS:* ns - Animals that spent less time looking around, spent more time feeding, spent less time moving from one feeder to another and had less self grooming events scored higher
2 (27.2%)	Relaxed (5), calm (5), bored (3), careless, satisfied, placid, confident	Tense (3), nervous (3), alert (2), interested (2), agitated (2), frightened, stressed, watchful, inquisitive, jumpy, distracted, edgy, flighty, defensive, curious, bothered, unsteady, attentive, restless, anxious, annoyed, erratic	*BCS:* ns - total time that cow's spent walking: Animals that spent more time walking scored lower
3 (11.7%)	Carefree, relaxed, aware, alert, distracted, casual, curious, happy, calm, nervous, angry, normal, disturbed, interested	Restless (2), uneasy, agitated, searching, erratic, annoyed, lonely, depressed, concerned	*BCS:* ns - number of bellows: animals that bellowed more scored higher - self grooming events: Animals that had fewer self grooming events scored higher

6.5.4 Discussion

This study found that observers did not detect differences in behavioural expression of different BCS cattle exposed to food rewards. The behavioural demand of the cattle in each BCS treatment was also not different, indicated by no significant difference in feed intake or number of feeding events between treatment groups [46].

Even though no differences were found between treatment groups, there was consensus between observers in their QBA. QBA scores were also correlated with quantitative behavioural assessments. The correlation of QBA scores (from footage recorded during the first 10 minutes of the behavioural demand test) with the number of feeding events of cows over the entire 23-hours of the behavioural demand test [46], suggests that the chosen clips are representative of the behaviour of cows during the behavioural demand period. Observers scored cows that had a higher number of feeding events during the behavioural demand period as more *hungry*, *purposeful* and *interested* by QBA and those that had a lower number of feeding events as more *bored, curious, watchful* by QBA. This demonstrates that quantitative and qualitative assessment methods could be meaningfully combined in interpreting behavioural responses. QBA therefore can add an interpretative element to the quantitative analysis, validating interpretation of quantified behaviour.

7 Success in achieving objectives

All objective or modified objectives (as per study plans) have been met and Milestone Reports delivered in a timely manner.

7.1 Primary objectives

The objectives of B.AWW.0130 were to:

1) Develop and validate a practical, convenient welfare assessment tool based on the identification and measurement of key animal behaviour that is indicative of animal welfare status in industry situations including:
    a. Identify and validate welfare-relevant behaviour in **sheep** that reflects psychological and physiological stress under transport conditions and validate the use of QBA for sheep welfare assessment.
    b. Identify and validate welfare-relevant behaviour in **cattle** that reflects psychological and physiological stress under transport conditions and validate the use of QBA for cattle welfare assessment.

2) Analyse suitable video recordings provided from other projects in the Animal Welfare Objective Measures (AWOM) program to determine the usefulness of QBA in assessing animal welfare under a range of conditions and challenge models

3) Training of 2 PhD students

7.2 Validating Qualitative Behavioural Assessment

We have tested three aspects of QBA as a measure of animal welfare. We have examined its reliability, objectivity and validity as a measure of animal welfare.

Previously, QBA had been **reliably** applied to pigs [7-9], horses [10,11], cattle [12,13], dogs [14], and poultry [15]. These previous studies have largely been carried out under experimental conditions. We have substantially expanded upon these studies, finding significant consensus in how observers interpret the behavioural expression of cattle and sheep under a wide range of experimental and industry conditions. For example, the application of QBA to the transport industry had never been examined previously, while industry situations such as pre-slaughter lairage demonstrate the range and versatility of situations under which QBA may be applied.

We have tested the **objectivity** of QBA by assessing whether we can use QBA scores to discriminate between treatment groups. Only two published studies had previously examined whether QBA can be used to distinguish between treatment groups [10,18]. We have demonstrated that using QBA, observers can distinguish between treatment groups under a wide range of experimental conditions.

We have demonstrated the **validity** of QBA as a measure of animal welfare. We recorded significant correlations between QBA scores and a range of physiological variables that indicate activation of the hypothamalo-pituitary-adrenal axis, commonly associated with a stress response. These results validate QBA as detecting and scoring behaviour significantly associated with a stress response, allowing its use as a tool to compare that response in individual animals. Only one previous study had compared QBA with the physiology of the animal [23] and we have therefore substantially increased this research field.

7.3 Research training

This project has provided financial support to one PhD student and one Honours student at Murdoch University, as well as involving undergraduate students and other interested parties as observers. Time commitments of the principal investigators and the nature of the contracting process necessitated the employment and further training of a post doctoral fellow rather than another PhD student, with that researcher providing valuable technical assistance and conduct of research.

We acknowledge the significant contribution from research students/fellows who have worked on various projects as part of the QBA research programme:

Veterinary student research experience:

Colette Sims: As part of carrying out her veterinary degree at Murdoch University, Ms Sims completed an experiment that examined different methods of measuring cortisol response in sheep.

Marion Farbos: As part of carrying out her veterinary degree in France, Ms Farbos travelled to Australia to complete an experiment at Murdoch University examining dietary preference of sheep. She also gained research experience through assisting us with our QBA experiments.

Honours:

Dr Kylie Snowden-Tucker (BVMSc Hons) completed her honours in *Veterinary Biology Science*, Murdoch University in 2007: *Qualitative Behavioural Assessment (QBA) in beef cattle.* Dr Snowden-Tucker has completed her veterinary science degree and is currently veterinary officer working with the Department of Agriculture and Food WA in regional animal disease surveillance.

Ms Cheree Dorman (BSc Hons) completed her honours in *Veterinary Biology Science*, Murdoch University in 2008: *Qualitative Behaviour Assessment in horses.* Ms Dorman is currently studying veterinary science at Murdoch University.

Doctoral:

Ms Sarah Wickham (BSc Hons) is completing her Doctor of Philosophy at Murdoch University: *The qualitative behavioural assessment of sheep during transport.* Ms Wickham is currently working with the Department of Agriculture and Food WA in animal welfare.

Postdoctoral:

Dr Catherine Stockman (BSc Hons PhD) worked as Postdoctoral Research Fellow on this research programme. Dr Stockman is continuing her role as Postdoctoral Research Fellow at Murdoch University on subsequent research projects (see Section 8.3.2).

7.3.1 Dissemination of research findings

Several presentations have been permitted at international conferences, where the work has been extremely well received. A number of papers are in preparation; two have been submitted for publication:

1. Stockman, C.A., T. Collins, A.L. Barnes, D. Miller, S.L. Wickham, D.T. Beatty, D. Blache, F. Wemelsfelder, P.A. Fleming. 2011. Qualitative behavioural assessment of cattle naïve and habituated to road transport. *Animal Production Science* 51 In press accepted 10 December 2010 51 (3) IN PRESS
2. Wickham, S.L., T. Collins, A.L. Barnes, D.W. Miller, D.T. Beatty, C. Stockman, D. Blache, F. Wemelsfelder, P.A. Fleming. 2011. Qualitative behavioural assessment of transport-naïve and transport-habituated sheep. *Journal of Animal Science* IN PRESS accepted 13/12/2010. Submitted 22 Aug 2010.

8 Impact on meat and livestock industry – Now and in five years time

8.1 The importance of objective animal welfare measures now and in the future

The development of objective and scientifically-based understanding and measurement of animal welfare is important to the future of red meat and wool industries. It will facilitate the industries' capacity to highlight good welfare and provide objective validation of management practices. Importantly, objective animal welfare measures can be used as a comparative measurement tool to guide development of management practices. As it is a quick and non-invasive assessment QBA will prove useful in interpretation of more detailed welfare measures or to highlight situations that require more intensive welfare assessment, particularly in animal production scenarios where more invasive welfare assessments are difficult to implement. Additionally, objective measures of animal welfare can provide sound evidence that the industries are working to maximise animal welfare, providing assurance to consumers of the animal welfare integrity of their products thus ensuring access to markets.

There is substantial interest and concern from the community regarding animal welfare. The increasing consumer interest in animal welfare and the recent responses of commercial providers (e.g. Coles, McDonalds, fabric producers etc) in providing products that have high animal welfare standards are significant drivers in our production industries. We predict there will be increasing interest and requirement from producers for methods that allow them to prove the integrity of their product.

8.2 QBA is a reliable, objective and valid animal welfare measure that is ready to be used NOW to improve animal welfare for the FUTURE

QBA can represent an animal's affective state

We have demonstrated that QBA is a reliable, objective and valid tool for researchers and animal producers to assess and compare animals. QBA may be a valuable contribution to objective measurement of animal welfare, providing a novel approach to the holistic, integrated assessment of animal behaviour with direct correlation to their physiology, and being one of very few measures that can effectively capture positive affective state.

QBA is a tool that is accessible and acceptable across the spectrum – both by stockman and by the general community

QBA is a readily-understood technique that can be used to provide scientific evidence of good practices as well as show how industry is already working, and responsive to, community concern in the area of animal welfare. QBA uses terms that people can readily relate to and will seek out in their approval of welfare-friendly products. In concert with other methods, QBA can play a significant role in providing the meat and livestock industry with the tools they need to objectively measure animal welfare.

Relevance of welfare measures requires that they are context-specific

The behaviour of cattle and sheep is particularly sensitive to context-specific disturbances, and individuals will respond completely differently to exactly the same stressors, depending on their experience and condition. Additionally, real-life situations for farm animals often involve a number of concurrent stressors (e.g. transport involves multiple factors). It is therefore important to develop welfare indicators that are context-specific. As part of this project, QBA was validated against common industry stressors in order to develop an understanding of welfare-relevant behaviour for the Australian cattle and sheep industries.

QBA is a versatile measure of animal welfare

QBA may be carried out for many situations that are not suitable for other methods of analyses. This project has been successful in using QBA under a range of industry-relevant situations. The technique is practical and convenient, in that it can be applied to a variety of situations where video footage can be collected and subsequently analysed in a robust and repeatable manner.

To illustrate the versatility of QBA as a welfare assessment tool, we have analysed footage from five experiments carried out under the AWOM research programme for QBA. QBA analysis of this footage provides additional information about the affective state of animals in those experiments and value-adds to their research findings.

QBA is currently being used as a measure of animal welfare

QBA has been included as one of 13 measures as part of the 2004-2009 European Commission's Welfare Quality® audit [72]. Importantly, QBA was the only measure which captured positive welfare. The research program was designed to develop European standards for on-farm welfare assessment and product information systems as well as practical strategies for improving animal welfare. Sub project 4 of this programme is ***"To implement a welfare monitoring and information system and the welfare improvement strategies developed".*** Welfare Quality 1 focused on three main species and their products: cattle (beef and dairy), pigs, and poultry (broiler chickens and laying hens) [72]. Welfare Quality 2 (seeking expressions of interest now) will continue the work to include sheep.

QBA has also been included as part of the auditing process carried out by Quality Meat Scotland [23].

QBA as a guide to best practice not as a test for good/bad welfare

We believe QBA can be used as an auditing tool to help producers identify areas in the production chain that may need improvement relative to other stages, or problems that occur in particular environmental conditions or seasons (e.g. drought). QBA could be done on a repeated basis (e.g. yearly) so that producers could identify the consequences (benefit/harm) of any intervention or change in management practice/facility design introduced. In this way, it provides the producer with feedback and allows some comparison with properties of similar enterprise.

8.2.1 Current research projects

With the success of these QBA validation studies, subsequent studies will focus on refining QBA as a method for implementation at an industry level. Further to discussions at the 2010 AWOM meeting, we identified a number of future studies that will be integral in the development of QBA for use in industry. The major emphasis of future work will be to trial QBA for use under industry conditions. Four current avenues for value-adding to existing processes and experiments will allow us to trial QBA at an industry level:

1. **Feedlotting and preparation for shipboard transport.** MLA W.LIV.0142 *'Backgrounding and feedlotting strategies to address Inanition in the Livestock Export Industry'* will include QBA studies to value-add to this new project:
2. **Working with the Pork industry.** Australian Pork Limited 2011/1018.347 '*Developing qualitative behavioural assessment as an objective measure of pig welfare'* will investigate the application of QBA under a range of experimental conditions.
3. **Goats:** MLA – WLIV0159 '*Preparation of rangeland goats for live export'* will include use of QBA for assessment of goat behaviour.
4. **Abattoir and lairage.** We have had a number of offers from industry bodies to trial QBA within their system. Harvey Beef has offered the use of their facilities to trial QBA as a welfare measure during lairage. They also have an interest in implementing QBA as a tool to be used by their stockpersons. It is hoped that integrating research of QBA at an industry level will assist in dispelling stigma associated with this new approach to welfare measurement.

8.2.2 Application of QBA to the Australian livestock industries

In conjunction with other measures of animal welfare, QBA may be applied under a number of industry situations. For instance:

Working under logistically-difficult conditions

QBA could be used in the live export process, where there can be cumulative effects of a number of stressors which are difficult to measure in isolation. A holistic measure of animal behavioural expression such as QBA will allow assessment that integrates these steps. QBA can also be applied under difficult logistical conditions, e.g. where it is not possible to collect blood samples from individual animals.

Assessing whether specific farm interventions improve welfare and therefore productivity

The link between good animal welfare and increased productivity has been clearly demonstrated for the poultry [73,74], pork [75] and cattle [76,77] industries (reviewed by [78-80]). Various farm interventions can improve an animal's performance later in life. Examples for the cattle and sheep industries include yard weaning of calves [81], habituation to people and handling [81] and early experience with feed [82]. We propose that QBA could be used at an industry level (at abattoir or feedlot) as one measure to compare between intervention treatments. Some on farm interventions could include:

- Exposure to various levels of novelty
- Low stress stock handling compared to other rougher and louder methods
- Length of time of transport curfew
- Yard weaning

QBA would be compared with productivity measures, in particular meat quality attributes (e.g. % dark cutters; cattle and sheep) and the risk of inanition in a feedlot (specifically for sheep within the live export process).

Comparison between farm management practices

Can we improve animal welfare by identifying best practice? For example:

- Comparison between different management practices, e.g. methods of dehorning, stocking rates, feeding practices

As a tool for farm assurance

QBA could be applied to animal welfare assurance at an industry-wide or individual farm level.

- Development of quality assurance programmes, i.e. follow the European model
- Comparisons between farms/enterprises to benchmark best practice
- Comparisons within farms/management enterprises at different time points

9 Conclusions and recommendations

9.1 What else needs to be tested/validated for use of QBA at an industry level?

Free Choice Profiling vs. fixed terms lists

We used QBA in association with Free Choice Profiling (FCP) to analyse observers' interpretation of the behavioural expression of individual animals. Free Choice Profiling (a technique that was developed in the sensory sciences discipline [8,83]) allows observers to develop their own descriptive terms to interpret what they see. Because observers use terms that they have ownership of, they understand the meaning that they attribute to each term.

The alternative to FCP is to use lists of Fixed Terms. QBA by Fixed Terms lists has been used successfully as part of the European Union's Welfare Quality audit [72], where research groups across Europe use the same species-specific lists of 20 behavioural expression terms, which are translated into varying languages. Each observer ('inspector') has to be trained through observation of video footage to ensure that each person has made the same interpretation of the meaning of each descriptive term. The advantage of the Fixed Terms method is that each inspector is working on the same terms and therefore, theoretically, is measuring behavioural expression on the same relative scale. Fixed Terms therefore have a very important place in comparative animal welfare assessment.

The disadvantage of Fixed Terms has been that some terms do not have a direct translation into every language. Additionally, subtle behaviour that is not captured by these 20 Fixed Terms may also not be captured by the QBA scores. For example, we have measured varying levels of 'engagement' and 'tiredness' in endurance horses at three stages of a 160km ride [18]; without including terms that address these subtle behavioural expressions in the scoring, these dimensions of behavioural expression would not be captured.

The Procrustes Statistic derived for many of the studies we report indicate that there is still a reasonable degree of variability in the behavioural assessments. This may be attributed to different numbers and types of descriptive terms being used by observers, as well as their different use. Because some observers did not generate terms that might be used to explain a particular dimension, individual observers maintain their different interpretations regarding animal behaviour, or use terms in a slightly different manner from each other. For example, with both the cattle and sheep transport studies, individual observers used the term *alert* slightly differently – it was correlated with either of two GPA dimension dimensions depending on the observer. The term *alert* was used by some observers to indicate a positive curiosity in their environment, while other observers used it to indicate a level of wariness or anxiety. Words that have a range of interpretations would be detrimental to Fixed Terms lists since they are open to a level of ambiguity and interpretation.

<u>We therefore recommend exploration of Fixed Terms lists for application of QBA to Australian livestock industries where the aim is to carry out comparison between farms or management practices, but note that FCP will provide more information for research applications.</u>

Limitations of QBA

There are no absolute measures for QBA. The scope of QBA assessments is dependent upon the footage/material included in the assessment since the dimensions are calculated according to the data that is inputted into the analysis. For example it may be possible to assess the welfare of animals over a range of intensive housing conditions, but adding footage from a completely different housing system will completely change the dimension calculations and relative position of each test scenario. This should not be seen as negative, however, since arguably no animal welfare measure has absolute limits – only those that are set by guidelines based on experience and practice. The key to successful use of QBA as an assessment tool is to ensure that the comparisons being carried out are realistic.

The application of QBA is necessarily restricted to comparative or relative measures. QBA is therefore useful as a tool where you want to compare two (or more) situations, e.g. husbandry practices, management strategies, housing conditions. For example, QBA would be extremely useful in allowing farmers to visualise where they lie in a spectrum of other farms that engage in the same practice (e.g. comparing across extensive sheep farms).

We recommend transparency at all times in respect to reporting the full range of data that have been included in each QBA analysis. Correct interpretation of QBA outputs would be limited without full knowledge of the comparisons being undertaken.

Is context relevant?

Qualitative measures are sensitive to environmental context, which is one of the main reasons that they can be so informative, but also makes qualitative measures vulnerable to observer's biased views of that context [9]. This is particularly a risk when different contexts have different moral connotations judged by observers in terms of good or bad. Wemelsfelder et al. [9] give the example that if pigs are observed lying and resting on a barren concrete floor in an empty pen, observers might be inclined to assess these animals as *bored*, whereas if one observed the same animals resting in an outdoor field, terms such as *content* may more easily come to mind. Equally, a pig sniffing and exploring its pen may be perceived as *fearful/restless* under barren conditions and as *curious/excited* under enriched conditions. This contextual bias is arguably the major reason for the criticism that qualitative measures can be prejudiced, unreliable or subjective. Understanding the contextual bias of qualitative measures is therefore important in ensuring that methods such as QBA can be implemented without risking negating the outcomes.

A recent study has indicated that observers viewing the same footage of pigs which is digitally projected onto either an indoor or outdoor background may have slightly shifted QBA scores, but their scoring of individual pigs retains the same pattern despite the different backgrounds [9]. The ranking of scores attributed to individual animals were highly correlated between the two backgrounds but observers assessed pigs as more *confident/content* and less *cautious/nervous* in outdoor than in indoor clips. The very high correlations between indoor and outdoor pig scores on both consensus dimensions indicate the stability of the pigs' ranking on these dimensions under different environmental conditions [9].

We had the opportunity to run a similar analysis ourselves as part of this research programme [84]. The same set of sheep footage (the habituated footage) was viewed by observers twice, once in comparison with the footage collected from their first transport event (the naïve event) and secondly in comparison with the non-grip flooring event. Observers showed significant correlation in their ranking of clips on both GPA dimension 1 (r^2_8=0.94, p<0.001) and GPA dimension 2 (r^2_8=0.509, p=0.021) (Table 1). Observers scored the habituated clips as more *anxious, agitated* and *worried* (RM-ANOVA: GPA dimension 1 $F_{1,9}$=42.55, p<0.001) and as more *nervous, alert* and *confused* (GPA dimension 2 $F_{1,9}$=371, p<0.001) when they were shown in juxtaposition with the naïve clips than when they were shown in juxtaposition with the non-grip flooring footage (Figure 1).

Table 1. Terms used by observers to describe behavioural expression of TRANSPORT-HABITUATED SHEEP WHERE THE SAME FOOTAGE WAS VIEWED TWICE IN SEPARATE QUANTIFICATION SESSIONS. The terms shown are those that had the highest correlation with each end of each GPA dimension axis (% of variation in behavioural expression accounted for by each dimension). Term order is determined firstly by the number of observers to use each term (in brackets if greater than one), and secondly by weighting of each term (i.e. correlation with the GPA dimension).

GPA dimension	Low values	High values	Treatment effect
1 (31.5%)	Calm (10), relaxed (7), content (6), bored (4), happy (3), sleepy (2), comfortable (2), trusting, doughy, quiet, steady, reassured, enduring, accepting, resigned, tolerating, chilled_out, sure, restful, mellow, chilled	Anxious (9), agitated (8), worried (3), concerned (3), scared (2), confused (2), distracted, upset, jittery, disturbed, fearful, stressed, vigilant, attentive	RM-ANOVA: $F_{1,9}$=42.55, p<0.001
2 (17.3%)	Happy (7), resigned (3), comfortable (3), at_ease (2), bored (2), agitated (2), curious (2), worried (2), alert (2), stoic (2), aware (2), inert, sure, placid, tranquil, peaceful, quiet	Nervous (7), alert (6), confused (5), anxious (5), tense (4), panicked (3), disorientated (3), frightened (3), aware (3), stressed (2), exhausted (2), afraid (2), aggressive (2), fearful (2), irritable (2), distressed (2), excited (2), mischievous, nervy, tired, angry, harassing, bewildered, irritated, aroused, panic, cautious, quiet, bored, bothered, restless, edgy, accepting, suspicious, peaceful	RM-ANOVA: $F_{1,9}$=371, p<0.001

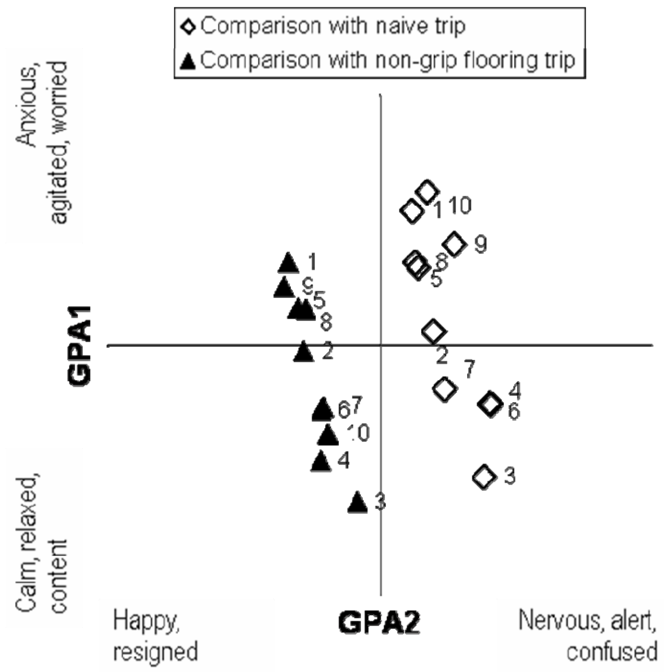


Figure 1. Observers shown the same footage of sheep habituated to transport rated these animals differently depending on the other footage that the clips were interspersed with. The numbers indicate the ID for each individual animal.

Studies such as these two serve to emphasise that, QBA, like all animal welfare measures, must be undertaken thoughtfully and with full cognisance of the terms of reference within which it can be applied. We recommend that future investigation of the effects of context could be undertaken, especially with respect to the full range of attitudes and experience present in the livestock industries. Understanding the effects of attitude towards animal welfare assessment will be important in finding wide acceptance amongst all stakeholders.

Does a person's background influence their ability to perform QBA?

Arguably, the degree of contextual bias and ability to read an animal's body language may differ between observers according to their experience, opinions, background etc. A person experienced with working with the animals may be less likely to misread the body language of the animals, while an observer with strong opinions on the welfare of a particular industry practice may rate animals in that context differently from another observer who does not share their opinion.

Wemelsfelder & Laurence [85] found that there was no difference in the ability to judge pig behaviour and welfare between veterinarians, animal protectionists or the animal's caregivers. Snowden-Tucker [86] also found no difference between a diverse range of observers (varying in sex, age, religion, habitat, diet and pet ownership) in their ability to judge the behavioural expression on cattle. Wickham [84] investigated whether observer demographics and their opinions towards animal welfare had an effect on the way they scored the behavioural expression of sheep during transport. Despite a wide range of backgrounds and attitudes, observers are still able to reach consensus in their assessment of the behavioural expression of sheep during transport.

<u>As part of industry and consumer engagement with the use of QBA, we recommend that QBA projects that actively engage with stakeholders of a range of backgrounds be undertaken</u>. This form of research would serve to demonstrate the validity and assure all parties of the merits of animal welfare assessment tools.

Does the method of assessment matter? QBA of groups of animals vs. individuals

The current research programme focussed on QBA of individual animals, allowing direct comparison with an individual's physiology. <u>To further develop the QBA method, we recommend extension of the current project to examine groups of animals, particularly within a lairage or feedlot environment</u>. The aim of this study would be to establish protocols for QBA use upon groups of animals. Pertinent questions to be addressed include:

- Does individual QBA correlate with assessment of the entire group?
- What is the optimum group size and should QBA be limited to a particular group size?
- Should an observer assess all animals in a group or just a percentage of them?
- The influence of individual animal temperament on other individuals within a group (i.e. group dynamics) would be compared, e.g. the influence of a 'nervous' animal in an otherwise 'calm' group.

9.2 Conclusions

This project demonstrates that Qualitative Behavioural Assessment is a valid method for evaluating differences in welfare between animals exposed to various industry-relevant conditions. We have demonstrated that:

- observers from a range of backgrounds have the capacity to detect and score differences between animals
- observers reach significant consensus in their interpretation of cattle and sheep under a range of circumstances
- observers are able to distinguish between groups of animals based on their behavioural expression
- and the QBA scores attributed are correlated with welfare-relevant physiological responses

We conclude that QBA is a reliable, objective and valid method of animal welfare assessment. QBA could valuably contribute to the skills of those working with livestock as they assess and respond to their animals.

9.3 Recommendations

QBA is an objective measure of animal welfare

QBA is a validated method of animal welfare assessment that is being increasingly used in other countries to evaluate and progress developments in animal management and welfare. As an integrated assessment having good correlation with physiology, it will be a very useful technique for evaluation of the welfare of animals undergoing multiple events or processes. <u>We therefore recommend that the Australian livestock industries investigate the application of QBA as one of their animal welfare assessment tools.</u>

10 Bibliography

[1] Fisher, A.D., D.M. Ferguson, I.G. Colditz. 2005. *MLA/AWI animal welfare objective measures workshop outcomes and future steps: Integrated framework to develop objective welfare measures. Project code: AHW.084*, Animal Health and Welfare, Meat & Livestock Australia Limited (MLA): North Sydney.

[2] Stankowich, T., D.T. Blumstein. 2005. Fear in animals: a meta-analysis and review of risk assessment*. Proceedings of the Royal Society B* **272**: 2627-2634.

[3] Welp, T. 2004. Vigilance as a measure of fear in dairy cattle*. Applied Animal Behaviour Science* **87**: 1-13.

[4] Forkman, B., A. Boissy, M.-C. Meunier-Salaün, E. Canali, R.B. Jones. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses*. Physiology and Behavior* **92**: 340-374.

[5] Munksgaard, L., A.M. DePassille, J. Rushen, M.S. Herskin, A.M. Kristensen. 2001. Dairy cows' fear of people: social learning, milk yield and behaviour at milking*. Applied Animal Behaviour Science* **73**: 15-26.

[6] Pajor, E.A., J. Rushen, A.M.B. de Passille. 2000. Aversion learning techniques to evaluate dairy cattle handling practices*. Applied Animal Behaviour Science* **69**: 89-102.

[7] Wemelsfelder, F., E.A. Hunter, M.T. Mendl, A.B. Lawrence. 2000. The spontaneous qualitative assessment of behavioural expressions in pigs: first explorations of a novel methodology for integrative animal welfare measurement *Applied Animal Behaviour Science* **67**: 193-215.

[8] Wemelsfelder, F., T.E.A. Hunter, M.T. Mendl, A.B. Lawrence. 2001. Assessing the 'whole animal': a free choice profiling approach*. Animal Behaviour* **62**: 209-220.

[9] Wemelsfelder, F., I. Nevison, A.B. Lawrence. 2009. The effect of perceived environmental background on qualitative assessments of pig behaviour*. Animal Behaviour* **78**: 477-484.

[10] Minero, M., M.V. Tosi, E. Canali, F. Wemelsfelder. 2009. Quantitative and qualitative assessment of the response of foals to the presence of an unfamiliar human*. Applied Animal Behaviour Science* **116**: 74-81.

[11] Napolitano, F., G. De Rosa, A. Braghieri, F. Grasso, A. Bordi, F. Wemelsfelder. 2008. The qualitative assessment of responsiveness to environmental challenge in horses and ponies*. Applied Animal Behaviour Science* **109**: 342-354.

[12] Rousing, T., F. Wemelsfelder. 2006. Qualitative assessment of social behaviour of dairy cows housed in loose housing systems*. Applied Animal Behaviour Science* **101**: 40-53.

[13] Brscic, M., F. Wemelsfelder, E. Tessitore, F. Gottardo, G. Cozzi, C.G. Van Reenen. 2009. Welfare assessment: correlations and integration between a Qualitative Behavioural Assessment and a clinical/ health protocol applied in veal calves farms. Proceedings of the 18th National Congrress ASPA Palermo, Italy*. Italian Journal of Animal Science* **8**: 601-603.

[14] Walker, J., A. Dale, N. Waran, N. Clarke, M. Farnworth, F. Wemelsfelder. 2010. The assessment of emotional expression in dogs using a Free Choice Profiling methodology*. animal welfare* **19**: 75-84.

[15] Wemelsfelder, F. 2007. How animals communicate quality of life: the qualitative assessment of behaviour*. animal welfare* **16**: 25-31.

[16] Dorman, C., A. Barnes, P.A. Fleming. *I 2009. *Quantifying behavioural expression of horses competing in a 160km endurance ride*, in *31st International Ethological Conference*: Rennes, France.

[17] Dorman, C., A. Barnes, P.A. Fleming. *I 2009. *Measuring the behavioural expression of horses competing in a 160 km endurance ride*, in *5th ISES Conference Ethical Equitation, International Society for Equitation Science*: Sydney.

[18] Dorman, C. 2008. *Qualitative Behaviour Assessment in horses*, in *School of Veterinary & Biomedical Sciences*, Murdoch University: Perth, WA.

[19] Grandin, T. 1997. Assessment of stress during handling and transport. *Journal of Animal Science* **75**: 249-257.

[20] Dwyer, C.M., H.L.I. Bornett. 2004. Chronic stress in sheep: assessment tools and their use in different management conditions. *animal welfare* **13**: 293-304.

[21] Fitzpatrick, J., M. Scott, A. Nolan. 2006. Assessment of pain and welfare in sheep. **62**: 55-61.

[22] De La Fuente, J., M.T. Diaz, M. Ibanex, E. Gonzalez de Chavarri. 2007. Physiological response of rabbits to heat, cold, noise and mixing in the context of transport. *animal welfare* **16**: 41-47.

[23] Wemelsfelder, F. 2008. *Qualitative Behavioural Assessment*, in *Presentation to the School of Veterinary & Biomedical Sciences*: Murdoch University, Perth, WA.

[24] Knowles, T.G. 1998. A review of the road transport of slaughter sheep. *Veterinary Record* **143**: 212-219.

[25] Knowles, T.G. 1999. A review of the road transport of cattle. *Veterinary Record* **144**: 197-201.

[26] Ferguson, D.M., R.D. Warner. 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants. *Meat Science* **80**: 12-19.

[27] Anon. 2002. *Model Code of Practice for the Welfare of Animals: Land Transport of Cattle*. Second Edition ed. Primary Industries Report Series 77: CSIRO Publishing/ PISC (SCARM). 30.

[28] Wickham, S.L., T. Collins, A.L. Barnes, D.W. Miller, D.T. Beatty, C. Stockman, D. Blache, F. Wemelsfelder, P.A. Fleming. 2011. Qualitative behavioural assessment of transport-naive and transport-habituated sheep. *Journal of Animal Science* **In press accepted 13 December 2010**.

[29] Stockman, C.A., T. Collins, A.L. Barnes, D. Miller, S.L. Wickham, D.T. Beatty, D. Blache, F. Wemelsfelder, P.A. Fleming. 2011. Qualitative behavioural assessment of cattle naïve and habituated to road transport. *Animal Production Science* **51 In press accepted 10 December 2010**.

[30] Burrow, H.M., G.W. Seifert, N.J. Corbet. 1988. A new technique for measuring temperament in cattle. *The Australian Society of Animal Production*: 154-157.

[31] Grandin, T. 1993. Behavioural agitation during handling of cattle is persistent over time. *Applied Animal Behaviour Science* **36**: 1-9.

[32] McGilchrist, e. al. in preparation.

[33] Pearson, A.M., R. Kilgour, H. de Langen, E. Payne. 1977. Hormonal responses of lambs to trucking, handling and electrical stunning. *Proceedings of the New Zealand Society for Animal Production* **37**: 243.

[34] Grandin, T. 1996. Factors which impede animal movement in slaughter plans. *Journal of the American Veterinary Medical Association* **209**: 757-759.

[35] Grandin, T. 1998. Solving livestock handling problems in slaughter plans, in *Animal Welfare and Meat Science*, N.C. Gregory, Editor CAB International: Wallingford, Oxon, UK. p. 42-63.

[36] Le Neindre, P. 1989. Influence of rearing conditions and breed on social behaviour and activity of cattle in novel environments. *Applied Animal Behaviour Science* **23**: 129-140.

[37] Grandin, T. 1993. Behavioural principles of handling cattle and other grazing animals under extensive conditions, in *Livestock Handling and Transport* T. Grandin, Editor CAB International: Wallingford, Oxon, UK. p. 63.

[38] Hemsworth, P.H., J.L. Barnett. 2001. Human-animal interactions and animal stress, in *The biology of animal stress - Basic principles and implications for animal welfare*, G.P. Moberg and J.A. Mench, Editors CABI Publishing: Oxon, UK. p. 309-336.

[39] McVeigh, J., P. Tarrant. 1981. The breakdown of muscle glycogen during behavioral stress in normal and beta-adrenoceptor blocked young bulls, in *The Problem of Dark-cutting in Beef. Current Topics of Veterinary Medicine and Animal Science*, D.E. Hood and P.V. Tarrant, Editors Martinus Niijhoff: The Hague, The Netherlands. p. 43&439.

[40] Kenny, F., P. Tarrant. 1987. The behavior of young Freisian bulls during social regrouping at an abattoir. Influence of an overhead electrified wire grid. *Applied Animal Behavior Science* **18**: 233.
[41] Boissy, A. 1995. Fear and fearfulness in animals. *The Quarterly Review of Biology* **70**: 165-191.
[42] Appleman, R.D., R.J. Gustafson. 1985. Source of stray voltage and effect on cow health and performance. *Journal of Dairy Science* **68**: 1554-1567.
[43] Stoebel. 1985. Source of stray voltage and luteinising surge in dairy heifers. *Journal of Dairy Science* **68**: 92 - 96.
[44] Rushen, J., A.A. Taylor, A.M. de Passille. 1999. Domestic animals' fear of humans and its effect on their welfare. *Applied Animal Behaviour Science* **65**: 285-303.
[45] Hemsworth, P.H. 2004. Human-Livestock Interaction, in *The well-being of farm animals: challenges and solutions*, G.J. Benson, Editor.
[46] Ferguson, D. 2009. in *AWOM annual review*. CSIRO Armidale, NSW.
[47] Adams, N.R., J.R. Briegel. 1998. Liveweight and woolgrowth responses to a Mediterranean environment in three strains of Merino sheep. *Australian Journal of Agriculture Research* **49**: 1187-1193.
[48] Dziuk, P.J., R.A. Bellows. 1983. Management of reproduction of beef cattle, sheep and pigs. *Journal of Animal Science* **57**: 355-379.
[49] Smith, J.F., T.W. Knight. 1998. Reproductive management of sheep, in *Reproductive management of grazing ruminants in New Zealand. Occasional publication 12*, E.D. Fielden and J.F. Smith, Editors New Zealand society of animal production: Hamilton, New Zealand. p. 113-144.
[50] Kingwell, R. 2002. Sheep animal welfare in a low rainfall Mediterranean environment: a profitable investment? *Agricultural Systems* **74**: 221-240.
[51] Baile, C.A., J.M. Forbes. 1974. Control of feed intake and regulation of energy balance in ruminants. *Physiological Reviews* **54**: 160-214.
[52] Sibbald, A.M. 1997. The effect of body condition on feeding behavior of sheep with different times of access to food. *Animal Science* **64**.
[53] Schutz, K., D. Davison, L. Matthews. 2006. Do different levels of moderate feed deprivation in dairy cows affect feeding motivation? *Applied animal behavior science* **101**: 253-263.
[54] Jackson, R.E., N.K. Waran, M.S. Cockram. 1999. Methods for measuring feed motivation in sheep. *animal welfare* **8**: 53-63.
[55] Coelho, J.S., J. Polivy, A. Fleming, D. Hargreaves, C.P. Herman, G. Lao. 2010. Inaccessible food cues affect stress and weight gain in calorically-restricted and ad lib fed rats. *Appetite* **54**: 229-232.
[56] Strack, A.M., S.F. Akana, C.J. Horsley, M.F. Dallman. 1997. A hypercaloric load induces thermogenesis but inhibits stress responses in the SNS and HPA system. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* **272**: R840-R848.
[57] Daniel, J.A., B.K. Whitlock, J.A. Baker, B. Steele, C.D. Morrison, D.H. Keisler, J.L. Sartin. 2002. Effect of body fat mass and nutritional status on 24-hour leptin profiles in ewes. *Journal of Animal Science* **80**: 1083-1089.
[58] Akana, S.F., A.M. Strack, E.S. Hanson, M.F. Dallman. 1994. Regulation of activity in the hypothalamo-pituitary-adrenal axisis integral to a larger hypothalamic system that determines caloric flow. *Endocrinology* **135**: 1125-1134.
[59] Jahng, J.W., J.G. Kim, H.J. Kim, B. Kim, D. Kand, J. Lee. 2007. Chronic food restriction in young rats results in depression- and anxiety-like behaviours with decreased expression of serotonin reuptake transported. *Brain Research* **1150**: 100-107.
[60] Tomiyama, A.J., T. Mann, D. Vinas, J.M. Hunger, J. DeJager, S.E. Taylor. 2010. Low calorie dieting increased cortisol. *Psychosomatic Medicine* **72**: 357-364.
[61] Gursoy, E., A. Cardounel, Y. Hu, M. Kalimi. 2001. Bioligcal effects of long-term caloric restriction: adaptation with simultaneous administration of caloric stress plus repeated immobilization stress in rats. *Experimental Biology & Medicine* **226**: 97-102.

[62] Higgs, A.R.B., R.T. Norris, R.B. Richards. 1991. Season, age and adiposity influence death rates in sheep exported by sea. *Australian Journal of Agricultural Research* **42**: 205-214.
[63] Norris, R. 2005. Transport of animals by sea. *Review Scientifique et Tecnique de L'Office International des Epizooties* **24**: 673-681.
[64] Richards, R.B., R.T. Norris, R.H. Dunlop, N.C. McQuade. 1989. Causes of death in sheep exported live by sea. *Australian Veterinary Journal* **66**: 33-38.
[65] Norris, R.T., R.B. Richards. 1989. Deaths in sheep exported by sea from Western Australia - analysis of ship master's reports. *Australian Veterinary Journal* **66**: 97-102.
[66] Yacob, H.T., C. Duranton-Grisez, F. Prevot, J.P. Bergeaud, C. Bleuart, P. Jacquiet, P. Dorchies, H. Hoste. 2002. Experimental concurrent infection of sheep with *Oestrus ovis* and *Trichostrongylus colubriformis*: negative interactions between parasite populations and related changes in the cellular responses or nasal and digestive mucosae. *Veterinary Parasitology* **104**: 307-317.
[67] Kent, J. 1997. Stress in transported sheep. *Comparative Haematology International* **7**: 163-166.
[68] Jones, M., R. Allison. 2007. *The ruminant complete blood count - evaluation and interpretation*. in *CanWest Veterinary Conference*. Banff, Alberta.
[69] Todd, S.E., D.J. Mellor, K.J. Stafford, N.G. Gregory, R.A. Bruce, R.N. Ward. 2000. Effects of food withdrawal and transport on 5- to 10-day-old calves. *Research in Veterinary Science* **68**: 125-134.
[70] Hogan, J., J. Petherick, C. Phillips. 2007. The physiological and metabolic impacts on sheep and cattle of feed and water deprivation before and during transport. *Nutrition Research Reviews* **20**: 17-28.
[71] Verbeek, E., J. Waas, L. McLeay, L.R. Matthews. 2011. The measurement of feeding motivation and associated endocrine responses in pregnant ewes with different body condition scores. *In Press*.
[72] European Union. 2011. *Welfare Quality®: Science and society improving animal welfare in the food quality chain*. Available from: http://www.welfarequality.net/everyone.
[73] Cransberg, P.H., P.H. Hemsworth, G.J. Coleman. 2000. Human factors affecting the behaviour and productivity of commercial broiler chickens. *British Poultry Science* **41**: 272-279.
[74] Moura, D.J., I.A. Naas, D.F. Pereira, R.B.T.R. Silva, G.A. Camargo. 2006. Animal welfare concepts and strategy for poultry production: a review. *Rev. Bras. Cienc. Avic.* **8**: 137-147.
[75] Hemsworth, P.H., J.L. Barnett, G.J. Coleman, C. Hansen. 1989. A study of the relationships between the attitudinal and behavioural profiles of stockpersons and the level of fear of humans and reproductive performance of commercial pigs. *Applied Animal Behaviour Science* **23**: 301-314.
[76] Hemsworth, P.H., G.J. Coleman, J.L. Barnett, S. Borg. 2000. Relationships between human-animal interactions and productivity of commercial dairy cows. *Journal of Animal Science* **78**: 2821-2831.
[77] Hemsworth, P., J.L. Barnett, L. Beveridge, L.R. Matthews. 1995. The welfare of extensively managed dairy cattle: A review. *Applied Animal Behaviour Science* **42**: 161-182.
[78] Hemsworth, P.H. 2007. Ethical stockmanship. *Australian Veterinary Journal* **85**: 194-200.
[79] Hemsworth, P.H., G.J. Coleman. 1998. *Human-livestock interactions: the stockperson and the productivity and welfare of intensively farmed animals*, Oxon, UK: CAB International.
[80] Grandin, T. 1998. Review: Reducing handling stress improves both productivity and welfare. *Professional Animal Scientist* **14**: 1-10.
[81] Petherick, C.J., V.J. Doogan, R.G. Holroyd, P. Olsson, B.K. Venus. 2009. Quality of handling and holding yard environment and beef cattle temperament: 1. Relationship with flight speed and fear of humans. *Applied Animal Behavioural Science* **120**: 18-27.

[82] Lynch, J.J., R.G. Keogh, R.L. Elwin, G.C. Green, B.E. Mottershead. 1983. Effects of early experience on the post weaning acceptance of whole grain wheat by fine wool merino lambs. *Animal Production* **36**: 175-183.
[83] Oreskovich, D.C., B.P. Klein, J.W. Sutherland. 1991. Procrustes Analysis and its applications to free-choice and other sensory profiling, in *Sensory Science: Theory and Aplications in Foods*, H.T. Lawless and B.P. Klein, Editors Marcel Dekker: New York. p. 353-393.
[84] Wickham, S.L. 2011. *The qualitative behavioural assessment of sheep during transport*, in *School of Veterinary and Biomedical Sciences*, Murdoch University: Perth, Western Australia.
[85] Wemelsfelder, F., A.B. Lawrence. 2001. Qualitative assessment of animal behaviour as an on-farm welfare-monitoring tool. *Acta Agriculturae Scandinavica, Section A, Animal Science* **51**: 21-25.
[86] Snowden-Tucker, K. 2007. *Qualitative Behavioural Assessment (QBA) in beef cattle*, in *Veterinary Biology Science*, Murdoch University.
[87] Dijksterhuis, G.B., W.J. Heiser. 1995. The role of permutation tests in exploratory multivariate data analysis. *Food Quality and Preference* **6**: 263-270.
[88] Arnold, G.M., A.A. Williams. 1985. The use of Generalized Procrustes Techniques in sensory analysis, in *Statistical Procedures in Food Research*, J.R. Piggott, Editor Elsevier Applied Science: London. p. 233-253.

11 Appendices

11.1 Appendix 1: Methodology of Qualitative Behavioural Assessment

Retrieval of video footage

From the video footage recorded in each study, one clip of each individual animal was selected from each treatment. These clips were between 30 seconds and 2 minutes in duration. Clips were edited to mask out the non-focal individuals, to obscure the background, or to obscure individual animal ear tag numbers – this was done to minimise observers' bias when viewing the footage. Trimming of the footage was performed with Adobe Premier Pro®; ear tags and backgrounds were masked with Adobe After Effects®. All trimmed and masked clips to be shown to the observers were converted as a windows media player file to enable footage to be screened in a PowerPoint presentation.

Footage of each animal was chosen using the process listed below;

1. This footage will be scanned and clips will be picked out that show view of the particular individual animal (e.g. the head needs to be clearly visible for the entire clip)
2. Footage that is not representative of that individual for that treatment was not be included (e.g. a clip of an animal showing unique or unusual interaction will not be chosen if this is a rare occurrence).
3. Clips (one per individual per treatment) were shown to observers in random order.

Observer sessions

The observer sessions were structured so that all observers worked independently to assess the behavioural expression of the animals. Careful instructions were given to observers before the sessions to ensure they completed the sessions correctly. There were two phases to the QBA sessions as detailed below:

Phase 1 – Term generation. Observers were shown video clips of the animals exhibiting as wide range of behaviour as possible. After watching each clip, observers were asked to write down in the following 2 minutes a series of words that described the animal's behavioural expression. The interposition of clips was based on trying to stimulate the observers to record a wide range of terms that describe the animals. Careful instructions were given at the beginning of the Phase 1 session to ensure that observes understood that:

- There was no right answer and that terms can vary between individuals.
- Behavioural expression is a layered effect (e.g. animals can be both nervous and distracted at the same time) and therefore there will be several terms that can be developed for each clip.
- Terms need to describe not <u>what</u> the animal is doing, but <u>how</u> the animal is doing it.

Following the term generation process, each observer's terms were edited as follows:

- Terms that were physical description of the animal (vocal, noisy) or describe what the animal is doing (chewing, tail flicking) were removed.
- Terms which pre-empt what the animal is thinking were removed (hungry, food seeking).

- Negative terms (e.g. those starting with un or non-) were changed to their positive meaning (e.g. uncomfortable to comfortable).

Phase 2 – Quantification. The observer's unique list of terms was printed with each term attached to a visual analogue scale of the same lengths with minimum on left hand side and maximum on right hand side. The terms were ordered alphabetically to randomise the order of terms and ensure that terms with a similar meaning will not be listed one after the other. In Phase 2, observers attended a series of sessions where they viewed a series of video clips of individual animals from the treatment. Observers were given detailed instructions on how to score using the visual analogue scale before session commencement. Observers viewed each clip and were given time following the clip to score on every one of their terms on the visual analogue scale.

Analysis of results

The observer scores generated from the video clips were analysed by Generalised Procrustes Analysis (GPA) using a specialised software edition written for Françoise Wemelsfelder (GenStat 2008). A more detailed description of this analysis can be found elsewhere [8].

The position of each mark (distance in millimetres from the start of the line to where the observer has marked was measured) were then inputted into individual observer excel files that are then analysed using Generalised Procrustes Analysis (GPA).

GPA calculates agreement between observer perceptions and therefore consensus dimensions. This is a multivariate statistical analysis that does not rely on fixed variables. GPA can be thought of as a pattern making mechanism. It is based on the assumption that each observer assessment in a treatment will be comparable to each other. Therefore, even though each observer has different terms, the assessment of each observer in a treatment will converge. Coordinates of the convergent configuration or 'best fit' consensus profile are calculated. GPA provides a statistic which indicates the level of consensus (i.e. the percentage of variation between observers that is explained by each GPA dimension) that has been achieved.

Through Principle Components Analysis (PCA), the number of dimensions of the consensus profile is reduced to one or more main dimensions explaining the majority of variation between the observed animals. Each animal receives a score on each of these main consensus dimensions. The calculation of the consensus profile takes place independently of the semantic information provided by the terminologies chosen by the observers (semantic interpretation of this consensus profile takes place after its calculation. This is an important aspect of the calculation, since it ensures that the dimensions are generated through a completely objective mathematical process; no interpretation of observers' terms is required to carry out this calculation.

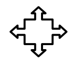
Each observer's terms are fitted against the consensus profile resulting in individual observer word charts. The dimensions are subsequently interpreted by viewing individual observer word charts and attaching descriptive names to each dimension. The individual subjects or animals are also fitted to the consensus profile and subject plots map the position of subjects (animals) against the GPA consensus dimensions. For a more detailed explanation see Wemelsfelder et al. (2000, 2001).

Stepwise example of GPA analysis process

Calculate the consensus between observers:

The scoring pattern of individual observers for all terms for all subject is determined (i.e. the scoring pattern of each individual observer scores in their excel spreadsheet) In this particular example there will be 2 observers:

Observer 1 | Observer 2

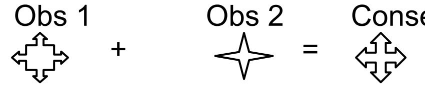
 N1: (scoring pattern for all terms for all animal subjects)  N1:

Each observer scoring pattern has dimensions (represented in the above scoring patterns by points on the shape=4). The number of dimensions is equal to the maximum number of terms recorded from an observer. To achieve this, zeros are added to make up extra number of dimensions that may be needed for each scoring pattern e.g. if one observer only had 3 terms; however the maximum number of terms developed out of any of the observers is four, zeros would be added to make up a fourth dimension for that particular observer.

The best fit of the scoring patterns between observers for all terms of all subjects is then determined (termed the consensus profile).

e.g.

Obs 1 + Obs 2 = Consensus profile or best fit scoring pattern



Calculate significance of consensus profile

GPA provides a statistic (called the Procrustes statistic) which indicates the level of consensus (i.e. the percentage of variation explained between observers) that has been 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 permutation or randomisation test [87]. This procedure rearranges at random each observer's scores and produces new permutated data matrices. By applying GPA to these permutated matrices, a 'randomised' profile is calculated. This procedure is repeated 100 times, providing a distribution of Procrustes statistics indicating how likely it is to find an observer consensus based on chance alone. Subsequently a one-way *t*-test (df=99) is used to determine whether the actual observer consensus profile falls significantly outside the distribution of randomised profiles.

$$t_{99} = \frac{(\text{mean consensus} - \text{mean simulation})}{\sqrt{\text{simvar} \times (1 + 1/100)}}$$

A significant result indicates the actual observer consensus profile falls significantly outside the distribution of randomised profiles and therefore the consensus between observers is real and not due to random chance. An observer plot (Figure 1) indicates agreement of scoring patterns of each observer with the consensus profile.

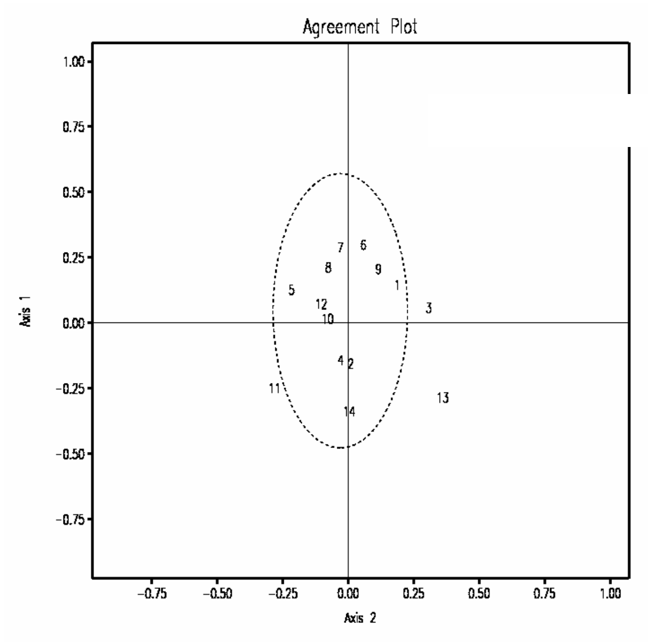
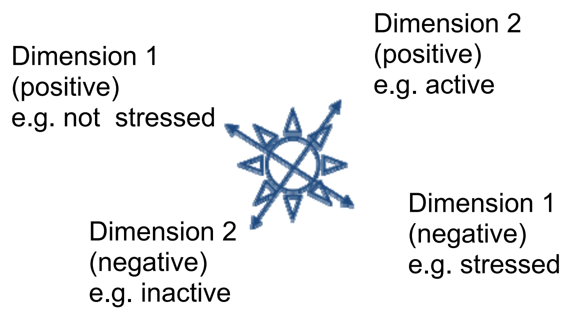


Figure 1. Observer Plot. One of the outputs of QBA.

Each point in Figure 1 indicates an observer. The dashed line indicates the 95% confidence interval and observer outliers are significantly outside this line. Axes indicate the distance observers scoring patterns are from the main consensus. This plot indicates that there are no extreme outliers.

Determining the dimensions of the consensus profile

The main dimensions in the consensus profile are then determined.



The main dimensions are determined using Principal Components Analysis (PCA). The outcome of this analysis is a GPA Scree Plot (Figure 2) that depicts the amount of variation in the data explained by each GPA dimension. The first 3 dimensions explained the majority of variation in all studies carried out as part of this project.

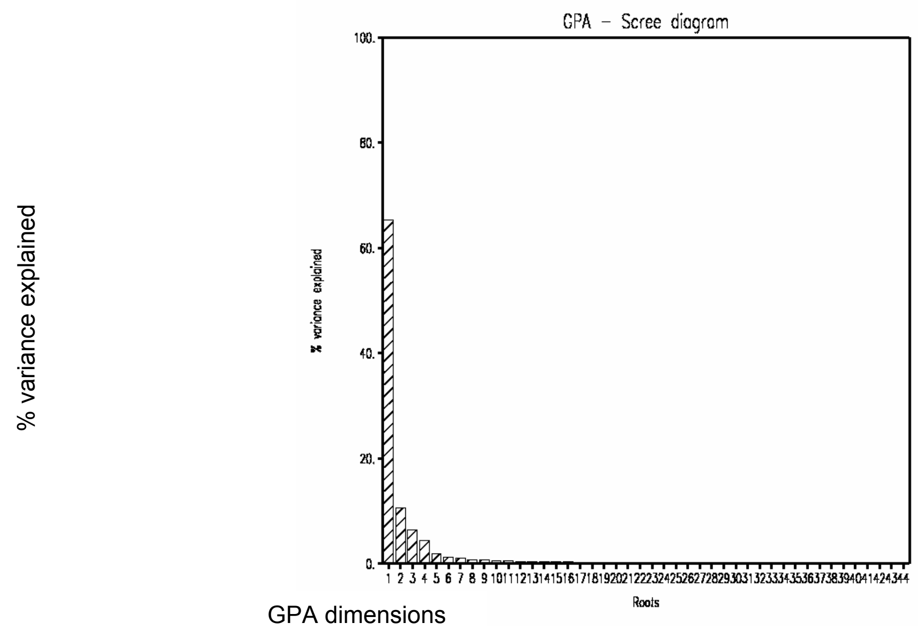


Figure 2. Scree diagram. The bars represent the degree of variation that can be explained by each GPA dimension.

Correlation of main dimensions with individual observer word charts

Individual observer word charts plot terms chosen by each observer against each of the GPA dimensions. This is shown for a single observer, in Figure 3, as an example.

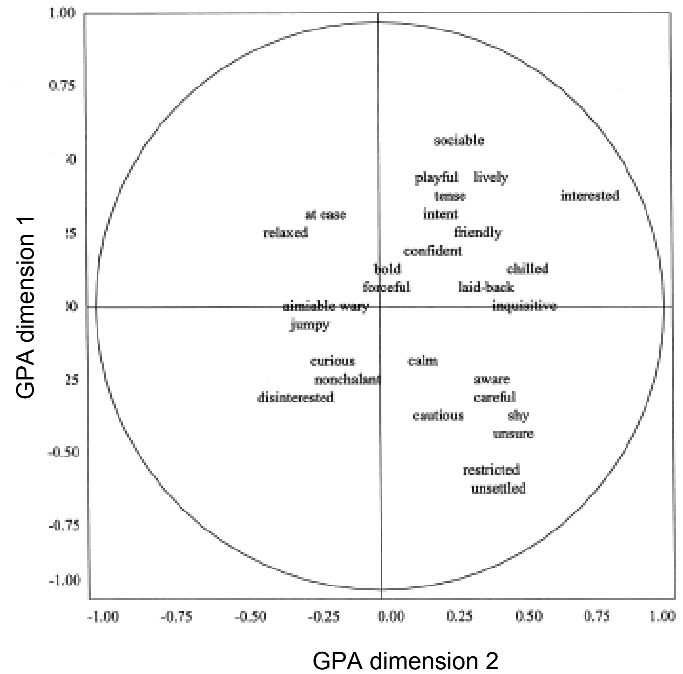


Figure 3. Observer word chart assembling pig behavioural expression from Wemelsfelder et al. (2000).

Figure 3 shows how this observer's terms correlate with GPA dimension 1 and 2. These word charts can be used to name the dimensions to make the GPA dimensions more accessible to understanding (<u>although we note that naming the GPA dimensions is done after they are determined, and therefore does not influence the integrity of the data in any</u>

way). In this particular chart you can see that within GPA dimension 1, the terms on the positive axis could be described as generally not stressed or positive welfare, while those on the negative axis describe terms associated with stressed or negative welfare. All observer word charts need to be viewed to determine if the terms in each dimension are similar in meaning for all observers. This will be the case if the dimension has a large amount of variance explained (see Figure 2). This is a transparent process because the highest weighting terms in each dimension for each observer and the most frequently used terms for each dimension are inputted into a table (see Table 3 for an example from this project). Therefore readers are free to attach preferred titles to each dimension. The process involves calculating how the coordinates of the consensus profile correlate with the coordinates of each of the individual observer matrices. This process shows the level of correlation of terms used by each observer with dimension axes. The higher a term's correlation with an axis, the more weight it has as a descriptor for that axis.

The final step is then to summarise these word charts and select specific terms/labels describing these main GPA dimensions. The details of this computational approach are published elsewhere [7,88].

Table 3. Terms used by observers to describe cattle behavioural expression during transport. Terms for all observers, showing the highest negative and positive correlation with generalised Procrustes analysis (GPA) dimensions 1, 2 and 3 of the consensus profile. Terms shown have a correlation of >0.7 (high values) and <–0.7 (low values) for GPA dimension 1 and >0.5 (high values) and <–0.5 (low values) for GPA dimensions 2 and 3. Order of terms is determined first by number of observers to use that term (in parentheses if > 1) and second by weighting of each term

GPA dimension	Negative correlation	Positive correlation
1	Calm (13), comfortable (7), relaxed (7), content (4), at ease (3), bored (2), settled (2), quiet, indifferent, predictable, happy, subdued, accepting, composed, fearful, controlled	Agitated (11), restless (7), stressed (7), anxious (6), flighty (5), nervous (5), alert (3), frightened (3), scared (3), worried (3), alarmed (2), concerned (2), fearful (2), frustrated (2), panicked (2), unsure (2), wants to escape (2), claustrophobic, confused, content, distressed, evasive, excitable, fidgety, hemmed in, impatient, inquisitive, lively, on edge, perplexed, tense, terrified, toey, trapped, twitchy unnerved, wants to leave
2	Sedate, upset, annoyed, frightened, weary, nervous, fatigued, sad, bored, happy	Alert (5), curious (4), aware (4), inquisitive (3), interested (2), focussed, quiet, relaxed, wary, shy, watchful
3	Weary, soothed, exhausted, depressed, irritated, alert, threatened, sad	Alert (3), questioning

Compare scores of individual subjects on dimensions of consensus profile

How each animal (e.g. each individual from each treatment) compares to the consensus profile is shown in the subject plots (Figure 4). This plot shows how each individual fits with the consensus profile dimensions. In the chart below the subjects are clearly divided by dimension 2, but demonstrate significant overlap in Dimension 1.

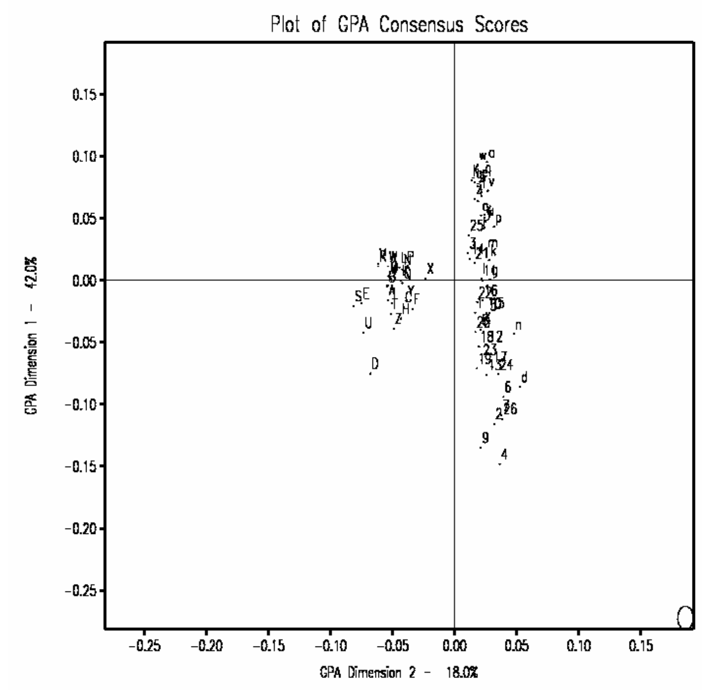


Figure 4. Individual subject plot (each number/letter represents an individual animal entered into the GPA). The circle in the lower right hand corner of the graph indicates the deviation between individuals entered into the GPA (the larger the circle the more deviation). The small size of this circle indicates there was little deviation for this particular analysis.

Determining how physiological responses of cattle are related to behavioural expression

The final step in the complete analysis is to carry out a Principal Components Analysis (PCA) to analyse the relationship between different physiological variables and the GPA dimensions (or the consensus profile). This will serve to determine which GPA dimensions are associated with known indicators of physiological stress or poor welfare.